Cure VCP Scientific Conference

Virtual

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Schedule

Day 1 Introduction

Welcome and brief introduction of event logistics with Nathan Peck

Keynote – Jerry Mendell MD – professor of pediatrics and neurology at Nationwide Children's Hospital

Session 1 – Introduction to VCP disease – Discussion of clinical symptoms, diagnostics tools and pathological features
Gerald Pfeffer MD, CM, FRCPC, PhD – Assistant professor Clinical Neurosciences, University of Calgary
Jordi Diaz-Manera MD, PhD - Professor of Neuromuscular disorders, Newcastle University
Virginia Kimonis MD MRCP - Professor, division of genetics and genomic medicine, Department of Pediatrics at UC Irvine

Session 2 – New Molecular and cellular mechanistic insight of VCP disease – Mechanistic insight, including the role of VCP in protein quality control, proteasome degradation, endolysomal function, autophagy regulation
Alyssa Johnson PhD – Assistant professor, Louisiana State university
Peter Shen, PhD – Assistant professor of Biochemistry, University of Utah
Hemmo Meyer PhD – Professor, Department Molekularbiologie, Universitat Duisburg-Essen

Session 3 – New tools and omics studies- New tools to facilitate collaborations. Various genomic, proteomics, and other omics studies, Cryo EM studies may yield new insight in structures of VCP
Kalina Paunovska PhD – Senior research scientist at Dahlman Lab, Georrgia Institute of Technology
Nicholas Seyfried PhD – Associate professor, Emory University School of medicine
Fabio Coppede PhD – Associate professor of medical genetics at Universita di Pisa

Session 4 – Resources for Research – NIH NeuroBioBank presentation and other biosample resources
Thomas Blanchard PhD JD – Director of the University of Maryland Brain and Tissue Bank

Highlighted Posters - Six selected posters from abstract submissions will be selected to present 1) Rod Carlo Columbres 2) Alyaa Shmara 3) Marianela Schiava 4) Po-Lin Chiu 5) Stephanie Moon 6) Brittany Ahlstedt

Day 2

Keynote – Translational topic
George Tolomiczenki PhD MPH MBA – Executive Director, Merkin Institute for Translational Research at Caltech
Barbara Wold PhD – Director, Merkin Institute for Translational Research at Caltech

Session 5 – Drug Discovery – New inhibitors, small molecules, and ASOs of VCP/p97 – Discussion on the recent drug discovery findings and mechanistic studies of VCP inhibitors including small molecule compounds and ASOs
Donna Huryn PhD ACSF – Professor, University of Pittsburgh School of Pharmacy
Tsui-Fen Chou PhD – Research professor in biology at Caltech

Oral Abstract Presentations - VCP Inhibitors - Protective or Deleterious? 1)Cheng Cheng 2) Jiang Shu 3) Feng Wang
Oral Abstract Presentations - Day 2 Clinical - Three selected posters from abstract submissions will be selected to present 1) Natalie Reash 2) Megan Iammarino 3) Eiman Abdoalsadig
Session 6 – Gaps in current research areas – Identify gaps in research and guide future research agenda
Michelle Arkin PhD – Department Chair and professor, department of pharmaceutical chemistry, UCSF
Eddie Lee MD PhD – Assistant professor, pathology and laboratory medicine, UPenn school of medicine

Session 7 – Gaps in current clinical care – Identify gaps in research and clinical knowledge and guide future research agenda
Bhaskar Roy, MBBS, MBMS, MHS – Assistant professor, Yale School of Medicine
Nupur Ghoshal MD PhD – Associate professor, Neurology at WashU school of medicine
Meredith James PT – Research physiotherapist, John Walton Muscular dystrophy research centre
Gerald Pfeffer, MD CM, FRCPC, PhD – Assistant professor Clinical neurosciences, University of Calgary
Manisha Korb MD – Assistant professor at Department of Neurology, UCI school of medicine
Session 8 – Clinical trial readiness – biomarker discovery, toxicology consideration and patient longitudinal studies
Nelson pace PhD SM – Director of epidemiology at AllStripes
Lindsay Alfano PT, DPT, PCS – PI at the Abigail Wexner research institute at Nationwide Children’s Hospital
Alison Skrinar, PhD – Vice president clinical outcomes research and evaluation at Ultragenyx

Session 9 – Look Forward – Where do we go from here
Michelle Arkin PhD – Department chair and professor, department of pharmaceutical chemistry, UCSF
Jordi Diaz-Manera MD PhD – Professor of Neuromuscular disroders, Newcastle University
Conrad Weihl MD PhD – Professor of Neurology at WashU school of medicine
Tahseen Mozaffar MD – Professor, department of neurology, UCI school of medicine

List of Abstracts

Structural and functional analyses of disease-linked p97 ATPase mutant

Purbasha Nandi(1,2), Shan Li(3), Rod Carlo A. Coulmbres(3), Feng Wang(3), Dewight R. Williams(4), Yu-Ping Poh(2), Tsui-Fen Chou(3), Po-Lin Chiu(1,2)

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IBMPFD/ALS is a genetic disorder caused by a single amino acid mutation on the p97 ATPase, which causes a three-fold increase in ATPase activity and cofactor dysregulation. The disease mechanism underlying p97 ATPase malfunction remains unclear. We assembled the full-length p97R155H and p47 proteins and visualized their complex structures in different nucleotide-binding states using single-particle cryo-EM. More than one-third of the molecular species in the mutant complex are the dodecameric form of the p97R155H mutant. The dodecamer dissociates into two hexamers in the presence of nucleotides and does not access the p47 cofactor. The C-terminal tails play an essential role in packing the two hexamers. The N-domains of the p97R155H mutants all show in the up configurations, except the ADP-bound p97R155H, leading to lower p47 binding affinity. The p47-bound or the mutated N-domains impact the p97R155H ATPase activities likely via changing the conformations of arginine fingers. These functional and structural analyses underline the ATPase dysregulation with the miscommunication between the functional modules of the p97R155H.

Coupling of translation quality control and mRNA targeting to stress granules

Stephanie L. Moon(1), Tatsuya Morisaki(2), Timothy J. Stasevich(2,3), Roy Parker(4,5)

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5. Howard Hughes Medical Institute, Chevy Chase, MD

Stress granules are dynamic assemblies of proteins and non-translating RNAs that form when translation is inhibited. Stress granules are similar to neuronal and germ cell granules, play a role in survival during stress, and aberrant, cytotoxic stress granules are implicated in degenerative diseases of the nervous, muscular and skeletal systems. Perturbations in the ubiquitin-proteasome (UPS) system also cause neurodegeneration, and alter the dynamicity and kinetics of stress granules. We used single mRNA imaging in living and fixed human cells to determine if defects in the UPS perturb mRNA translation and partitioning into stress granules during acute stress. We observe ribosomes stall on mRNAs during arsenite stress, and the release of transcripts from stalled ribosomes for their partitioning into stress granules requires the activities of valosin-containing protein (VCP) and the proteasome. Moreover, we demonstrate members of a specialized complex in the UPS that targets aberrant nascent proteins for decay upon ribosome stalling, referred to as ribosome-associated quality control complex (RQC), are also required for mRNA release from ribosomes and partitioning into stress granules. VCP alleles that increase segregase activity and cause neurodegeneration and inclusion body myopathies increase mRNA recruitment to stress granules, suggesting aberrant mRNA localization to stress granules in disease contexts. This work identifies a new type of stress-activated RQC (saRQC) distinct from canonical RQC pathways in mRNA substrates, cellular context and mRNA fate. Current research in the Moon lab aims to elucidate the mechanisms and mRNA specificity of the saRQC pathway and uncover the mechanistic basis of stress granule regulation by VCP and other UPS factors.

