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Report

Exome Sequencing Reveals *VCP* Mutations as a Cause of Familial ALS

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SUMMARY

Using exome sequencing, we identified a p.R191Q amino acid change in the *valosin-containing protein* (*VCP*) gene in an Italian family with autosomal dominantly inherited amyotrophic lateral sclerosis (ALS). Mutations in *VCP* have previously been identified in families with Inclusion Body Myopathy, Paget disease, and Frontotemporal Dementia (IBMPFD). Screening of *VCP* in a cohort of 210 familial ALS cases and 78 autopsy-proven ALS cases identified four additional mutations including a p.R155H mutation in a pathologically proven case of ALS. VCP protein is essential for maturation of ubiquitin-containing autophagosomes, and mutant VCP toxicity is partially mediated through its effect on TDP-43 protein, a major constituent of ubiquitin inclusions that neuropathologically characterize ALS. Our data broaden the phenotype of IBMPFD to include motor neuron degeneration, suggest that *VCP* mutations may account for ~1%–2% of familial ALS, and provide evidence directly implicating defects in the ubiquitination/protein degradation pathway in motor neuron degeneration.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease clinically characterized by upper and lower motor neuron dysfunction resulting in rapidly progressive paralysis and death from respiratory failure. The pathological hallmarks of the disease include pallor of the corticospinal tract due to

loss of motor neurons, the presence of ubiquitin-positive inclusions within surviving motor neurons, and the deposition of pathological TDP-43 aggregates (Neumann et al., 2006). Median survival is 3 years from symptom onset, reflecting the devastating nature of the disease and the lack of effective disease-modifying therapies for this disorder.

The identification of genes underlying rare familial forms of ALS has had significant impact on our understanding of the molecular mechanisms underlying typical ALS (Rowland and Shneider, 2001). Much of the ongoing molecular biology work in the ALS field is based on the discovery of mutations in genes encoding *SOD1*, *TDP-43*, and *FUS* (Kwiatkowski et al., 2009; Rosen et al., 1993; Sreedharan et al., 2008; Vance et al., 2009). Each new gene implicated in the etiology of ALS provides fundamental insights into the pathogenesis of motor neuron degeneration and facilitates disease modeling and the design and testing of targeted therapeutics; hence, there is much interest in the identification of novel genetic mutations.

Population-based epidemiological studies estimate that approximately 5% of ALS cases are familial in nature (Chiò et al., 2008). Of these, approximately 15% are caused by mutations in the *SOD1* gene (Chiò et al., 2008), and a further 3%–4% of cases are due to pathogenic variants in either the *TDP-43* or *FUS* gene (Kabashi et al., 2008; Chiò et al., 2009; Mackenzie et al., 2010). Linkage and positional cloning studies aimed at finding additional familial ALS genes have been complicated by a lack of samples from large, multigenerational families, mainly due to the dramatically shortened lifespan associated with the diagnosis.

Whole-exome sequencing is a new technique that exploits the massively parallel sequencing capabilities of next-generation platforms to rapidly identify rare variants in the ~1% of the genome that codes for proteins. The power of exome sequencing stems from the fact that the majority of monogenic diseases arise from mutations within this protein-coding portion of the genome, and the ability of this technology to find new causative genes has already been demonstrated (Choi et al., 2009; Ng et al., 2009, 2010). Furthermore, whole-exome sequencing is now a realistic strategy for detecting pathogenic variants in small families where linkage analysis would not be possible due to a shortage of DNA samples from affected individuals.

In this report, we describe exome sequencing of a family with an autosomal dominant ALS phenotype, in which *SOD1*, *TDP-43*, and *FUS* mutations were previously excluded, in an attempt to identify the underlying genetic lesion responsible for disease.

