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Valosin-containing protein mutations in sporadic amyotrophic lateral sclerosis

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Abstract

We recently reported that mutations in the *VCP* gene are a cause of 1–2% of familial amyotrophic lateral sclerosis (ALS) cases, but their role in the pathogenesis of sporadic ALS is unclear. We undertook mutational screening of *VCP* in 701 sporadic ALS cases. Three pathogenic variants (p.Arg159Cys, p.Asn387Thr, and p.R662C) were found in three US cases, each of whom presented with progressive upper and lower motor neuron signs consistent with definite ALS by El

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None of the other authors report any conflicts of interest. Informed consent for genetic analysis was obtained from each individual, and appropriate institutional review boards approved the study.

Escorial diagnostic criteria. Our data indicate that *VCP* mutations may underlie apparently sporadic ALS, but account for less than 1% of this form of disease.

Keywords

Amyotrophic lateral sclerosis; valosin-containing protein; mutations; sporadic disease

1. Introduction

Using an exome sequencing approach, we recently found that mutations in the *Valosin containing protein* (*VCP*) gene on the short arm of chromosome 9 are a cause of familial amyotrophic lateral sclerosis (ALS) (Johnson, et al., 2010). Mutations in this gene were previously known to cause an unusual clinical syndrome characterized by inclusion body myopathy with early-onset Paget's disease and fronto-temporal dementia (IBMPFD, OMIM# 167320) (Watts, et al., 2004). Our data extend the phenotype associated with *VCP* mutations to include progressive upper and lower motor neuron degeneration consistent with ALS (without concomitant muscle or bone involvement), and provide fundamental insight into the pathogenesis of motor neuron degeneration.

Approximately 5% of ALS cases are familial in nature, whereas the bulk of patients have no family history of ALS and are presumed to represent sporadic disease (Chiò, et al., 2008). Although *VCP* mutations account for 1–2% of familial cases, the role of this gene in the more common sporadic form of ALS remains unclear. To address this gap in our knowledge, we undertook mutational screening of *VCP* in a large cohort of patients diagnosed with sporadic ALS.

2. Methods

2.1 Study population

Case samples consisted of 459 US and 242 Italian individuals diagnosed with ALS based on El Escorial criteria (Brooks, 1994). The US samples were obtained from the NINDS Human Genetics DNA and Cell Line Repository at Coriell (catalog numbers for the precompiled panels of DNA samples used in this study: NDPT025, NDPT026, NDPT100, NDPT103 and NDPT106, see www.coriell.org). The Italian samples were collected from the Piedmont region having been identified by the population-based ALS Registry that has been operating in that region since 1995 (Chiò, et al., 2009a). Control samples consisted of neurologically normal individuals obtained from Coriell (US, n = 569) and Italy (n = 636). An additional 364 samples that are part of the Human Genome Diversity Panel (HGDP) (Cann, et al., 2002) were included in the mutational analysis as controls to evaluate the genetic variability of *VCP* in non-Caucasian populations (Johnson, et al, 2010). Demographics and clinical features of these samples are summarized in Supplementary Table 1, whereas the frequencies of *SOD1*, *TDP-32*, and *FUS* mutations, as well as the *C9ORF72* hexanucleotide repeat expansion in the Italian and US patients are shown in Supplemental Table 2.

2.2 Mutational screening

All patients underwent mutational analysis for *VCP* mutations. Specifically, all the coding exons and 30bp of the flanking intron-exon boundaries of *VCP* (NM_007126.3) were PCR amplified, sequenced using the Big-Dye Terminator v3.1 sequencing kit, run on an ABI 3730xl genetic analyzer (Applied Biosystems Inc.), and analyzed using Sequencer software version 4.2 (Gene Codes Corp., Ann Arbor, MI, USA).

3. Results

We performed sequence analysis of the *VCP* gene in 701 Caucasian cases diagnosed with sporadic ALS. This identified three missense mutations, namely a p.Arg159Cys (c.864C>T) mutation, a p.Asn387Thr (c.1549A>C) mutation and a p.Arg662Cys (c.2373C>T) mutation, each of which were observed in single cases (Table 1). The three cases with these *VCP* mutations did not carry a *FUS* mutation or the pathogenic hexanucleotide expansion of the *C9ORF72* gene (Lai, et al. 2011; Majounie, et al., 2012). The p.Arg159Cys variant is known to be pathogenic, in that it has been previously found in patients with IBMPFD (Bersano, et al., 2009, Spina, et al., 2008). The p.Asn387Thr variant represents a novel amino acid shift, though a different mutation (p.Asn387His) involving the same codon has also been described as causative for IBMPFD (Watts, et al., 2007). The p.Arg662Cys variant also represents a novel mutation. None of the variants were found in control samples screened in our own laboratory (n = 1,569 individuals, equating to 3,138 chromosomes), or in the dbSNP (Build 133, http://www.ncbi.nlm.nih.gov/projects/SNP/) and the 1000 genomes online databases (accessed 11th April 2011, n = 629 individuals, www.1000genomes.org) of human population polymorphisms.