High Prevalence of Frontotemporal Dementia in Females in Five Hispanic Families with R159H VCP Multisystem Proteinopathy

Alyaa Shmara(1), Liliane H. Gibbs(2), Ryan Patrick Mahoney(1), Kyle Hurth(3), Vanessa S. Goodwill(4), Alicia Cuber(1), Regina Im(1), Ashley Castan(1), Donald Pizzo(4), Jerry Brown(5), Shalini Mahajan(6), Virginia Kimonis(1)

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5. Diagnostic Radiology, Tripler Army Medical Center, Honolulu, Hawaii
6. Department of Neurology, Cedars Sinai Medical Center, Los Angeles, CA

Missense variants of the valosin-containing protein (*VCP*) gene cause a progressive, adult-onset, autosomal dominant disease also assigned the term VCP multisystem proteinopathy (MSP1). The clinical characteristics of the disease are a constellation of clinical features including inclusion body myopathy (IBM), Paget's disease of bone (PDB), frontotemporal dementia (FTD) (IBMPFD) and amyotrophic lateral sclerosis (ALS), typically reported at a frequency of 90%, 42%, 30% and 9% respectively. We report the clinical findings in five Hispanic families with the c.476G>A, p.R159H *VCP* variant. FTD was the most prevalent feature reported, most frequently in females. PDB was only seen in one patient in contrast to the earlier reported cohorts. The overall frequency of the different manifestations: myopathy, PDB, FTD and ALS in these five families was 36%, 3%, 72% and 8% respectively. The atypical phenotype and later onset of manifestations in these families resulted in a significant delay in the diagnosis of VCP disease. Studying each unique family is pivotal in increasing awareness of the variability of VCP-related diseases across ethnic backgrounds and in understanding the mechanism for these genotype-phenotype correlations.

A Clinicopathologic Study of Malignancy in VCP-Associated Multisystem Proteinopathy

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Valosin-containing protein (VCP) is an important protein with many vital functions mostly related to the ubiquitin-proteasome system that provides protein quality control. VCP has also been strongly involved in cancer, with over-activity of VCP found in several cancers such as prostate and pancreatic cancers, endometrial and esophageal carcinomas, and osteosarcoma. The overexpression of VCP has been associated with poor prognosis and increased metastasis, proving it valuable as a marker for the advancement of these cancers. The inhibition of VCP has been suggested as a treatment of metastasis in certain cancers.

VCP-associated inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD or VCP multisystem proteinopathy) is an autosomal dominant disorder caused by mutations in the VCP gene on human chromosome 9. VCP mutations are also associated with amyotrophic lateral sclerosis (ALS), Parkinson's disease and Charcot-Marie-Tooth disease type 2. Since the disease is caused by gain of function mutations in VCP, our hypothesis was that we would find an increased incidence of malignancies amongst our patients. We present cases of unusual tumors in patients with classic features of VCP associated disease including malignant peripheral nerve sheath tumor, anaplastic pleomorphic xanthoastrocytoma with multiple recurrences and thymoma. However, we did not find an increased incidence of common cancers compared to the general population. These findings expand the phenotype of VCP disease to potentially include unusual cancers which could potentially be treated with VCP inhibitors. Further research is needed on whether VCP mutations are implicated in cancer development.

VCP/p97 inhibitor CB-5083 modulates muscle pathology in a mouse model of VCP inclusion body myopathy

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Pathogenic mutations in Valosin-containing protein (VCP) lead to VCP disease, characterized by inclusion body myopathy with early-onset Paget disease of bone and frontotemporal dementia (also known as Multisystem proteinopathy). Previous studies in drosophila models of VCP disease indicate that the pathogenic mutations have a gain-of-function effect, and treatment with VCP inhibitors mitigates disease pathology. Earlier-generation VCP inhibitors display relatively low inhibitory potency for VCP and exhibit off-target effects, leading to toxicity and generally poor molecular properties for therapeutic use such as low solubility. In this study, we tested the safety and efficacy of a novel and potent VCP inhibitor, CB-5083, in an animal model of VCP disease and with VCP patient-derived myoblast cells. Our results indicate that chronic CB-5083 treatment is well tolerated in the mouse model of VCP disease, and can ameliorate the muscle pathology characteristic of the disease. At the cellular level, VCP-associated pathology biomarkers, such as elevated TDP-43 and P62 levels, were significantly alleviated. In vitro analyses using patient-derived myoblasts confirmed that CB-5083 can modulate expressions of disease biomarkers and autophagy pathways. Finally, to address the potential adverse effect of CB-5083 on visual function observed in a previous oncology clinical trial, we analyzed retinal function in mice treated with moderate doses of CB-5083 for 5 months. These experiments documented the absence of permanent ocular toxicity. Altogether, our results indicate that chronic use of CB-5083 is safe and efficacious in the mouse model of VCP-inclusion body myopathy.

Bone density studies using DEXA in patients with VCP myopathy and/or Paget disease of bone

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Background: Inclusion Body Myopathy (IBM), associated with combinations of Paget's Disease of the Bone (PDB), Frontotemporal Dementia (FTD) (IBMPFD, VCP disease or VCP multisystem proteinopathy), and amyotrophic lateral sclerosis (ALS) is a rare, autosomal dominant, adult-onset, progressive, and multisystem disorder caused by mutations in VCP gene. The disorder is characterized by progressive axial and proximal muscle weakness, bone pain and fractures, and FTD manifestations such as behavioral, cognitive and language impairment. Dual-energy X-ray absorptiometry (DEXA) is a gold standard for body composition measurements. It generates values for bone mineral densities (BMD), Z- scores and T scores from left hip, lumbar spine (L1-4) and whole-body scans.

Purpose of the Study: This cross-sectional study of individuals with VCP disease aims to accurately detect differences in lean mass %, total body fat mass %, bone mass %, and bone mineral density (BMD): 1) at different stages of the myopathic disease 2) in subjects who additionally had Paget disease of bone, 3) and to establish the effect of changes in lean mass on bone mass in the different cohorts.

Results: Our cohort included nineteen subjects with VCP disease, twelve had VCP myopathy and seven additionally had PDB (11 males, 8 females), three carriers, and six controls. The mean ages of the groups were (mean ± SD): 53.8 ± 9.4 years, 49.0 ± 9.2 years, 36.7 ± 1.7 years, 45.8 ± 15.8 years, respectively. The mean disease onset for myopathy and Paget's group were (mean ± SD): 43.3 ± 9.1 years and 39.2 ± 6.9 years, respectively. Osteopenia was found in all groups (myopathy: 33%, Paget's: 14%, carrier: 33%, control: 17%), while osteoporosis was only seen in one female (8%) within the myopathy group. The VCP disease group exhibited a significant reduction in lean mass % ($p=0.03$) and increased total body fat % ($p=0.01$) compared to the control group. Bone mass and lean mass in kgs positively correlated in all groups (Control: $r=0.9$, $p=0.04$; Paget's: $r=0.5$, $p=0.2$; Myopathy: $r=0.2$, $p= 0.5$). The left hip BMD value for the VCP disease group showed a significant decrease compared to the control ($p=0.03$). In addition, the left hip z-score of the VCP disease group showed a significant decrease ($p=0.02$) compared to the control group.

Conclusion: Thus far, this is the very first DEXA study assessing individuals with VCP disease. Using DEXA, we found that 33% had osteopenia and 8% had osteoporosis within the myopathy

group, while 14% had osteopenia within Paget's group. DEXA also showed a reduction in lean mass and increased fat mass with increasing age, as expected for VCP myopathy. DEXA also showed a direct association between lean mass and bone mass. In addition, the generated DEXA left hip z-score value is lower for the VCP disease group. Overall, DEXA is a valuable method in evaluating BMD, total fat mass, and lean mass estimation in individuals with VCP disease. However, a larger cohort would be needed to show its value in monitoring the progression of the disease and the effect on BMD.

Analysis of quantitative MRI features and functional assessment in IBMPFD patients

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Introduction: Magnetic Resonance Imaging (MRI) is a pivotal imaging tool for diagnosis and monitoring progression of several inherited and acquired neuromuscular disorders, such as inclusion body myopathy (IBM) associated with Paget's Disease of the Bone (PDB) and Frontotemporal dementia (FTD) (IBMPFD) and mutations in the valosin-containing protein (VCP) gene, in both clinical and research setting. However, the use of MRI in such application has been relatively qualitative in nature, limited to visual assessment of chronic alterations such as fatty infiltration and muscle volume decrease.