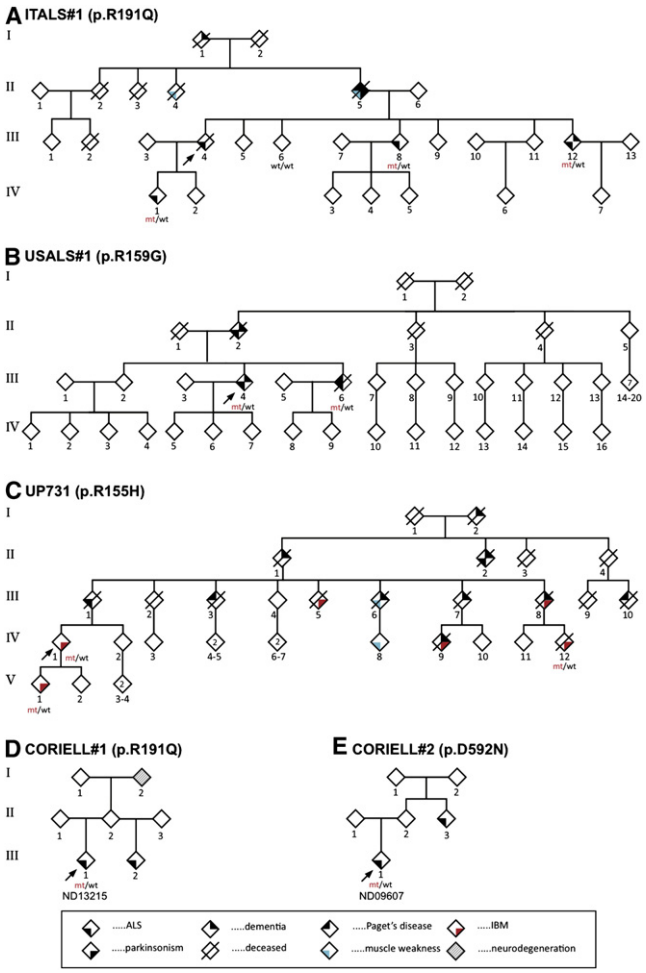

Figure 1. Pedigrees of Patients with *VCP* Mutations

(A–E) Pedigrees of patients with VCP mutations. Mutant alleles are indicated by *mt*, whereas wild-type alleles are indicated by *wt*. Probands are indicated by arrows. Sex of the pedigree members is obscured to protect privacy. Pedigrees of the familial samples from the NINDS repository at Coriell have been constructed based on available family history information from the Coriell website (http://www.coriell.org/).

RESULTS

Description of the ITALS#1 Pedigree

We studied a four-generation Italian family (ITALS#1) in which four individuals had been diagnosed with ALS. The pedigree of this family is shown in Figure 1A, and the clinical features are detailed in the Supplemental Information (available online) and are summarized in Table 1. Briefly, all four patients with ALS presented with limb-onset motor neuron symptoms (Figure 1A, individuals III:4, III:8, III:12, IV:1). A parent of the proband (II:5) died at age 58 with dementia, parkinsonism, Paget disease, and upper limb muscle weakness, and a sibling of this individual (II:4) used a wheelchair prior to death at age 51 from respiratory failure. The clinical course of the four ALS patients was characterized by unequivocal upper and lower motor signs affecting all four limbs and bulbar musculature resulting in progressive quadriparesis and disability. Neuropsychological testing of individual III:12 performed within 1 year of symptom onset was suggestive of mild frontal lobe dysfunction. Nerve conduction studies and electromyography in each case demonstrated widespread ongoing denervation and chronic reinnervation changes consistent with ALS. Alkaline phosphatase was within normal limits, thereby excluding the diagnosis of concomitant Paget disease. DNA samples were available from three individuals of generations III and IV who had been diagnosed with ALS by neurologists specializing in ALS (A. Chiò and J.M.). Autopsy

Table 1. *VCP* Mutations in ALS Cases with Onset and Disease Duration Data

Pedigree[a]	Individual ID	Mutation[b]	Diagnosis	Age at Onset	Site of Onset	Cognitive Impairment	Disease Duration from Onset (Vital Status)	ALP (U/L)	CPK (U/L)
ITALS#1	III:4	p.R191Q c.961G > A[c]	ALS	51	upper limb	no impairment; not formally tested	ventilated at 11 months (died at 29 months)	normal	normal
	III:8	p.R191Q c.961G > A	ALS	53	upper and lower limbs	no impairment on formal testing	ventilated at 34 months (alive)	normal	normal
	III:12	p.R191Q c.961G > A	ALS-FTD	50	lower limb	impaired on formal testing	alive at 4.5 years	normal	385
	IV:1	p.R191Q c.961G > A	ALS	37	lower limb	no impairment on formal testing	alive at 4 years	normal	500–900
USALS#1	III:4	p.R159G c.864C > G	ALS-FTD	53	lower limb	impaired on formal testing	ventilated at 24 months (alive)[d]	no data	no data
	III:6	p.R159G c.864C > G	ALS	46	lower limb	no impairment; not formally tested	died after 5 years	elevated	no data
Coriell#1	ND13215	p.R191Q c.961G > A	ALS	42	lower limb	no impairment; not formally tested	alive at 12 years	no data	no data
Coriell#2	ND09607	p.D592N c.2163G > A	ALS	52	bulbar	no impairment; not formally tested	died within 1 year	no data	no data
UP731	III:1[e]	c.853G > A p.R155H	ALS	53	upper limb	no impairment; not formally tested	died at 39 months	normal	normal