In addition to the three missense mutations, we also found variants for which the pathogenicity is less clear. These are listed in Supplementary Table 3, and consist of the synonymous variant p.Tyr755 (c.2654T>C, n = 1), and intronic variants IVS12+9 T>C (n = 1) and 3′UTR *12 C>T (n = 1). Again, none of these variants were found in controls sequenced in our own laboratory, or in the dbSNP or 1000 genomes databases of human population polymorphisms.

All of the patients carrying *VCP* mutations developed lower limb weakness in middle to late age, which progressed to involve all four limbs over a number of years. Apart from the mother of ND12329, who was diagnosed with unspecified dementia, there was no reported personal or family history of muscle disease, frontotemporal dementia or bone disease. Examination at the time of sampling revealed upper and lower motor neuron signs in all four limbs, and widespread ongoing denervation and chronic reinnervation changes on EMG. One of the patients had evidence of bulbar involvement at the time of sampling. Revised ALS Functional Rating Scale (ALSFRS-R) scores revealed moderate disability. The clinical pictures of the patients carrying mutations of the *VCP* gene were consistent with definite ALS by El Escorial diagnostic criteria, and they had been treated with Riluzole.

4. Discussion

By definition, it is not possible to prove causation by showing segregation of a mutation with disease in sporadic ALS cases. Despite this, the three missense mutations of *VCP* identified in our cohort are likely to be pathogenic for several reasons: first, one of them has previously been described in patients with IBMPFD (Kimonis, et al., 2008), and another involves the same codon as another mutation known to be pathogenic; none of the variants were found in large numbers of controls nor have been described as a population polymorphism; furthermore, the p.Arg159Cys variant lies in the known mutational hotspot of the gene, a region of the VCP protein that is thought to be essential to its proper cellular function (Weihl, et al., 2009).

We found that mutations of the *VCP* gene account for less than 1% of sporadic cases in our cohort (3 mutations out of 701 cases screened = 0.43%). Thus, mutations in this gene account for a smaller percentage of sporadic cases compared to the 1–2% rate of *VCP* mutations seen in familial ALS (Johnson, et al., 2010). A similar pattern has been observed with other familial ALS genes: *SOD1* mutations accounts for ~13% of familial ALS cases in Italy, but are found in less than 1% of sporadic cases (Chiò, et al., 2008); *TDP-43* and *FUS*

mutations are each found in ~3–4% of familial cases, but have been described in less than 1% of sporadic cases in the general European population (Chiò, et al., 2009b, Guerreiro, et al., 2008, Kabashi, et al., 2008, Lai, et al., 2011, Mackenzie, et al., 2010).

The question arises as to whether the sporadic cases with mutations in *VCP* or any other familial ALS gene are truly sporadic cases or whether they represent cryptically-related cases. This scenario may occur for many reasons: lack of knowledge of the pedigree on the part of the patient or neurologist; previous generations dying at a young age prior to the onset of neurological symptoms; decreased penetrance of genes where not all individuals carrying the mutation manifest a clinical phenotype, which may be particularly relevant in any late-onset disease, such as ALS (Lai, et al., 2011). Indeed, *VCP* mutations are known to be highly variable with respect to both penetrance and phenotype expressivity: 90% developing weakness, 51% having osteolytic lesions, but only one third of cases manifesting FTD, and a smaller percentage developing ALS (Johnson, et al., 2010, Weihl, et al., 2009). Despite these caveats, it is clear that mutations of *SOD1* and *FUS* can underlie truly sporadic ALS, as there are documented *de novo* mutations in both these genes (Alexander, et al., 2002, Chiò, et al., 2011, DeJesus-Hernandez, et al., 2010). Furthermore, recent data has eroded the artificial barrier between familial and sporadic disease, and suggest that the definition of sporadic disease should be considered operational, rather than definitive (Majounie, et al., 2012).

We found a number of variants that were present only in cases, which did not obviously alter amino acid structure of the VCP protein, or were located in the intron close to the splice site. Both types of mutations can occasionally cause disease by altering splice patterns of genes. For example, a synonymous mutation (p.G608) that introduces a cryptic splice site in the *LMNA* gene is responsible for a large proportion of Hutchinson-Gilford progeria cases (Eriksson, et al., 2003). Furthermore, mutations in the 5′-splice site of exon 10 of the *MAPT* gene are known to be pathogenic in families with frontotemporal dementia with parkinsonism (Hutton, et al., 1998). Although these variants were not found in controls, it would be inappropriate to label them as pathogenic at this stage, especially as RNA is not available from these cases to experimentally confirm aberrant splicing. It will be interesting to see if other groups find these variants in their cohorts of familial and sporadic cases, thus providing additional evidence pointing toward their pathogenicity.