Purpose: To investigate the correlation between quantitative features observed in MRI and thigh muscle strength measured with isokinetic dynamometer and to determine any correlation between the degree of MRI burden with function.

Methods: MRI and biophysical evaluation were performed on 25 subjects (affected=14, carriers=2, control=9). MRI was performed on a 3T scanner (Philips Medical Systems). Bilateral T2w thigh scan was performed in 25 contiguous axial slices with the following parameters: TR/TE=6957/100 (ms) and voxel- size=0.8x0.8x5 (mm3). Offline image processing including ROI drawing and texture analysis based on Gray-Level Co-occurrence Matrix (GLCM) were performed using MIPAV (NIH) and custom program developed in Matlab (The MathWorks) on exported DICOM images. The maximal muscle strengths of the knee extensors and flexors were measured unilaterally using a dynamometer (Biodex System 3). With respect to knee extension, subjects were required to produce maximal contractions at angular velocity of 60 /sec. Dynamometer measurements (n=16) were then correlated with imaging features obtained from 2 muscle groups: rectus femoris (RF) and vastus lateralis (VL). Pearson's correlation coefficient, linear regression analysis and Mann-Whitney test were performed in SPSS and Prism.

Results: Two imaging features including normalized maximum probability (MAX) of and sum average (SAG) of GLCM were first averaged over the two muscle groups before being correlated separately to the dynamometer measurements. Statistically significant correlation was observed with following Pearson's r values: 0.793 ($P<0.0001$) and -0.800 ($P<0.0001$) for MAX, and SAG, respectively. Correlation between 6 minute-walk test (6MWT) with MAX and

SAG were significant for the affected group (Pearson r= 0.825, P=0.006; Pearson r= -0.854, P=0.003, respectively). Disease duration and SAG exhibited a significant correlation in the affected group (Pearson r= 0.723, P=0.003), but not with MAX (P=0.122). In similarity, a significant correlation between MRC and SAG was observed for affected groups (Pearson r= -0.857, P=0.002), but not with MAX (P=0.113). No significant correlation between age at visit and SAG and MAX were observed in both affected and non-affected groups (P>0.05, for all).

Although muscle loss and/or fat-infiltration of myopathy can be easily seen in MRI, quantitative analytic approach based on MRI is currently lacking in this field. A superior soft-tissue provided by and lack of ionizing radiation in this imaging modality, however, makes MRI an ideal tool that could provide quantitative imaging markers in the study of the disease progression and/or treatment intervention.

Preliminary results presented here clearly demonstrate a potential of such quantitative approach based on MRI.

Semi-quantitative MRI and functional analysis of thigh muscles in IBMPFD patients

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Introduction: Inclusion body myopathy (IBM) with Paget's disease (PDB) and frontotemporal dementia (FTD), referred collectively as IBMPFD, VCP Disease or Multisystem proteinopathy 1 (MSP1), is a rare autosomal dominant disease associated with mutations in valosin-containing protein (VCP) gene. Patients with IBMPFD often experience progressive and severe muscle degeneration, and fatty replacement of muscle fibers as inclusion bodies accumulate. MRI is the pivotal non-invasive imaging tool to support clinical diagnosis and monitoring progression of several inherited and acquired neuromuscular disorders, revealing anatomic details and changes in signal intensity within the muscles. T1-weighted images (T1W) disclose chronic alterations including fatty infiltration and muscle volume decrease. Semi-quantitative scoring systems exist for the evaluation of fatty infiltration, MRI quantification methods have been recently applied to evaluate fatty infiltration in clinical research.

Purpose: The purpose of this study are: 1) to correlate semi-quantitative scores of fatty infiltration with MRI-based fat fraction analysis, 2) demonstrate the association of such scoring with functional measures in patients and non-affected subjects, and 3) characterize specific patterns of chronic VCP myopathy with semi-quantitative methods.

Methods: The study protocol was approved by the Institutional Review Board, and all subjects gave written informed consent. A total of 21 individuals were recruited for the study, 12 have varying degrees of VCP myopathy (7 women and 5 men; mean age, 50.3 years; range, 28-64 years) and 8 healthy volunteers (4 women and 4 men; mean age, 43.5 years; range, 28-65 years). Mean onset of myopathy was around 39.9 years (range 25-51 years) while the mean myopathy duration at enrollment was 10.4 years (range 3-31 years). All MR studies were performed on a 3T scanner (Achieva, Philips Healthcare, Netherlands) using a body coil. Axial images of the thighs were acquired including T1WI generating 20-25 slices. For the semi-quantitative assessment, the degree of fatty infiltration in the mid section of the thigh was

graded by a radiologist according to 5-point scale (Table 1) and subsequently the MR images were quantitatively analyzed for fat fraction. Patients underwent a 6-minute walk test to measure gross motor function and dynamometry testing. Statistical analysis was done in SPSS and Prism using Pearson correlation and simple linear regression analysis.

Results: The fat fraction of muscle showed a strong and statistically significant positive correlation with semi-quantitative scale for all muscles ($r=0.928$; $p < 0.001$). Fat fraction values showed a stepwise increase in higher point semi-quantitative scales ($p <0.001$ for all). Vastii, sartorius and adductor magnus muscles in patients demonstrated significantly higher fatty infiltration while adductor longus and rectus femoris were relatively spared. Statistically significant and strong negative association between 6MWT and 5-point scores in left and right flexors and extensors in VCP disease patients were noted (LF: $r=-0.80$, p-value=0.002; LE: $r=-0.76$, p-value=0.006; RF: $r=-0.78$, p-value=0.003; RE: $r=-0.76$, p-value=0.004). No asymmetric effects in fatty infiltration were observed in thigh muscle groups. Knee flexor compartments were more severely impaired relative to extensor groups.

Conclusion: We demonstrate that the 5 point semi-quantitative scale can provide equivalent accuracy as quantification of fatty infiltration. In addition, the muscle-specific pattern of fatty infiltration delineates supporting evidence in the differential diagnosis of VCP myopathy relative to other acquired and genetic neuromuscular diseases. This method and associated muscle-specific pattern present a promising non-invasive and widely accessible strategy in clinical practice to initial and follow-up of VCP myopathy.

Modeling p97 ALS using isogenic IPSC-derived motor neurons

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Background:

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by upper and lower motor neuron degeneration. In the majority of ALS cases, degeneration is preceded by hallmark molecular events including TAR DNA-binding protein 43 (TDP-43) mislocalization and endoplasmic reticulum (ER) stress.

In the last decade, p97/VCP/CDC48 (p97) has been discovered as cause of ALS as well as other protein aggregation diseases. P97 is an ATPase that binds and extracts ubiquitinated substrates from membranes or multi-protein complexes. Through this action, p97 participates in a myriad of cellular processes including ER-associated degradation, autophagy, and mitophagy, among others; however, the specific process(es) which lead to neurodegeneration in patients with p97 mutations remains unknown. Uncovering the pathways that are altered and the salient proteins in these pathways will provide insight into potential therapeutic targets. Creating an isogenic model will allow for an “-omics” approach without the variability of genetic background present in patient cell lines.

Methods:

We have developed a novel isogenic model of p97 ALS using CRISPR gene editing to introduce the most common p97 mutations, R155H and R159H, into the KOLF2 IPSC line. Wildtype and mutant cells were differentiated and characterized using immunoblot, immunocytochemistry, live-cell calcium imaging, whole-cell patch clamp electrophysiology, and unbiased, quantitative proteomics.

Results:

Using a novel differentiation paradigm, we can produce spontaneously active, TUJ1/ChAT positive, motor neurons within 3 weeks. These cells produce a train of action potentials when depolarized and exhibit KCl stimulated calcium transients. p97 mutations do not alter the efficiency of differentiation; however, these mutations increase the proportion of spontaneously active neurons, suggesting inherent hyperexcitability. Additionally, mutant neurons display induction of ATF4, increased LC3b, and TDP-43 mislocalization. Unbiased quantitative proteomics of IPSCs (9-plex with 3 replicates per genotype) uncovered proteins and processes altered by p97 mutations. Gene ontology analysis revealed significantly depleted ER stress pathways and lipid biosynthetic processes. Further, several salient p97 interactors were depleted from the homozygous mutant including UBXD2, UBXD8, and derlin-1/2.