ALP, alkaline phosphatase; *CPK*, creatine phosphokinase. See also Table S2 for demographics and clinical details of ALS cases and controls screened for *VCP* mutations.
[a] See Figure 1 for pedigrees.
[b] Nucleotide position is based on NM_007126.3, and amino acid numbering is based on NP_009057.1.
[c] III:4 of family ITALS#1 is an obligate carrier of this mutation.
[d] Respiratory failure hastened by coexisting pulmonary disease.
[e] III:1 of family UP731 is an obligate carrier of this mutation.

material was not available from any members of the ITALS#1 pedigree.

Exome Sequencing of the ITALS#1 Pedigree

Exome sequencing was performed on DNA obtained from two affected members of the ITALS#1 family (Figure 1A, ITALS#1, III:12, and IV:1) using SureSelect Exome target enrichment technology followed by paired-end sequencing on a Genome Analyzer IIx. This generated 3.48 gigabases of alignable sequence data for patient III:12 (median read depth = 40, base pairs with >10 reads = 79.9%) and 3.53 gigabases for patient IV:1 (median read depth = 37, base pairs with >10 reads = 76.1%). The same portion of the exome was sequenced in both patients (r^2 = 0.92 comparing the number of reads per targeted sequence [bait] in each sample). After filtering, there were 75 heterozygous coding variants and 13 heterozygous coding indels that had not previously been identified as variants in the 1000 Genomes or dbSNP databases, and which were shared by both patients (Figure 2). Sanger sequencing of these variants in an additional affected member (III:8) reduced this list to 24 variants and nine indels (see Table S1 available online). Of these, only four variants were within the 18 genomic regions with a LOD score greater than zero based on linkage analysis of the ITALS#1 pedigree (see Figure S1 available online; Sobreira et al., 2010), were not present in the exome data of 200 neurologically normal control samples, and were predicted to be damaging to protein structure using SIFT software analysis.

Each of these variants segregated with disease within the family, and therefore were plausible candidates for the genetic defect responsible for disease. Within this list, we identified a c.961G > A nucleotide change that resulted in a p.R191Q amino acid change in the *VCP* gene. This particular mutation has been previously described as the cause of an unusual syndrome characterized clinically by the triad of Inclusion Body Myopathy with early-onset Paget disease of the bone and Frontotemporal Dementia (IBMPFD) (Watts et al., 2004), and characterized pathologically by the presence of TDP-43 staining ubiquitin inclusions in muscle and frontal cortex neurons (Ju and Weihl, 2010). Since ALS is also characterized by the deposition of TDP-43 inclusions (Neumann et al., 2006), and the identified mutations are known to alter VCP structure and impair VCP function and to be pathogenic in humans (Custer et al., 2010; Fernández-Sáiz and Buchberger, 2010; Ju et al., 2009; Ritson et al., 2010; Tang et al., 2010; Tresse et al., 2010; Watts et al., 2004), we postulated that mutations in the *VCP* gene could also give rise to an ALS phenotype.

Mutational Screening of VCP in Additional Familial ALS Samples

To test our hypothesis and to establish the frequency of *VCP* mutations in ALS, we sequenced 210 cases from unrelated

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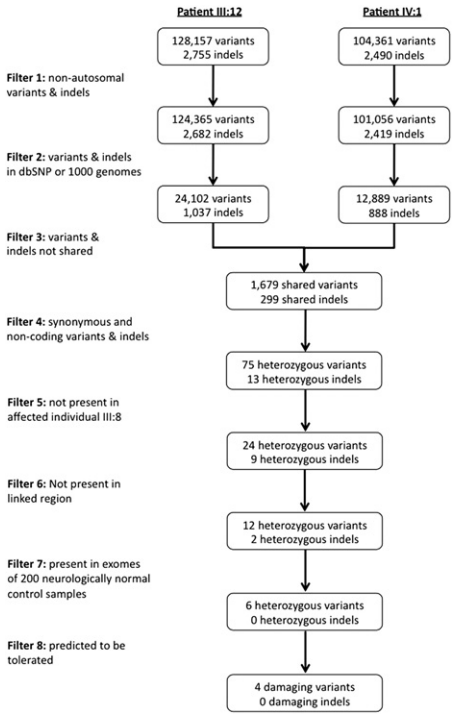