In summary, our data indicate that mutations of *VCP* can be responsible for occasional cases of sporadic ALS, but their low frequency means that there is little need to routinely screen the gene in such cases in the absence of additional clinical features or family history of concomitant bone disease, muscle disease or frontotemporal dementia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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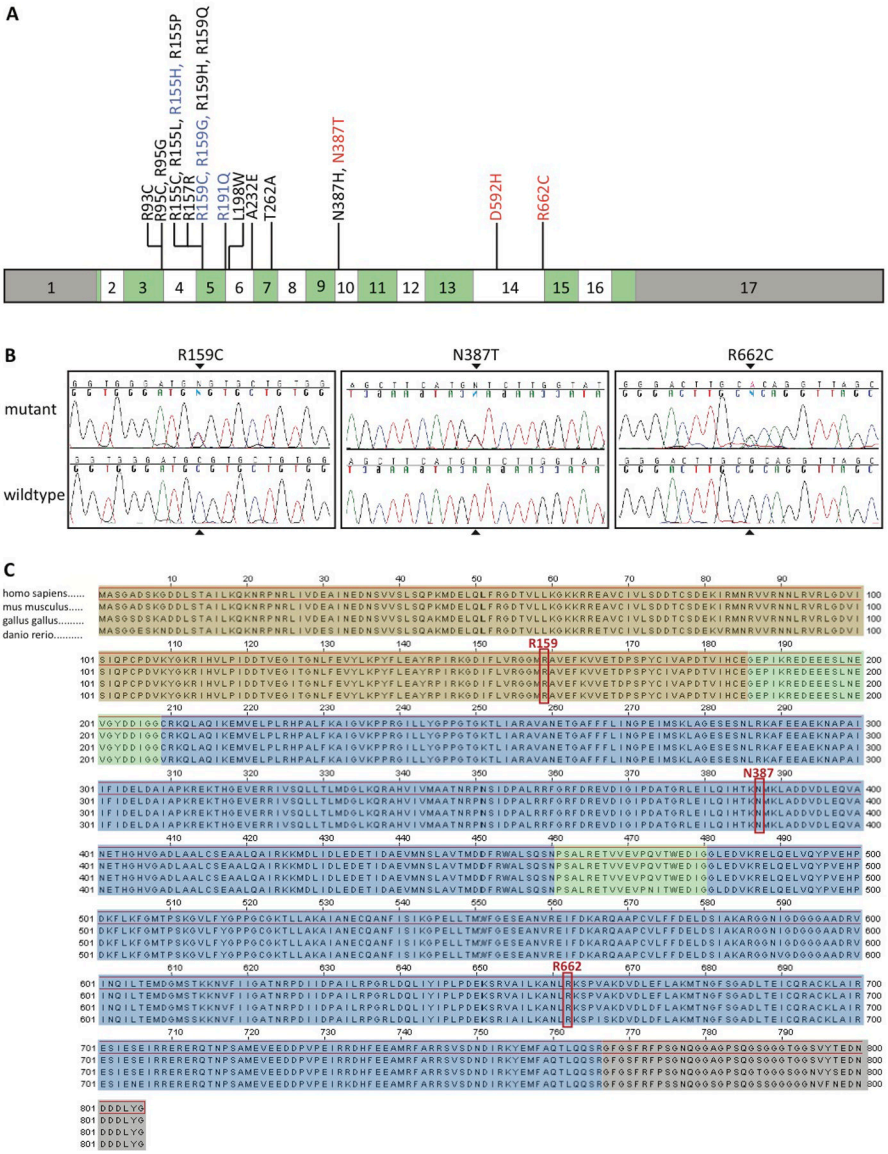

Figure 1.

(A) Graphical representation of the *VCP* gene showing its 17 exons. Coding exons are colored in alternating green and white, and non-coding regions are shown in grey. Mutations known to cause IBMPFD are shown in black, mutations detected in both IBMPFD and ALS cases are shown in blue, and mutations that have been found in only ALS cases are displayed in red. (B) Chromatograms showing mutant and wild-type alleles of the three variants found in sporadic ALS cases. (C) Sequence alignment demonstrates near complete protein conservation across species (brown shading, N-terminal domain; green, linker domains; blue, AAA-domains; grey, C-terminal domain). Mutated residues are highlighted in red.

Table 1

VCP mutations in sporadic ALS cases with clinical data

Coriell Sample ID	Mutation	Age at Onset	Gender	Race (Origin)	Site of Onset	Cognitive Impairment	ALSFRS-R	Neurological Examination	Duration from Onset
ND11807	p.Arg159Cys (c.864C>T)	68	Female	Caucasian (US)	Lower limb	No impairment reported	40/48	UMN and LMN signs in 4 limbs	Alive at 5 years
ND12329	p.Asn387Thr (c.1549A>C)	57	Male	Caucasian (US)	Lower limb	No impairment reported	38/48	UMN and LMN signs in 4 limbs	Alive at 5 years
ND10069	p.Arg662Cys (c.2373C>T)	67	Male	Caucasian (US)	Lower limb	No impairment reported	38/42 *	UMN and LMN signs in 4 limbs; Bulbar LMN signs	Alive at 2 years

Additional phenotype data is available for these samples at www.coriell.org; ALSFRS-R, Revised ALS Functional Rating Scale;

* this value represents the ALSFRS score (maximum value = 42);

UMN, upper motor neuron; LMN, lower motor neuron; base pair coordinates are based on *VCP* transcript NM_007126.3.