Conclusion:

Our novel isogenic model of VCP ALS recapitulates the phenotypes seen in p97 ALS patients including hyperexcitability and ER stress. Further, proteomic analysis uncovered altered pathways in IPSCs, phenotypically normal cells. Additional study of proteome changes in motor neurons may uncover cell-type specific changes that engender ALS pathogenesis and render motor neurons susceptible to degeneration.

Insight in VCP mutants pathological mechanisms: disruption of lysosomal stability, multilamellar bodies formation and autophagy activation

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7. Department of Pediatrics, Children’s Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.
8. Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA.
9. Department of Experimental Medicine, Human Anatomy, University of Genova, Genova, GE, Italy.

Valosin Containing Protein (VCP) has a key role in many crucial cellular pathways, including the maintenance and regulation of proteostasis. Indeed, VCP mutations cause alterations in proteostasis in muscle and brain tissues of VCP patients allowing accumulation of ubiquitin-positive inclusions, TDP-43 mislocalization and aggregation, formation of abnormal rimmed vacuoles. By electron microscopy (EM) analysis, we found the existence of previously unidentified multilamellar bodies (MLBs) in muscles of two animal models of VCP disease, a Drosophila and a murine model expressing a common VCP-pathogenic variant. Similar MLBs were present in motoneuron cell lines expressing VCP R155H and VCP R191Q. Interestingly, we noticed that these MLBs are also detectable in tissue specimens of VCP patients and that their morphology recalled peculiar structures that characterize lysosomal storage disorders. We demonstrated that these MLBs are formed by aberrant lysosomes that present alterations in size, morphology, and activity. These aberrant lysosomes display membrane damage and rapture, which is known to be highly detrimental for cells. Indeed, cells to prevent lysosomal damage toxicity, activate lysosomal removal through autophagy. We found that VCP mutants trigger autophagy via a specific PPP3CB- and TFE3- (but not TFEB-) dependent pathway that promotes MAP1LC3B conversion, SQSTM1 relocalization, and functional autophagosome

formation. Altogether, our findings provide insight into VCP pathogenesis. We propose a novel mechanism of MLBs formation induced by VCP mutants, which involves lysosomal damage and induction of lysophagy.

Loss of Valosin-Containing Protein (VCP) activity enhances proteopathic seeding

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VCP (also called p97, or cdc48 in yeast) is a ubiquitin-directed AAA-ATPase implicated in multiple forms of neurodegeneration. Dominantly inherited mutations in VCP cause multisystem proteinopathy (MSP) which is associated with multiple variably penetrant phenotypes that include inclusion body myopathy, frontotemporal dementia, ALS, and Parkinsonism. One of the shared features of VCP induced MSP is protein inclusions such as tau, α-synuclein (αS), and TDP-43. These proteins are prone to misfold, aggregate, and template inclusion body formation. Accumulating evidence suggests that proteins in their oligomeric form can serve as a “seed”, spread through an interconnected brain network, and induce new inclusions. This transmission correlates with disease progression. Insofar, those proteins are recognized as proteopathic seeds.

Using a genome-wide CRISPR-Cas9 screen, we identified VCP as a suppressor of αS seeding. VCP inhibition or disease mutations increased αS seeding in cells or neurons. This was not associated with an increase in seed uptake and αS expression level. Intrastriatal injection of αS seeds into VCP disease mice (VCPR155H/WT and VCPR155C/FL; CREERT2) enhanced seeding efficiency compared with controls. A similar phenomenon was seen with temporal LLoME treatment, a lysosomal damaging agent. In addition, we screened different VCP cofactors and found UBXD1 modifies the α-synuclein seeding similar to VCP. UBXD1 is known to facilitate VCP in endolysosomal sorting. Our data suggested VCP involved in seeding via endolysosomal-related pathway.

This was not specific to αS since VCP inhibition or disease mutations increased tau and TDP-43 seeding in cells and neurons. These data support that VCP protects against proteopathic spread of pathogenic aggregates. The spread of distinct aggregate species may dictate pleiotropic phenotypes and pathologies in VCP associated MSP.

Utilization of CoRDS Registry to Monitor Quality of Life in Patients with VCP Disease

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Objective:

The purpose of this study is to utilize the Coordination of Rare Diseases at Sanford (CoRDS) Registry Database as a tool for monitoring the quality of life (QOL) in patients with VCP disease over an extended period of time ranging from one to three years.

Background:

VCP disease, characterized as multisystem proteinopathy (MSP1), is a rare, adult-onset, neuromuscular disease that is caused by autosomal dominant variants in the valosin-containing protein (VCP) gene. VCP disease may exhibit one or more of the following primary features: Inclusion Body Myopathy, Paget's Disease of Bone, Frontotemporal Dementia and Amyotrophic Lateral Sclerosis. Because it is a progressive disease, death normally occurs in the 50s and 60s due to respiratory and cardiac failure. The purpose of this study is to utilize the CoRDS database to conduct a prospective natural history study over the course of three years to explore the progression of disease using QOL surveys from participants.

Methods:

Eighty participants signed up for the CoRDS registry and answered QOL questionnaires provided by Cure VCP Disease, Inc., Registry, and the National Institute of Neurological Disorders and Stroke (NINDS) on an annual basis. We conducted a genotype-phenotype comparison by observing the correlation between mutation groups and physical diagnosis. Additionally, we investigated the progression of cognitive function, lower extremity function, and upper extremity function in males (n=38, 47.5%) and females (n=42, 52.5%) by utilizing the 5-point Likert scale with a (range of 0 to maximum total raw score of 40, which represents no difficulty in performing specific tasks). Furthermore, we analyzed the participants' reported pain levels on a scale from 0 to 10 with 10 being the worst pain imaginable. We assessed the correlation between the individual's age and sex with the overall rate of deterioration of cognitive function, mobility, and pain using linear regression. Participants also reported their overall

quality of life and their answers were converted to a 5-point Likert scale and compared across different age groups.

Results:

Participants reported R155H as the most prevalent mutation group and myopathy as the most prevalent phenotype affecting 84% of symptomatic participants. There was a significant decline in cognitive function, lower extremities, and upper extremities with advancing age. Female patients exhibited a more rapid decline than males for cognitive function (females: slope = -0.18, R^2=0.06, $p<0.01$; males: slope=-0.06, R^2=0.01, $p<0.01$), upper extremity function (females: slope = -0.43, R^2=0.30, $p<0.01$; males: slope = -0.33, R^2=0.14, $p<0.01$), and pain (females: slope = -0.03, R^2=0.03, $p<0.01$). Males displayed worsening progression in lower extremity function (females: slope = -0.41, R^2=0.22, $p<0.01$; males: slope = -0.52, R^2=0.34, $p<0.01$) and an increase in pain levels (males: slope = 0.08, R^2=0.13, $p<0.01$) with advancing age.

Conclusion:

CoRDS was found to be a valuable tool for monitoring the quality of life in patients with VCP disease. The patients with VCP disease experienced worsening progression in quality of life symptoms over time. Interestingly, declines in cognitive function and mobility were greater in women. Men experienced a greater increase in pain as the disease progressed. Future studies should continue to monitor VCP disease patients over an extended period of time in order to better understand the factors such as the VCP genotype, age of diagnosis, including other comorbidities that contribute to the progression of the quality of life symptoms.