Figure 2. Filters Applied to Variants and Indels Detected by Exome Sequencing in Affected Individuals of the ITALS#1 Pedigree

Regions with LOD score greater than zero were based on linkage analysis of ITALS#1 pedigree (see also Figure S1). Variants that were found in exome data of 200 neurologically normal control subjects were filtered. Prediction of effect of variant on protein function was based on SIFT analysis (http://sift.jcvi.org/).

families and 78 pathologically proven ALS cases, and found four additional mutations in five individuals diagnosed with ALS (Table 1). None of these mutations were found in 569 U.S. control samples, in 636 Italian control samples, in 364 African and Asian samples that are part of the Human Gene Diversity Panel (HGDP), or in either the dbSNP or 1000 Genomes databases, and all of them affected amino acids that were highly conserved across species (Figure 3).

The additional mutations included a p.R159G mutation that segregated with disease in a U.S. family whose various members have carried diagnoses of ALS, frontotemporal dementia (FTD), and Paget disease (see Figure 1B for pedigree structure and Supplemental Information for clinical description). Though to our knowledge mutation p.R159G has not been previously identified, different mutations involving the same codon (p.R159H and p.R159C) have been previously described as a cause of IBMPFD (Daroszewska and Ralston, 2005; Kimonis et al., 2008a; Schröder et al., 2005). We also detected a second example of the p.R191Q mutation found in the ITALS#1 family (Figure 1C). This patient (ND13215) carried the same 199.8 kb haplotype across the *VCP* locus as affected members of the ITALS#1 pedigree, and identity-by-descent analysis based on genome-wide genotyping data confirmed that he was cryptically related to the Italian kindred (PI_hat between ND13215 and individual IV:4 of ITALS#1 = 0.251, indicating that these cases were second degree relatives). A novel p.D592N mutation was detected in a separate ALS family (Figure 1D). Protein structure analysis revealed that residue D592 is directly adjacent to the central pore formed by the VCP hexamer (see Figure S2).

Finally, we identified a p.R155H mutation in a large, multigenerational family with IBMPFD (Watts et al., 2004), in which an obligate carrier of the *VCP* mutation (Figure 1C, III:1) had been clinically diagnosed with ALS without evidence of IBM or Paget disease. Autopsy 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 (Figure 4, and Supplemental Experimental Procedures for detailed clinical summary and pathological description).

The clinical phenotypes of the patients carrying *VCP* mutations are summarized in Table 1. Consistent with *VCP* as a known cause of frontal lobe dysfunction, at least one member of the ITALS#1 family and two affected members of the family carrying the p.R159G mutation were diagnosed with significant cognitive impairment. Apart from the deceased parent of the proband in the ITALS#1 family and an affected member of the USALS#1 pedigree, none of the other patients reported personal or family history of bone disease or myopathy. Histological examination of a tibialis anterior muscle biopsy from patient III:4 of family ITALS#1 was consistent with denervation and reinnervation, and did not show pathological features of IBM (see Figure S3). At least five patients with *VCP* mutations had a rapidly progressive disease course in that they either died or required mechanical ventilation within 3 years of symptom onset.

DISCUSSION

In this paper, we used whole-exome sequencing to identify a pathogenic *VCP* variant in an autosomal dominant Italian family with an ALS phenotype, and subsequently found that *VCP* mutations were present in ~1%–2% of our large cohort of familial ALS cases from unrelated families. Though the frequency of *VCP* mutations in familial ALS will have to be confirmed in independent cohorts, this mutational frequency is comparable to that reported for *TDP-43* and *FUS* mutations (Kabashi et al., 2008; Chiò et al., 2009; Mackenzie et al., 2010), highlighting the relative significance of this gene as a cause of familial ALS. Furthermore, our study shows that this new genomics technique can successfully be applied to find causative mutations in autosomal dominant neurodegenerative disease, where DNA is available from such a limited number of cases that linkage and positional cloning would not be feasible.

