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Elevated VCP ATPase Activity Correlates With Disease Onset in Multisystem Proteinopathy-1

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Abstract

Objectives

Multisystem proteinopathy-1 (MSP1) is a late onset disease with >50 pathogenic variants in p97/VCP. MSP1 patients have multiple phenotypes that include inclusion body myopathy, Paget disease of the bone, amyotrophic lateral sclerosis, and frontotemporal dementia. There have been no clear genotype-phenotype correlations. We sought to identify genotype-phenotype correlations and associate these with VCP intrinsic ATPase activity.

Methods

Patients with MSP1 were identified from the literature and the Cure VCP patient registry. Age at onset and at loss of ambulation were collated. VCP intrinsic ATPase activity was evaluated from recombinant purified protein.

Results

Among the 5 most common pathogenic *VCP* variants in MSP1 patients, R155C patients had the earliest average age at onset (38.15 ± 9.78). This correlated with higher ATPase activity. Evaluation of 5 variants confirmed an inverse correlation between age at onset and ATPase activity ($r = -0.94$, $p = 0.01$).

Discussion

Previous studies have reported that VCP pathogenic variants are "hyperactive." Whether this elevation in VCP ATPase activity is relevant to disease is unclear. Our study supports that in vitro VCP activity correlates with disease onset and may guide the prognosis of patients with rare or unreported variants. Moreover, it suggests that inhibition of VCP ATPase activity in MSP1 may be therapeutic.

Introduction

Autosomal dominant variants in valosin-containing protein (*VCP*) gene cause a rare inherited syndrome with varied phenotypic penetrance of 4 main phenotypes: inclusion body myopathy (IBM), Paget disease of the bone (PDB), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS).[1] This clinical constellation resulting from a pathogenic variant in *VCP* has recently been termed multisystem proteinopathy-1 (MSP1).[2] Over 50 missense variants in VCP have been associated with an MSP1 phenotype.[3] Moreover, the phenotypic spectrum has broadened to include parkinsonism and Charcot-Marie-Tooth disease (CMT).[3,4] Unique to MSP1, patients with the same *VCP* variant can present with 1 or more MSP1 phenotypes, and this variability exists even within the same family. No clear genotype-phenotype correlations exist. Recently, a distinct autosomal dominant VCP syndrome with intellectual disability and developmental delay (VCP-IDDD) was associated with de novo missense and loss of function variants in *VCP*,[5] further expanding the phenotypic spectrum of VCP variants.

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VCP is an AAA-ATPase essential for multiple ubiquitin-dependent cellular processes including chromatin remodeling, protein degradation, vesicular trafficking, and autophago-lysosomal function.[6] Functionally, VCP hydrolyzes ATP to generate a conformational change between its N and D1 domains leading to substrate engagement and/or protein unfolding. Several studies have demonstrated that pathogenic MSP1 *VCP* variants have an increase in basal ATP hydrolysis activity.[7,8] These data have supported that MSP1 variants may have increased functional activity and that VCP inhibition may be therapeutic.[9,10] This contrasts with VCP-IDDD variants. Recombinant purified VCP protein with VCP-IDDD missense variants have a reduction of ATPase activity supporting a loss of function.[5] VCP-IDDD may have a different mechanism of action from MSP1 that is due to haploinsufficiency and hypomorphic activity of VCP rather than VCP hyperactivity. In the case of VCP-IDDD, approaches aimed at enhancing VCP activity may be therapeutic.

Several studies have described large cohorts of MSP1 patients but have not demonstrated a clear genotype-phenotype correlation regarding the presentation of myopathy, PDB, FTD, or ALS. However, it has been suggested that patients with the p.Arg159Cys variant have a later age at onset[11] and patients with a p.Arg155Cys have an earlier age at onset[3] in their respective cohorts. No correlation was made with intrinsic VCP ATPase activity. In this study, we reasoned that intrinsic VCP activity may correlate with disease onset or severity and evaluated this in a large patient cohort of adult onset MSP1 patients.

Methods

VCP ATPase

Activity was measured by an in vitro assay using purified VCP protein as previously described.[12] *VCP* variants were generated by site-directed mutagenesis of a VCP plasmid (TCB197) to create the 5 studied variants.

Clinical Data

Retrospective data was pulled from the literature (130 patients) including the research groups' previous work[3,12] (174 patients) and Cure VCP patient registry database (49 patients). Patient variant, age at onset (defined as age at first symptom), and availability of VCP ATPase activity measurements were the minimum inclusion criteria. Age at loss of ambulation was included when available. All datapoints were combined into a single database for analysis.