Development of a standard of care for patients with valosin-containing protein associated multisystem proteinopathy

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Valosin-containing protein (VCP) associated multisystem proteinopathy (MSP) is a rare inherited disorder that may result in multisystem involvement of varying phenotypes including

inclusion body myopathy, Paget's disease of bone (PDB), frontotemporal dementia (FTD), parkinsonism, and amyotrophic lateral sclerosis (ALS), among others. An international multidisciplinary consortium of 40+ experts in neuromuscular disease, dementia, movement disorders, psychology, cardiology, pulmonology, physical therapy, occupational therapy, speech and language pathology, nutrition, genetics, integrative medicine, and endocrinology were convened by the patient advocacy organization, Cure VCP Disease, in December 2020 to develop a standard of care for this heterogeneous and under-diagnosed disease. To achieve this goal, working groups collaborated to generate best evidence recommendations in 10 key areas: genetic diagnosis, myopathy, FTD, PDB, ALS, Charcot-Marie-Tooth disease (CMT), parkinsonism, cardiomyopathy, pulmonology, supportive therapies, nutrition and supplements, and mental health. In April 2021, facilitated discussion of each working group's conclusions with consensus building techniques enabled final agreement on the proposed standard of care for VCP patients. Timely referral to a specialty neuromuscular center is recommended to aid in efficient diagnosis of VCP MSP via single-gene testing in the case of a known familial VCP variant, or multi-gene panel sequencing in undifferentiated cases. Additionally, regular and ongoing multidisciplinary team follow up is essential for proactive screening and management of secondary complications. The goal of our consortium is to raise awareness of VCP MSP, expedite the time to accurate diagnosis, define gaps and inequities in patient care, initiate appropriate pharmacotherapies and supportive therapies for optimal management, and elevate the recommended best practices guidelines for multidisciplinary care internationally.

Examining Interactions Between Autophagy and Stress Granules Dysfunction in a Digenic VCP and TIA1 Distal Inclusion Body Myopathy

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Background

Mutations in valosin-containing protein (VCP) cause inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPFD). IBMPFD is characterized by incomplete penetrance, which may be due to digenic or polygenic interactions. Digenic disease occurs when a secondary variant in another gene is necessary for manifestation of disease, or when the secondary variant modifies the disease phenotype. In this study, we characterize the molecular mechanism underlying a digenic interaction between heterozygous VCP (p.R159H) and a heterozygous T-cell Intracellular Antigen (TIA1) (p.N357S). The presence of TIA1 modifies the disease phenotype, patients with mutant TIA1 present with distal weakness, but only if the VCP variant is also present. TIA1 induces dynamic cytosolic aggregation of RNA-binding molecules, RNA, and translation machinery, deemed stress granules (SGs). SGs selectively inhibit translation under many conditions. For instance, SGs will form when exposed to oxidative stress, viral infection, starvation, or heat shock. Notably TIA1 (p.N357S) is located on the same C-terminal disordered domain as the Welander distal myopathy variant (p.E384K). The C-terminal disordered domain is essential for SG formation. VCP is a multifunctional protein, whose most prominent function is to remove protein from organellar structures and non-membrane bound structures such as SGs and is essential for autophagy. In previous work, we identified TIA1 (p.N357S) as digenic modifier with heterozygous Sequestosome 1 (SQSTM1) (p.P392L) mutation. Like VCP, SQSTM1 plays a critical role in autophagic processes. The objective of our study is to characterize the molecular mechanism and interactions between altered SG dynamics and autophagy defects in mtTIA1/mtVCP mutants. We hypothesize that mtTIA1/mtVCP double mutants exhibit stress granule dysfunction and impaired autophagy.

Methods

Human fibroblasts were extracted from the following patient skin biopsy samples; TIA1 (p.N357S), VCP (p.N91Y), TIA1(p.N357S)/SQSTM1(p.P392L), TIA1(p.N357S)/ VCP(p.R159H). Trypan blue viability assays were performed on patient fibroblasts and were treated with oxidative stressor sodium arsenite (0.5mM) for one hour with or without autophagy inhibitor bafilomycin, or serum starved for 8 hours with or without bafilomycin. Protein was extracted from these cells and autophagic flux western blotting assays were performed. Cells were stained with anti-LC3B and and-SQSTM1 antibodies.

To quantify features of stress granule recovery after insult by oxidative stress, fibroblasts were treated with 0.5mM of sodium arsenite for 45 minutes followed by a 30 and a 60-minute

recovery period. Samples were stained with an anti-G3BP1 antibody. Percent cells with stress granules average number of stress granule per cell and stress granule volume were measured at each time point.

Genetic constructs were generated for wild-type and mutant isoforms of TIA1-GFP (TIA1a and TIA1b), VCP-V5 and SQSTM1-V5 epitopes. HEK293FT cells were transfected with different combinations of wild-type and mutant constructs to examine colocalization in untreated or with 1 hour arsenite (0.5mM) treated cells. Cells were then stained with anti-V5 antibodies.

Predictions and Results

Untreated mtTIA1/mtVCP exhibit a 50% reduction in viability compared to male and female controls, mtTIA1 alone, mtVCP alone, and mtTIA1/mtSQSTM1. Serum starved mtTIA1/mtVCP cells appeared to also experience a slightly greater reduction in viability (40% viable), however this was not statistically significant. We predict that western blot autophagy assays will illustrate impairments in carrier flux with mtTIA1/mtVCP and reduced clearance of SQSTM1.

After 1 hour recovery from arsenite, 20% of all cells with at least one mutant had SGs, compared to controls (<10%). During the 45 minute arsenite treatment, mtVCP, mtTIA1/mtVCP, and mtTIA1/mtSQSTM1 had significantly more SGs per cell. While the average volume of SGs was two-fold higher at 30 minutes recovered in mtVCP and mtTIA1/mtVCP only. We predict that there will be reduced colocalization of mtVCP to SGs.

Conclusion

This is the first time that TIA1 has been shown to act as a modifier variant alongside VCP. Currently results indicate that there is clear impairment of SG clearance and abnormal SG features in cells with mtVCP, as well as a large reduction in viability of mtTIA1/mtVCP cells.

Regulation of ER-mitochondrial Contact Sites by the p97 AAA-ATPase

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Inter-organelle contacts are appreciated to regulate a number of fundamental biological processes, by serving as platforms for the synthesis of specific biomolecules, transfer of metabolites and organelle division. In particular, the close apposition of the endoplasmic reticulum (ER) and mitochondria is important for calcium transfer and signaling, membrane lipid synthesis and exchange, mitochondrial division, and autophagy. Aberrant formation and dissolution of contacts between these organelles is emerging as an important contributor to the pathobiology of a number of human disorders. However, how contact sites are dynamically re-modeled in a spatio-temporal manner is largely unknown.

We sought to explore the role of the ubiquitin proteasome system (UPS) in the regulation of proteins localized to ER-mitochondria contacts and identified a novel role for the p97 AAA-ATPase and its ER membrane-anchored adaptor UBXD8 in regulating the abundance of contacts between the ER and mitochondria. p97 (also known as valosin containing protein, VCP) is an evolutionarily conserved ATP-driven unfoldase, that functions in the UPS to mediate degradation of ubiquitylated substrates. Substrate identification in many instances requires dedicated ‘adaptor’ proteins (such as UBXD8) that recruit p97 to ubiquitylated targets. Our studies indicate that loss of the p97-UBXD8 complex leads to an increase and the abundance of contacts between the ER and mitochondria. Quantitative proteomics of purified ER-mitochondria contacts from wildtype and UBXD8 knockout (KO) cells identified widespread changes in enzymes involved in lipid synthesis. These findings were verified by lipidomic studies that indicated that UBXD8 KO cells had elevated levels of specific phospholipids and an increase in very long chain fatty acids. We show that loss of p97-UBXD8 prevents activation of master lipogenic transcription factor sterol regulatory element binding protein (SREBP1/2) in the ER and contributes to defective lipid metabolism.

Importantly, aberrant contacts observed in p97 or UBXD8 depleted cells can be rescued by supplementing cells with specific fatty acids. In summary, our findings suggest that perturbed lipid metabolism may impact inter-organelle contacts and have identified a new role for p97 and its adaptor UBXD8 in modulating ER-mitochondria contact sites.

Establishing clinical trial readiness for valosin containing protein-associated multisystem proteinopathy: baseline demographics from a 1-year prospective study

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Introduction: Valosin containing protein-associated multisystem proteinopathy (VCP MSP), previously referred to as IBMPFD, is a rare disorder of multisystemic involvement resulting in progressive weakness, cardiac, respiratory, and/or bulbar dysfunction. Presentation is heterogeneous, highlighting the need for a prospective natural history study.

Objective: To describe the baseline and functional characteristics of a cohort of 24 patients with VCP MSP.