Although we have nominated *VCP* as the causative mutation in our Italian family, it remains possible that one of the other three shared variants identified by the whole-exome sequencing process is the true cause of disease. In addition, although the depth of sequencing coverage in our samples was adequate to identify several thousands of variants, other mutations may have been missed, either due to stochastic variations in sequence capture or coverage, or because they lie outside of coding regions.

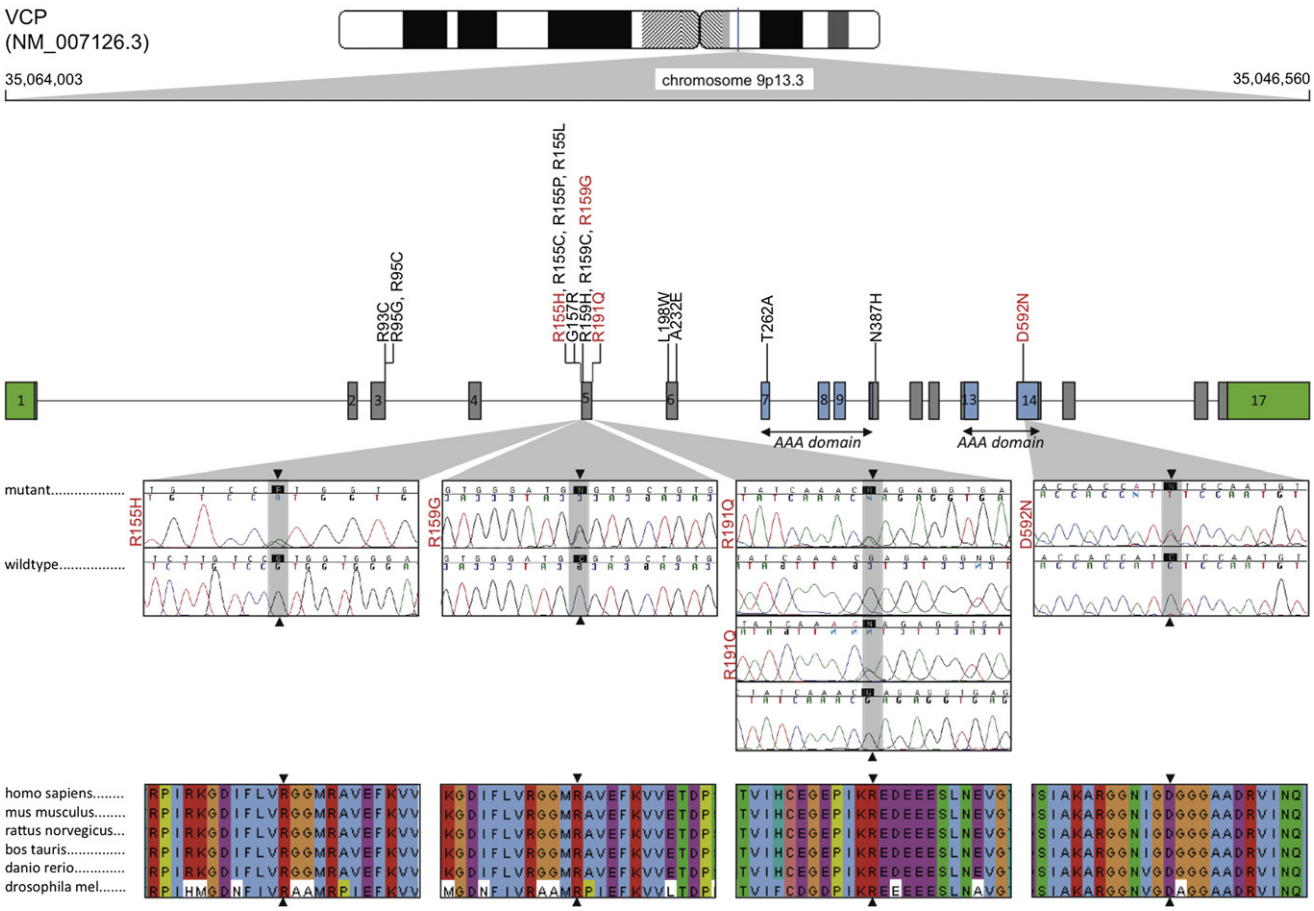

Figure 3. Distribution of *VCP* Mutations Detected in Familial ALS Patients

An ideogram of chromosome 9 is shown at the top. The 17 exons of *VCP* are numbered. Mutations detected in familial ALS cases are indicated in red, whereas mutations previously described to cause IBMPFD are shown in black. Corresponding chromatograms showing mutant and wild-type alleles are as indicated, and conservation of amino acid residue across species is highlighted at the bottom (generated using the ClustalW2 online tool, http://www.ebi.ac.uk/clustalw/). See also Figure S2 for location of mutations on VCP protein structure.