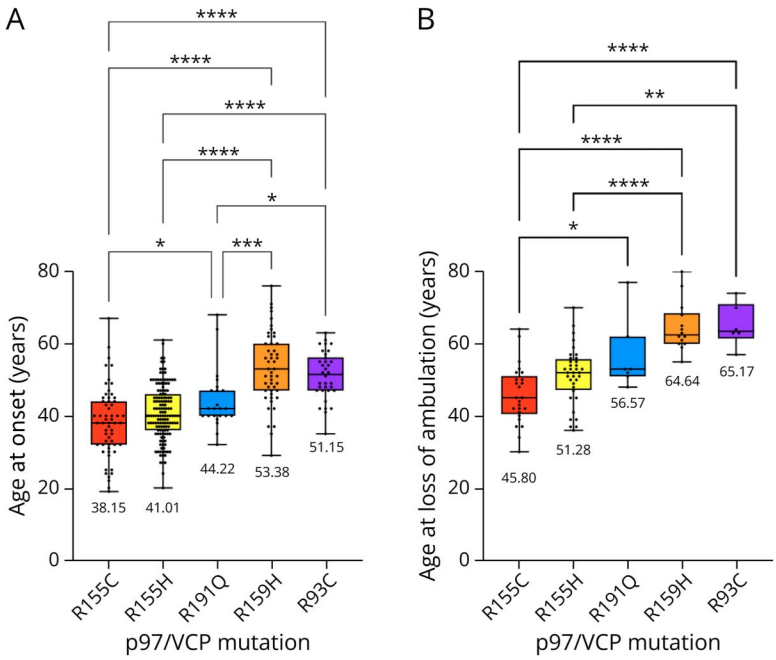
Statistics

A one-way analysis of variance (ANOVA) was run to detect differences between mutations for ATPase activity and age of symptom onset. Correlation between ATPase activity and age at onset was assessed using Pearson correlations, as was ATPase activity with age at loss of ambulation.

Results

The average age at onset for patients with MSP1 was 43.32 ± 10.44. We reasoned that some *VCP* variants may have an earlier age at onset. To explore this, we collated data

Figure 1 Average Age at Onset for 5 Most Common VCP Variants



(A) Box and whisker plot of age at onset (AAO) and (B) age at loss of ambulation (LOA) for the 5 most prevalent VCP variants. Each dot represents an individual patient. The value below is the average for that genotype (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and ****$p \leq 0.0001$).

from patients with the 5 most common *VCP* variants from our recent study, a comprehensive literature review, and a VCP patient registry (Figure 1A). Across all samples, VCP patients with an R155C variant had an earlier age at onset (38.15 ± 9.78) and patients with a VCP R93C or R159H had a later age at onset (51.15 ± 6.67) and (53.38 ± 9.79), respectively. Since the predominant phenotype in MSP1 is muscle weakness, we performed a similar analysis to determine the average age at loss of ambulation which we defined as becoming wheelchair bound.[3] These data similarly demonstrated that patients with R155C variant lose ambulation at an earlier age (42.80 ± 8.09) compared with patients with a R93C variant (65.17 ± 5.98) (Figure 1B). The average age for loss of ambulation is 53.22 ± 10.45 when all 5 variants are combined.

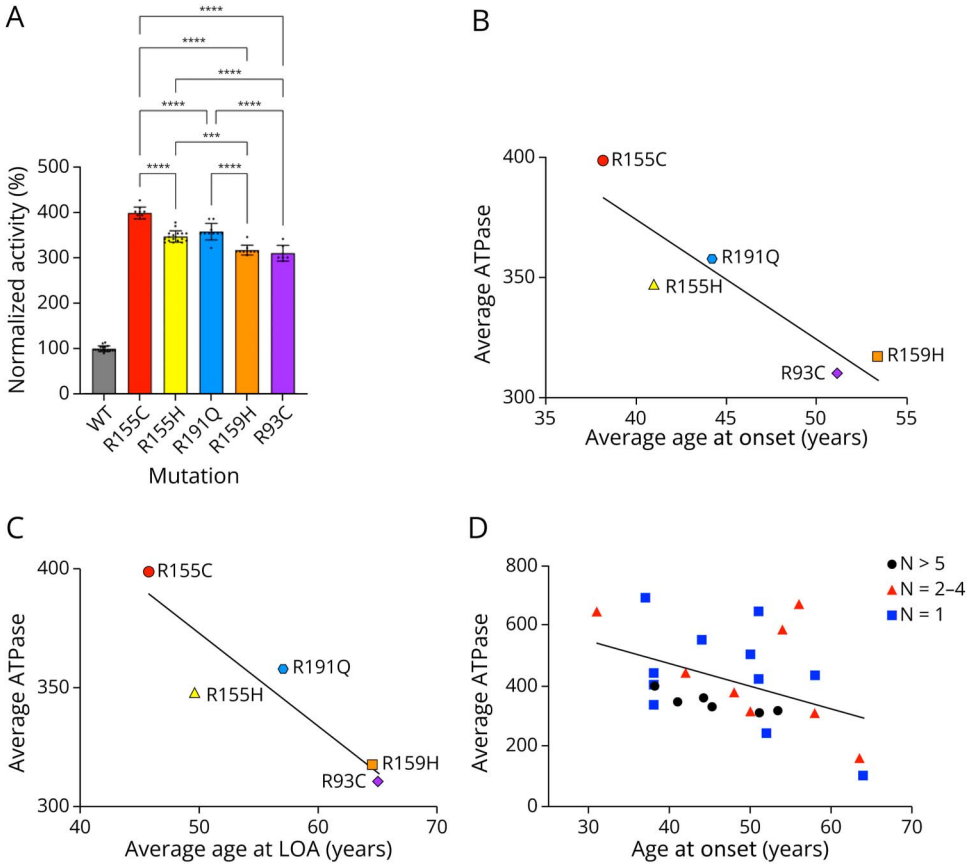
To see if the age at onset or loss of ambulation for different *VCP* variants correlated with in vitro VCP ATPase activity, we purified VCP-WT and each of the 5 VCP variants in Figure 1 and performed basal ATPase assays. Consistent with previous studies, all MSP1 variants had an increase in ATPase activity relative to VCP-WT. Surprisingly, VCP variants associated with early onset or earlier age at loss of ambulation had a higher ATPase activity than those with a later age at onset. For example, the VCP-R155C variant had an activity of 398.9% ±12.86% and the VCP-R93C variant had an activity of 310% ±17.38% of VCP-WT that was arbitrarily set to 100% (Figure 2A). To explore the relevance of these findings, we performed a Pearson correlation by plotting VCP ATPase activity vs average age of onset (Figure 2B) or average age at loss of ambulation (Figure 2C) using the 5 most common *VCP* mutations and found significant correlation for both.