Methods: This natural history study is a single-site, prospective study of this disease, evaluating patients with genetically confirmed VCP MSP over 1 year. We present a cross-sectional analysis of baseline visits. A complete medical and family history, physical exam, and functional testing were completed.

Results: Twenty-four patients (mean age 51.2 years, 58% female, 100% Caucasian, 21% Hispanic) completed Baseline assessments. Myopathy-type weakness was present in 96% of the cohort with an average onset at 42.5 years of age (18 – 59 years). The amount of time since symptom onset was 8.8 years (1.9 – 23.8). A majority of the cohort (88%) were considered ambulatory, which is defined as able to complete the 10-meter walk/run in <30 seconds without an assistive device. Upper extremity function was also examined with 33% of the cohort able to raise both arms overhead without compensations, 25% could complete the task but used compensatory motions, and 42% were unable to raise both arms overhead but could bring hands to mouth-level. Patients reported other system involvement in addition to myopathy-type weakness in a subset of the cohort: frontotemporal dementia 4%, Paget's disease of the bone 21%, cardiomyopathy 13%, cataracts 17%, respiratory involvement requiring BiPAP/CPAP 21%, and bowel/bladder urgency and reduced control 67%. Upon physical exam, peripheral neuropathy was present in the lower extremity in 63%, 21% had both upper and lower extremity sensory impairments; whereas only 13% had co-morbidities likely to cause sensory impairment including diabetes and pre-diabetes (but did not account for neuropathy in the majority). Additional findings and mutation-specific performance on functional assessments will be presented.

Conclusions: This ongoing study will provide an opportunity to further understand patterns in disease progression and identify outcome measures that are most sensitive to change over time in individuals with VCP MSP to inform future clinical trial design.

Overview of Valosin Containing Protein from the Perspective of a Rare Disease Advocacy Organization

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Valosin-containing protein (VCP) is a ubiquitous protein and a member of the AAA ATPase superfamily (ATPases Associated with a variety of cellular Activities). It has a myriad of functions in the cell including protein quality control, membrane fusion, and maintenance of the cell cycle, and apoptosis. VCP Disease is a devastating disorder caused by mutation of the gene encoding for the valosin-containing protein. This disease results in several pathologies including inclusion body myopathy, Paget's disease of bone, ALS, and frontotemporal dementia. Considered a rare disease, people who develop this disorder experience life-altering symptoms and often feel disenfranchised with their diagnosis, being unable to access appropriate treatments or support systems. Another complication of its rare nature is that this disease is frequently misdiagnosed, due in large part to lack of awareness. Advocacy programs like Cure VCP Disease, Inc. seek to educate caregivers, patients, and medical professionals about the disease and advocate for increased research and resources devoted to its cure. These groups are an integral facet of the rare disease community. They serve as a reminder that this research is essential for people who have experienced real pain and hardship as a result of diseases, they advocate for and protect the interest of people who suffer from these diseases, and they can even influence research by raising awareness. It is imperative that the scientific community bolster and encourage the work of these groups. This review will examine existing literature concerning VCP Disease and will present what is currently known of this multisystem proteinopathy through the lens of patient advocacy. Additionally, the unpublished and deidentified testimonials of several patients and caregivers will be presented to emphasize the importance of patient advocacy work.

CRISPR/Cas9-engineered Drosophila knock-in models to study VCP diseases

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Mutations in Valosin Containing Protein (VCP) are associated with several degenerative diseases, including multisystem proteinopathy (MSP-1) and amyotrophic lateral sclerosis. However, patients with VCP mutations vary widely in their pathology and clinical penetrance, making it difficult to devise effective treatment strategies. A deeper understanding of how each mutation affects VCP function could enhance the prediction of clinical outcomes and design of personalized treatment options. The power of a genetically tractable model organism coupled with well-established in vivo assays and a relatively short life cycle make Drosophila an attractive system to study VCP disease pathogenesis. Using CRISPR/Cas9, we have generated individual Drosophila knock-in mutants that include nine hereditary VCP disease mutations. Our models display many hallmarks of VCP-mediated degeneration, including progressive decline in mobility, protein aggregate accumulation and defects in lysosomal and mitochondrial function.

We also made some novel and unexpected findings, including nuclear morphology defects and sex-specific phenotypic differences in several mutants. Taken together, the Drosophila VCP disease models generated in this study will be useful for studying the etiology of individual VCP patient mutations and testing potential genetic and/or pharmacological therapies.

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Regulation of ER-protein homeostasis by the p97-UBXN1 complex

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The AAA+ ATPase p97 mediates the ubiquitin-dependent proteasomal degradation of a wide variety of substrates through interaction with pathway specific adaptor proteins. Mutations in p97 that are proposed to disrupt p97-adaptor associations are implicated in a number of neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS) and Charcot-Marie Type-IIB. There are currently about forty known adaptor proteins, but most remain poorly characterized. Our lab has previously identified a novel role for a poorly understood p97 adaptor, UBXN1, in protein quality control. We found that in association with the BAG6 complex, p97-UBXN1 regulates specific substrate degradation of proteins that fail to target to and enter the endoplasmic reticulum (ER) and are rerouted to the cytosol. Recently, we have identified a new role for p97-UBXN1 in preserving ER-protein homeostasis by preventing ER stress induction and subsequent activation of the unfolded protein response (UPR). We have shown through tandem-mass-tag (TMT) proteomics that CRISPR-Cas9 mediated knock-out of UBXN1 results in significant expression of proteins involved in ER-protein quality control and response to ER stress. We have utilized cell viability assays to show that CRISPR-Cas9 UBXN1 knock-out cells have decreased viability and are highly susceptible to ER-stress inducing agents. Furthermore, we demonstrate through immunoblot, real-time PCR, and immunofluorescent imaging that there is increased activation of all three UPR arms upon loss of UBXN1. These data suggest that p97-UBXN1 dependent ER-quality control is crucial to preserve ER-homeostasis. We are interested in determining how cytosolic UBXN1 regulates protein homeostasis within the ER. In support of this role in quality control at the ER, we have shown that UBXN1 localizes to ER-derived microsomes. Notably, this new function of p97 in ER-quality control is distinct from the role of p97 in ER-associated degradation (ERAD), as we previously found that UBXN1 depletion does not impact the degradation of several ERAD reporters. We hypothesize that p97-UBXN1 functions as a pre-emptive quality control pathway that precludes protein misfolding in the ER, highlighting a novel role for p97-UBXN1 in not only alleviating ER stress by ERAD but preventing ER stress through pre-emptive quality control. Preliminary data from our lab alludes to increased influx of several substrates into the ER when UBXN1 is depleted. Disbalance between the protein folding load and folding machinery in the ER generates ER stress and can lead to apoptosis if not alleviated. Our work investigates the

overarching hypothesis that the p97-UBXN1 complex prevents ER stress and UPR induction by preventing aberrant protein insertion into the ER, and that this process is dysregulated upon loss of UBXN1. This work has important clinical implications as numerous studies suggest that aging cells experience progressive decline in ER-quality control machinery and begin to favor the apoptotic cascade of the UPR. Our work will provide insight into mechanisms that regulate ER homeostasis and will provide rationale for therapeutic avenues that prevent protein misfolding in the ER. It is an attractive option to generate therapies that could prevent ER stress rather than attempting to alleviate it.

Structural insights into the p97 disease mutant complex with p47 and its functional implications

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The Human p97/VCP (valosin-containing protein) is a hexameric AAA+ (ATPase associated with diverse cellular activities) plays a pivotal role in the regulation of multiple cellular activities by interacting with various co-factors and adapter proteins. Critical roles of p97 involve ubiquitin-dependent protein quality control and regulation of membrane fusion in the Golgi apparatus and endoplasmic reticulum-associated degradation (ERAD) pathway in the presence of cofactor p47. Heterozygous mis-sense mutations of human p97 have been implicated in numerous neurodegenerative diseases, such as IBMPFD (Inclusion body myopathy with early-onset Paget's disease and frontotemporal dementia)/ALS (amyotrophic lateral sclerosis). The disease mutations of the p97 are mostly clustered on the N-domain or the connection between N and D1-domain. The single point mutation of R155H on the N-domain is one of the highest mutated sites, leading to a rare degenerative disease multisystem proteinopathy 1 (MSP1) and resulting

in abnormal ATPase activity and cofactor dysregulation. The structural details of the p97 R155H malfunction are unknown. Our study aims to characterize the protein interactions and identify the key complex structure to answer disease relevance of the p97 R155H -p47 complex.