Against this, there are four pieces of genetic data supporting the pathogenicity of the p.R191Q *VCP* variant in our family. First, the p.R191Q variant was found in three affected individuals within the ITALS#1 family, and a fourth affected member (patient III:4) was an obligate carrier. Second, we did not find this mutation in the dbSNP database, in the 1000 Genomes project database, or in 1569 control subjects (3138 control chromosomes) sequenced in our laboratory. Third, the same amino acid change has been previously described as pathogenic in two large, multigenerational IBMPFD families (Watts et al., 2004; S. Spina et al., 2008, FASEB J., abstract). Fourth, we found several other instances of *VCP* mutations in familial ALS cases. These included a second example of the same p.R191Q variant in an apparently unrelated familial case (Coriell#1) that carried a 199.8 kb haplotype across the *VCP* locus identical to those of affected members of the ITALS#1 pedigree, suggesting that these individuals shared a common ancestor. In addition to these genetic data, ALS and IBMPFD share a common pathology in that both conditions lead to the deposition of ubiquitin-positive TDP-43 inclusions in diverse tissue types including neurons of the frontal cortex (Ince et al., 1998; Weihl et al., 2009). Furthermore, a missense mutation in *vacuolar protein sorting 54*, the mouse homolog of *VCP*, is responsible for motor neuron degeneration in the wobbler mouse, an animal model of ALS (Schmitt-John et al., 2005).

Although it may be retrospectively argued that *VCP* was an obvious candidate gene for the causation of ALS, there are no prior publications of mutational screening of this gene in ALS patients. Furthermore, weakness in affected individuals carrying *VCP* mutations has been uniformly ascribed to muscle involvement by IBM, even when their clinical features were clearly consistent with motor neuron degeneration (Kimonis et al., 2008b; Kumar et al., 2010). Indeed, until now, a diagnosis of ALS in patients carrying *VCP* mutations was regularly considered to be erroneous (Kimonis et al., 2008a; Weihl et al., 2009). It is noteworthy that the samples used in our study for exome sequencing and subsequent mutational screening of *VCP* were selected because they had been diagnosed as having familial ALS. The presence of Paget disease and FTD in the ITALS#1

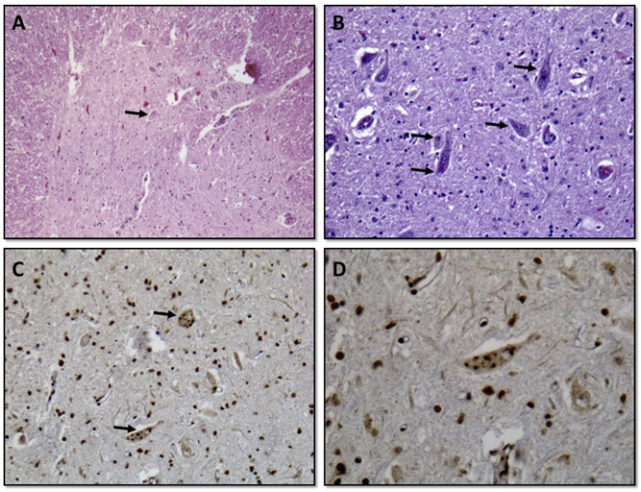

Figure 4. Histopathological Images from Autopsy Tissue, Family UP731, Individual III:1

(A) Haematoxylin-eosin (H&E) stained low-power view (100×) of a lumbar spinal cord section demonstrates extensive motor neuron loss in the anterior horn, with a few remaining motor neurons (arrow). (B) Motor neurons from the hypoglossal nucleus in the medulla with abundant round eosinophilic cytoplasmic inclusions consistent with Bunina bodies (arrows), H&E, 200×. (C and D) 200× and 400×, respectively: TDP-43 immunohistochemistry on the hypoglossal nucleus shows TDP-43 positive punctate cytoplasmic inclusions with concomitant loss of nuclear stain in two motor neurons (arrows). Normal nuclear staining is preserved in the surrounding normal cells. Of note, additional tissue was not available from this autopsy. The current images were generated by removing the coverslip from selected old slides, followed by destaining and processing for TDP-43 immunohistochemistry. See also Figure S3 for histopathological images of a muscle biopsy from patient III:4 of the ITALS#1 family.

and the USALS#1 families was only established after the *VCP* mutations were discovered.