In many cases, pathogenic variants in *VCP* are found in a few families or singleton cases. Our correlation model supported an association between ATPase activity and disease onset. Therefore, we performed a correlation using 25 *VCP* variants from 353 patients. For visual purposes, we categorized the *VCP* variants into variants in which patient level data were available for >5 patients, 2–4 patients, or *VCP* variants with onset data from a singleton patient (Figure 2D). These combined data demonstrated r = −0.42 and $p = 0.034$.

Discussion

MSP1 is a heterogenous genetic disease due to dominant pathogenic variants in *VCP* with varied penetrance of IBM, PDB, ALS, and FTD.[3] At present, no genotype-phenotype correlation has been established for the penetrance of these

Figure 2 Intrinsic VCP ATPase Activity From Individual Variants Correlates With Age at Onset and Loss of Ambulation



(A) Bar graph of normalized VCP ATPase activity for VCP WT and 5 VCP variants. Each data point represents individual replicates. Error bars represent SD. VCP-WT was arbitrarily set to 100%. (B) Scatter plot of variant ATPase activity and average patient age at onset with linear regression ($r^2 = 0.82$, $p = 0.034$). (C) Scatter plot of variant ATPase activity and patient age at loss of ambulation ($r^2 = 0.86$, $p = 0.023$). (D) Scatter plot of variant ATPase activity and age at onset for 31 VCP variants from 379 patients. Patient-level data were acquired from >5 patients (n = 318), 2–4 patients (n = 24), or singleton patients (n = 11) and linear regression of all patient data (r = −0.42 and $p = 0.034$). Patient data from combined database (eTable 1).

4 features.[3] However, using patient data, we find significant differences in the average ages of onset for the 5 most common pathogenic *VCP* variants (VCP-R155C, R155H, R191Q, R159H, and R93C). Specifically, the VCP-R155C variant has an earlier onset with the VCP-R159H and VCP-R93C having later onsets. Notably, the average age at onset inversely correlated with VCP variant ATPase activity suggesting that VCP activity may serve as a proxy for disease severity. It is important to note that our study is not an exhaustive description of every VCP variant or MSP1 patient reported. Study inclusion required that the age at onset of any phenotypic symptom was clearly indicated and that we had performed an in vitro ATPase assay on the indicated VCP variant.

How an increase in VCP ATPase function leads to disease pathogenesis is unclear. Several studies have demonstrated that MSP1 variants in VCP have elevated ATPase activity and in vitro unfoldase activity.[7,8] Whether this translates to its function in vivo is not clear. Genetic knockdown of VCP in mouse models recapitulates several features of MSP1 including ubiquitinated inclusions, vacuolation, and TDP-43 pathology supporting a loss of function for VCP mutations.[13,14] By contrast, several cell and animal models of MSP1 are rescued by VCP inhibition suggesting that normalizing VCP activity in the setting of hyperactive MSP1 mutations is therapeutic.[9,10,15] Our data correlating enhanced VCP ATPase activity with disease onset further support that the MSP1 mechanism of action is due to a gain of function.

While most MSP1 variants have an elevation in ATPase activity, there are some exceptions from families with *VCP* variants and distinct phenotypes that deviate from MSP1. A family with a late onset axonal neuropathy, CMT2Y and no other phenotypic features carried a VCP-E185K variant that had normal in vitro VCP ATPase activity.[4] In addition, 2 families with a pathologically distinct form of dementia termed a vacuolar tauopathy were found to have a VCP-D395A variant that segregated with disease.[16] In vitro ATPase and unfoldase assays found this variant to have reduced activity supporting a distinct mechanism of disease.[16] Whether these 2 variants clearly cause MSP1 as defined by varied penetrance of IBM, PDB, ALS, and FTD remains to be established with the identification of more patients. Nonetheless, our recent studies support that a three-fold increase in basal ATPase activity using recombinant protein of an MSP1 variant as compared with VCP-WT may be helpful to assess pathogenicity in the case a variant of uncertain significance.[12]

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