Effects of VCP mutations on endolysosomal damage and TDP-43 aggregation in skeletal muscle and neurons

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Mutations in valosin-containing protein (VCP) are the most common cause of multisystem proteinopathy (MSP), a disease that involves combinations of inclusion body myopathy, Paget's disease of the bone, amyotrophic lateral sclerosis, and frontotemporal dementia. VCP is a ubiquitously expressed ATPase involved in many cellular processes including mitosis, apoptosis, membrane fusion, and protein degradation. However, it is unknown specifically how mutations in VCP cause neurodegeneration in a subset of patients, myopathy in others, and in some cases both. To investigate tissue-specific pathology, VCP patient induced pluripotent stem cell (iPSC) lines and isogenic controls were differentiated into both cortical neurons and skeletal myocytes and characterized by immunostaining and western blot. Preliminary results show increased ubiquitin and p62-positive inclusions in both skeletal myocytes and neurons differentiated from the VCP lines compared to healthy or isogenic control lines. Previous work from our group has shown that VCP is recruited to damaged lysosomes and facilitates their clearance. To test whether lysosomal homeostasis is altered in VCP cell lines, iPSC-derived skeletal myocytes were treated with L-leucyl-L-leucine methyl ester (LLOMe) to induce endolysosomal damage. Skeletal myocytes from VCP patients treated with LLOMe showed an increase in ubiquitin and p62-positive inclusions as well as accumulation of Lamp2-positive endolysosomes. In addition, iPSC-derived skeletal myocytes with the VCP mutation showed an increase in insoluble TDP-43, a protein commonly found aggregated in MSP patients and several other neurodegenerative diseases and myopathies. The amount of insoluble TDP-43 was further increased when myocytes were treated with arsenite to induce cell stress. In addition, C2C12 mouse myoblasts which were treated with arsenite followed by a VCP inhibitor had a persistence of insoluble TDP-43 compared to arsenite-only controls. Together, these results suggest that mutations in VCP may facilitate TDP-43 aggregation as previously suggested, or lead to decreased clearance. Future work will investigate whether mutations in VCP influence TDP-43 aggregation through dysregulation of lysosomal processes or another mechanism.

Mechanisms of stress granule dysregulation caused by VCP mutations

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An important biological question is what factors regulate stress granule dynamics in human cells, as aberrant stress granule behavior is associated with a growing class of neurodegenerative diseases. Stress granules are dynamic mRNA and protein-containing complexes that rapidly form in response to cellular stressors that inhibit mRNA translation. They then dissociate when the stress is resolved and translation resumes. In neurodegeneration, stress granules become less fluid and more persistent, and are hypothesized to help seed the pathological protein inclusions that are a hallmark of these disorders. Valosin containing protein (VCP) is an important stress granule regulator. Depletion or inhibition of VCP impairs stress granule disassembly, as does overexpression of VCP mutant alleles that cause amyotrophic lateral sclerosis, frontotemporal dementia, Paget's disease of the bone, and inclusion body myopathy. Thus, understanding how VCP regulates stress granules is important to understanding its role in disease. To this end, we are using fluorescence microscopy to investigate how VCP inhibition or mutations alter stress granule dynamics. As VCP is involved in diverse cellular processes, from ER stress to ribosome-associated quality control, defining the precise mechanism through which it regulates stress granules will advance our understanding of how stress granule behavior becomes impaired in degenerative diseases.

Evaluating consistency of subject performance between 2-day visits in subjects with valosin-containing protein multisystem proteinopathy

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Introduction: Valosin- containing protein multisystem proteinopathy (VCP-MSP), is a genetically-inherited neuromuscular disorder that impacts multisystem function. A variant in the VCP gene leads to various phenotypes such as inclusion body myopathy, Paget's Disease of the bone, and Frontotemporal Dementia, among others.

Objective: To evaluate the consistency of individual and group performance on clinical outcome assessments (COA) between 2-day visits performed in clinic.

Methods: We evaluated 24 individuals, mean age 51.2 years, with a genetically confirmed diagnosis of VCP-MSP. Subjects were asked to complete 2-day back to back functional assessments in clinic. Clinical outcome assessments include 100 meter timed test (100m),10 meter walk/run (10m), Performance of Upper Limb 2.0 (PUL), North Star Ambulatory Assessment for limb girdle-type muscular dystrophies (NSAD), and Timed Up and Go (TUG). Comparative analysis was performed between COA for day 1 and 2 for in- clinic assessments.

Results: Group performance between days 1 and 2 for all outcomes was statistically significantly correlated ($r=0.93- 0.99$, $P<0.001$). Mean change in performance for the NSAD between days 1 and 2 was 0.33 ± 2.24 points,100m was 1.51 ± 22.76 seconds (2.5% average change in performance between day 1 and day 2), TUG 0.26 ±1.61 seconds, PUL 0.04 ±1.72 points, and 10m 0.35 ±1.06 seconds. ICC for all COA were > 0.96 ($P<0.001$) indicating excellent test-retest reliability. Of note, some variability was identified in individual patient performance across and will be presented in more detail.

Conclusion: In conclusion, group performance appears consistent in all COA across visits. Individual patient performance can be variable for some due to other contributing factors such as endurance and fatigue, comfort, confidence, and safety. Use of 2-day visits in clinical trials is recommended to understand variability existing at baseline both within individuals and the group, as well as factors associated with that variability. Further research is ongoing to evaluate sensitivity to change over time across all COA in patients with VCP-MSP.

Utility and reliability of patient-reported outcome measures for valosin containing protein-associated multisystem proteinopathy: baseline results from a 1-year prospective study

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Introduction: Valosin containing protein-associated multisystem proteinopathy (VCP MSP), also IBMPFD, is a rare disorder of multisystemic involvement resulting in progressive weakness, cardiac, respiratory, and/or bulbar dysfunction. Presentation is heterogeneous, highlighting the need for a prospective natural history study. As a growing number of experimental treatments move toward clinical trial in rare disease populations, The Federal Drug Administration (FDA) has placed increasing emphasis on the importance of documenting a patient's perception of their disease and the relationship of change with treatment. Currently, there are no disease-specific patient-reported outcome measures (PROs) that have been developed for or validated for use in individuals with VCP MSP.

Objective: To determine the utility and reliability of a selection of patient-reported outcome measures and their correlation to functional assessments.

Methods: In this prospective, one-year natural history study subjects were asked to complete 9 patient-reported questionnaires following functional testing during 2-day remote and onsite baseline visits. The PROs completed have been previously validated in other populations and focused on domains of interest in VCP MSP including PROMIS Upper Extremity, Mobility, Cognitive, and Global Health scales, Communicative Participation Item Bank (CPIB), Rasch Overall ALS Disability Scale (ROADS), IBM Functional Rating Scale (IBMFRS), EAT-10: A Swallowing Screening Tool, and Patient Global Impression of Change Scale.

Results: Twenty-four subjects (mean age: 51.2 years (range: 28-66)) with genetically confirmed VCP MSP completed 96 total visits. Inter-visit reliability of patient report between and within visit types was strong to excellent across all PROs (ICC=0.72-0.99, $p<0.001$; and confirmed using Pearson correlation $r>0.78$, $p<0.001$). There was a moderate to strong correlation between PROMIS Mobility and assessments of ambulatory and gross motor function (spearman rho>0.62, $p<0.005$) and a moderate correlation between PROMIS Upper Extremity and assessment of upper extremity function (spearman rho=0.64; $p<0.005$). A ceiling effect was noted with the CPIB, with 75% of subjects (18/ 24) receiving a score of 93% or above.

Additionally, the CPIB was found to have the greatest variability of scoring between inter-day onsite visits. Four subjects required additional e-mail reminders to complete PROs following one of their visits; two subjects did not complete PROs for one visit.