In contrast to the previous publications in which motor neuron degeneration was misdiagnosed, our data clearly indicate that mutations in the *VCP* gene can be associated with a classical ALS phenotype. In this study, all of the cases in which we found *VCP* mutations displayed upper and lower motor neuron signs consistent with a diagnosis of ALS on the basis of the El Escorial diagnostic criteria, which are known to be highly specific for ALS (Chaudhuri et al., 1995). Apart from a single member of the USALS#1 family who developed Paget disease prior to onset of muscle weakness, none of the analyzed cases displayed atypical features, such as biochemical or radiographic signs of bone disease. EMG studies in each patient revealed widespread ongoing denervation and chronic reinnervation changes, which are the pathognomonic neurophysiological features of ALS. Two of the affected individuals in the original Italian family followed a rapidly progressive course, requiring mechanical ventilation within 2 years of symptom onset. Such rapid progression is not seen in IBM patients, where life expectancy is typically not significantly shortened (Amato and Barohn, 2009). Finally, autopsy data from an affected individual carrying the p.R155H mutation demonstrated brainstem and spinal cord motor neuron loss in the presence of TDP-43 staining and Bunina bodies, thereby pathologically confirming the clinical diagnosis of ALS (Ince et al., 1998; Neumann et al., 2006).

Despite these clinical data supporting the presence of fulminant motor neuron degeneration, the possibility that these patients had coexisting IBM cannot be excluded. Muscle involvement in the pathogenesis and progression of ALS has long been debated (Abmayr and Weydt, 2005), and *VCP*-associated disease may represent an instance in which muscle and nerve are simultaneously affected, thus offering an opportunity to dissect this relationship. At the very least, our data widen the clinical symptomatology associated with *VCP* mutations to include an ALS phenotype, and suggest that familial ALS patients should be monitored for features of IBM and osteoclast dysfunction (for example, by screening for elevated serum alkaline phosphatase).

VCP is a highly conserved AAA+-ATPase that mediates ubiquitin-dependent extraction of substrates from multiprotein complexes for subsequent recycling or degradation by the proteasome. Through this activity, VCP regulates a variety of cellular functions including cell signaling, cell cycling, organelle biogenesis, autophagy, and certain aspects of proteostasis. Mutant VCP toxicity is mediated in part through altered TDP-43 metabolism (Ritson et al., 2010). TDP-43 pathology has been observed in spinal cord motor neurons of mouse models of *VCP* mutations (Custer et al., 2010), and mutant VCP expression leads to redistribution of TDP-43 from the nucleus to the cytoplasm in vitro and in vivo (Custer et al., 2010; Gitcho et al., 2009; Ritson et al., 2010).

Despite this, the molecular mechanisms by which mutations in the *VCP* gene cause motor neuron degeneration and the various features of the IBMPFD syndrome are not clear. It is known that VCP is essential for maturation of ubiquitin-containing autophagosomes, and that disease-causing mutations in *VCP* impair this process (Alexandru et al., 2008; Halawani and Latterich, 2006; Ju et al., 2009; Ju and Weihl, 2010). It is plausible that these mutations disrupt its protein removal function, leading to the accumulation of degraded proteins observed as ubiquitinated inclusions within the cell. It is noteworthy that the known mutations of *VCP* predominantly cluster within the cleft that separates the D1 and N domains of the protein (see Figure S2), and may interfere with VCP function by impairing the relative movement of these domains that occurs in response to ATP hydrolysis (Dai and Li, 2001; Tang et al., 2010). Our data provide evidence directly implicating defects in the cellular machinery of the ubiquitination/protein degradation pathway in motor neuron degeneration. Additional studies will be required to confirm this putative pathophysiological function of mutant VCP, and to determine if the location of the mutations within the VCP protein influence which tissues are affected by ubiquitin deposition and account for the heterogeneous clinical symptomatology known to exist within this syndrome (van der Zee et al., 2009).