Conclusions: Patient reported outcome measures are becoming increasingly important to the FDA when determining the impact of study drugs on patient outcome. Cross-sectional baseline analysis indicates strong reliability and correlation to functional outcomes of nine PROs in individuals with VCP MSP. Further analysis is needed to determine sensitivity to change across time and to explore mutation-specific trends by PRO domain.

Benefits of Remote Inspiratory Exercise Training in Familial Myopathy

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VCP disease is a lethal, autosomal dominant disorder caused by a mutation in the VCP gene. Pathogenic mutations in the VCP gene lead to a variety of phenotypes such as inclusion body myopathy, Paget's disease of bone, frontotemporal dementia, amyotrophic lateral sclerosis, and Parkinson's disease. Of these, inclusion body myopathy is the most common affecting approximately 90% of individuals with a pathogenic mutation. As a result of their variant, it is believed that individuals with VCP disease are unable to degrade proteins properly which can result in the late onset of proximal muscle weakness which eventually progresses throughout the rest of the body. Because of their advancing muscle weakness, complications of respiratory failure are known to be the most common causes of death amongst patients with advanced VCP disease. In order to combat this progressive respiratory muscle weakness, we are researching the link between daily inspiratory muscle exercises and their effect on a VCP patient's maximum inspiratory pressure (MIP).

As the COVID-19 pandemic greatly restricted in person patient research and care, a remote study protocol was developed for a cohort of VCP patients to follow. Due to the rare nature of VCP disease, each participant in the study will be used as their own baseline measurement to strengthen the data collected. For the first eight weeks of the study, each patient would partake in biweekly quality of life surveys, hand grip dynamometry, and two types of walking tests. In addition to these, their forced vital capacities and MIPs were measured using handheld spirometers and inspiratory training devices respectively. Once their baseline data is collected, each participant will be placed on a unique inspiratory exercise regimen using an inspiratory threshold trainer set to 50% of the highest MIP recorded throughout the eight week baseline period. Each participant will then train six times a week with their inspiratory threshold trainer; 25 inhalations through their devices each morning and evening for 6 days a week. Their perceived exertion following these exercises will also be determined using the Borg Scale modified to fit this study's focus on inspiratory effort and exertion. Once the participants have completed eight weeks of exercising at 50% of their MIP, they will be prescribed a new exercise regimen consisting of 60% of the highest MIP recorded from the 50% training period, thus progressively increasing the effort required and theoretically strengthening the intercostal muscles and diaphragms of each subject participating. This same practice of increasing the pressure they must overcome by 10% every eight weeks will continue for a total of forty weeks, ideally having patients exercising at 80% of their MIP as the study comes to a close. Although the study is planned to be forty weeks in total, early termination of the study may be an option if a significant increase in the MIP is observed regularly throughout the cohort.

We are hoping to observe at least a 25% increase in each subjects' MIP respective to their highest recorded baseline measurement. Many other studies have examined the benefits of inspiratory resistance training in a variety of patient populations and have demonstrated significant results, something that we are also hoping to achieve in those affected by VCP disease. If significant results are obtained, it may be advisable to include inspiratory training as a standard of care for all VCP patients experiencing muscle weakness.

Clinical classification of variants in the valosin containing protein gene associated with multisystem proteinopathy

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Introduction

Dominant mutations in Valosin Containing Protein (VCP) cause a disorder that includes myopathy, motor neuron disease, Paget's disease of the bone (PDB), fronto-temporal dementia (FTD) and parkinsonism termed multisystem proteinopathy (MSP). This pleotropy in MSP makes the systematic classification of VCP variants challenging.

Objective

To describe the novel variants in the VCP gene identified in the VCP international multicentric study and to develop an integrated framework to assess variant pathogenicity.

Methods

The VCP international multicentric study collected 233 adults with a confirmed mutation in the VCP gene. Fifty-four variants were identified. A scoring system (0-5 points) to evaluate phenotypic level of evidence to support pathogenicity of the novel variants was developed. A single point was given to each of the following: a MSP core phenotype (myopathy, motor neuron disease, dementia, PDB or Parkinsonism), a muscle biopsy evidence of rimmed vacuoles or protein inclusions and a family history of a 1st degree relative with an MSP core phenotype. Variant segregation in the affected family member earns two points. Two variants, p.I216M and p.I369F were identified in more than one unrelated family earning an additional point. A half point was given if the variant was present in residue that has been previously described in an MSP patient including a patient in our cohort or evidence of a symmetric or patchy asymmetric fat replacement on an axial T1-w MRI of the tights and/or legs. A score ≥3 was deemed as high likelihood of pathogenicity.

Results

Eighteen unreported variants in 24 patients from 21 unrelated families were identified. All were heterozygous, and the variants were missense coding except for one small deletion-insertion. All patients manifested with at least one MSP core phenotype (myopathy, PDB, FTD, ALS or Parkinsonism). Muscle weakness was reported in the 95.2% (20/21), cognitive impairment in 26% (6/23), and PDB in 16.8% (7/24). The classical IBMPFD triad was reported in only one patient and one patient presented with isolated PDB. In 60.8% of the families (14/23) a first degree relative reported to have an MSP core phenotype the most common being a history of dementia (7/23). The Pathogenic Degree Score strategy defined the variants p.I216M, p.I241S, p.M158T, p.N90D, p.I369T, and p.V123M as high-likelihood pathogenic variants. p.I369F, p.R144H, p.E66K, p.K164Q, p.I27T and p.R155G were deemed probable pathogenic variants whereas p.K663R, p. L96V, p.I353V, p.C209G, p.R89G and p.I233V were deemed uncertain pathogenicity.

Conclusion

This study describes novel variants associated with MSP in VCP and provides guidance for clinicians in its evaluation. We are currently carrying out enzymatic and cell-based assays to identify the functional defect in the VCP protein function as a result of the novel variants.

Genotype-phenotype correlations in Valosin Containing Protein disease: Results of an International Multicentric study

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Introduction

Valosin-containing protein (VCP) disease caused by mutations in the VCP gene leads to disabling weakness, Paget's disease of bone (PBD) and Fronto temporal dementia (FDT) and is frequently misdiagnosed with other neuromuscular diseases.

Objectives

To describe the clinical and genetic features of a large international cohort of patients with mutations in the VCP gene.

Methods

Retrospective descriptive study collecting clinical and genetic data from patients with confirmed mutations in the VCP gene in 51 centres from 24 countries.

Results

233 patients were collected and 223 included in the final analysis (175 families, 71% males, mean age 56.6 + 9.7 y). Mean age at symptom onset 45.6 + 9.4 y. and median time to a genetic test confirming VCP 8.9 y. 99% were heterozygous. 58 variants were identified, 4 were the most frequent: c.464G>A (n=63), c.463C>T (n=26), c.476G>A (n=18) and c.277C>T (n=15). First symptoms frequency: 32.7% proximal symmetric lower limbs (LL) weakness, 14.8% symmetric distal LL weakness and 6.7% asymmetric weakness. At last assessment, 89% proximal LL, 81% proximal Upper limb, 74% distal LL, 49% scapular winging and 48% axial weakness. 27% had respiratory impairment, 25% PBD and 14% FDT. Only 9 patients showed the classic triad (4%). Other clinical features: lower motor neuron signs (22%), dysautonomia (19%), dysphagia (17%) and upper motor neuron signs (13%). The variant c.463C>T had the earliest age of onset (37.8+7.5 $p<0.01$) and a greater distal UL and axial weakness in comparison to c.464G>A ($p<0.05$). 24% of the patients required a full-time wheelchair at a mean of 10.3+5.9 y. from onset and it was associated to the presence of axial weakness and dysautonomia ($p<0.05$). Thirty-five (15%) patients died (causes: 8 respiratory impairment) at a mean of 15.3 + 6.1 y. from onset and it was associated to the presence of FDT and dysphagia ($p<0.05$).

Conclusion

VCP disease resembled a LGMD, however, the presence of PDB, FDT, dysautonomia, LMN, UMN signs and the rapid loss of ambulation should raise awareness of VCP. c.463C>T variant had a more severe phenotype.