In summary, our data demonstrate the utility of exome sequencing in determining the genetic causes of familial neurodegeneration, and indicate that mutations of the *VCP* gene are a cause of familial ALS. Another interpretation of our data, which is compatible with the conclusion that mutations in *VCP* are

a cause of familial ALS, is that the phenotypic spectrum of IBMPFD is broader than previously recognized and extends to include ALS. Our study also potentially widens the clinical spectrum associated with ALS to include bone dysfunction and myopathy, and provides further insight into the importance of cellular protein degradation pathways in this fatal neurodegenerative disease.

EXPERIMENTAL PROCEDURES

Patient Samples

Exome sequencing was performed on a four-generation Italian family in which four individuals had been diagnosed with ALS (ITALS#1, Figure 1A). The clinical details of this family are summarized in Table 1 and a detailed description is available in the Supplemental Information.

For subsequent mutational screening of *VCP*, an additional 210 DNA samples were obtained from affected individuals in unrelated ALS families (169 US cases and 41 Italian cases), and from 78 ALS cases for which autopsy material was available. Control samples consisted of 569 neurologically normal U.S. individuals obtained from the NINDS repository at the Coriell Cell Repositories (equivalent to 1138 control chromosomes), and 636 neurologically normal Italian individuals (1272 chromosomes). An additional series of 364 anonymous African and Asian samples that are part of the HGDP (Cann et al., 2002) were included in the mutational analysis as controls to evaluate the genetic variability of *VCP* in non-Caucasian populations. Demographics and clinical features of these samples are described in the Supplemental Information and are summarized in Table S2. Appropriate institutional review boards approved the study.

Exome Sequencing

DNA from affected individuals III:12 and IV:1 of the ITALS#1 family was enriched using SureSelect Exome target enrichment technology according to the manufacturer's protocol (version 1.0, Agilent, CA). The enriched DNA was paired-end sequenced on a Genome Analyzer IIx (Illumina, CA). Sequence alignment and variant calling were performed against the reference human genome (UCSC hg 18) using the Genome Analysis Toolkit (http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit). PCR duplicates were removed prior to variant calling using Picard software (http://picard.sourceforge.net/index.shtml). Based on the hypothesis that the mutation underlying this rare familial disease was not present in the general population, SNPs identified in the 1000 Genomes project (www.1000genomes.org/) or in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/, Build 131) were removed. We then excluded variants that were not shared by both patients. Next, synonymous changes were identified and filtered from the variant list using SIFT software (version 4.0, http://sift.jcvi.org/). Sanger sequencing using customized primers was performed to determine the presence of the remaining variants in the other affected member of the ITALS#1 family (patient III:8). As an additional step, variants and indels detected in the ITALS#1 family were filtered against exome data generated for 200 neurologically normal control subjects.

Linkage Analysis

Linkage analysis was performed on the ITALS#1 pedigree by genotyping four family members (Figure 1A; individuals III:6, III:8, III:12, and IV:1) using Illumina Human660W-Quad genotyping arrays. We selected ~2% of the genotyped SNPs (12,092 autosomal SNPs), and used them for parametric linkage in the Merlin linkage software assuming a dominant model (Abecasis et al., 2002; Sobreira et al., 2010). Linkage analysis identified 18 regions across the autosomes with a LOD score greater than zero (see Figure S1) (Abecasis et al., 2002; Sobreira et al., 2010).

Mutational Screening

All 17 exons and flanking introns of the *VCP* gene (NM_007126.3) were sequenced using the Big-Dye Terminator v3.1 chemistry (ABI, CA), run on an ABI 3730xl analyzer, and analyzed using Sequencer software version 4.2 (Gene Codes, MI). PCR primers and conditions are listed in Table S3. Mutations of *ANG*, *DCTN1*, *FUS*, *OPTN*, *SETX*, *SOD1*, and *TDP-43* were similarly excluded in cases carrying *VCP* mutations.

Haplotype Analysis

Haplotypes across the *VCP* locus in the two unrelated families carrying the p.R191Q mutation were compared by genotyping individuals IV:1 from the ITALS#1 pedigree and ND13215 from the Coriell#1 pedigree using Illumina Human660W-Quad genotyping arrays, and analyzing the resulting SNP data using Haploview 4.2 (Barrett et al., 2005). The degree of relatedness of the samples (quantified as the PI_hat metric) was determined using identity-by-descent algorithm within the PLINK software (Purcell et al., 2007).

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes three figures, three tables, Supplemental Experimental Procedures, and list of additional members of the ITALSGEN Consortium and can be found with this article online at doi:10.1016/j.neuron.2010.11.036.

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