ENVIRONMENTAL AND GENETIC INFLUENCE ON COGNITION IN ALS-FTD SPECTRUM DISEASE

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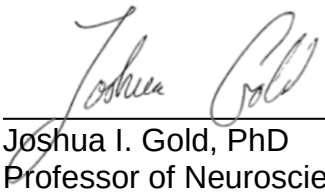

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ABSTRACT

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Amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) are earlier-onset, fatal neurodegenerative diseases that progressively rob affected individuals of their cognitive faculties and their ability to move freely, produce coherent speech, and be their former selves. Diagnosis precedes death by <10 years, and current interventions are limited to palliative or disease-slowing therapies. Recent seminal research has revealed that ALS and FTD are phenotypic extremes on a continuous spectrum with shared symptoms, pathobiology, and genetics. Despite increased knowledge of ALS-FTD spectrum disease, prognostication and therapeutic development are limited by immense phenotypic heterogeneity. One source of this heterogeneity is cognition: individuals across the ALS-FTD disease spectrum suffer varying degrees of decline in cognition due to neurodegeneration in the frontal and temporal lobes. Cognition is inextricably linked to functional ability and survival, and is thus a promising candidate for a prognostic marker and therapeutic target. With this in mind, the goal of my thesis work is to elucidate factors that influence the heterogeneity of cognitive impairment and corresponding frontotemporal neurodegeneration in ALS-FTD spectrum disease. I pursue this goal by studying

clinical, demographic, genetic, anatomic, and biologic data from deeply-phenotyped patient populations and applying robust statistical approaches including multimodal, nonparametric, and machine learning analyses. My work demonstrates strong environmental and genetic contribution to cognition and frontotemporal disease anatomy in ALS-FTD spectrum disease, and suggests their importance to advancements in clinical care and therapeutic development.

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CHAPTER 1: INTRODUCTION

In this chapter, I summarize prior scientific contributions to the study of factors influencing cognition in ALS-FTD spectrum disease. I begin by providing a broad overview of ALS-FTD spectrum disease with a focus on shared clinical, biologic, and genetic features. I then turn to the specific issue of heterogeneity in cognitive impairment, and review preliminary evidence for environmental and genetic influence on its presentation. I finally discuss how analytic approaches incorporating multimodal data sources and nonparametric and data-driven techniques can be used to study outstanding questions regarding environmental and genetic influence on cognition in ALS-FTD spectrum disease.

ALS-FTD SPECTRUM DISEASE

Amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) are earlier-onset, fatal neurodegenerative diseases that progressively rob affected individuals of their cognitive faculties and their ability to move freely, to produce coherent speech, and to be their former selves. Diagnosis precedes death in typically less than ten years, and currently-available treatments are only palliative or serve to slow disease course. Historically, ALS and FTD comprised separate areas of research due to their respective neurological classifications as diseases of the motor system or of higher order cognition and behavior. These conditions are modernly thought of as two extremes on a continuous ALS-FTD disease spectrum rather than distinct neurodegenerative conditions due to seminal research from the past two decades demonstrating considerable overlap in clinical presentation, common underlying pathobiology and neuroanatomy, and shared genetic architecture. In this section, I will first briefly review the classic disease profiles of ALS and FTD, and then discuss key clinical, pathobiologic, neuroanatomic, and genetic evidence for the existence of these neurodegenerative conditions along a continuous disease spectrum.

Disease Profile of ALS

ALS, which is also referred to colloquially as Lou Gehrig's disease in the United States and as motor neuron disease (MND) in the European Union, is characterized by progressive degradation of the voluntary motor system resulting in paralysis, and ultimately death. Symptom onset occurs from ages 40 - 70 but

is also noted in individuals in the 20s and 30s, and a recent meta-analysis found that 1.75 (1.55-1.96)/100,000 people per year worldwide are newly affected (Marin et al. 2017). Diagnosis requires clinically-evident symptoms of upper motor neuron (UMN) degeneration (e.g. spastic tone, deep tendon reflex) and clinical, neuropathological, or electrophysiological evidence of lower motor neuron (LMN) degeneration (e.g. weakness, muscle atrophy) (Brooks et al. 2000). Symptoms can occur in bulbar or spinal musculature and on ipsilateral or contralateral sides of the body. Because ALS is a progressive disease and patients may come to clinical attention with a sub-clinical syndrome, the internationally recognized El Escorial criteria provide four categories of ALS based on diagnostic certainty: Suspected, Possible, Probable, or Definite ALS. Worsening of neuromuscular function over time is accompanied by progressive gray matter (GM) loss in the motor cortex and white matter (WM) degeneration in the corticospinal tracts and corpus callosum as evident through magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) (Foerster, Welsh, and Feldman 2013; Bede and Hardiman 2018), although extra-motor neurodegeneration is also observed. An estimated 10% of patients with ALS have a family history of disease often associated with a genetic mutation in *C9ORF72* (Renton et al. 2011; DeJesus-Hernandez et al. 2011), *SOD1* (D. R. Rosen et al. 1993), or *TARDBP* (Kabashi et al. 2008; Sreedharan et al. 2008); and a minority of patients (~10%) without a family history also have *C9ORF72* mutations (Taylor, Brown, and Cleveland 2016). Disease mechanism in patients with and without a genetic mutation is yet unknown; abnormal protein inclusions

composed of TDP-43 are found *post mortem* in ~97% cases, with the remaining showing fused in sarcoma (FUS) pathology associated with point mutations in the *FUS* gene (~1%) or showing superoxide dismutase pathology associated with point mutations in the *SOD1* gene (Al-Chalabi et al. 2012). Death typically occurs 20 - 48 months from initial symptom onset (Chiò et al. 2009), and is most frequently due to respiratory failure but can also result from other causes including cardiac failure (Corcia et al. 2008; Gil et al. 2008; D. R. Rosen et al. 1993). Currently, Riluzole (brand name: Rilutek) and Edavarone (brand name: Radicava) are the only two treatments for ALS approved by the Food and Drug Administration (FDA), and have mild impact on disease course and nonspecific mechanisms of action. Riluzole targets excitotoxicity as a purported cause of neuronal death (Van Den Bosch et al. 2006), and its potential mechanisms of action include the inhibition of glutamatergic transmission, inactivation of voltage-gated sodium channels, and interference with events following transmitter binding at excitatory amino acid receptors (Doble 1996). Meta-analysis of four clinical trials indicate that Riluzole usage mildly prolongs survival by 2-3 months (R. G. Miller et al. 2007). The exact mechanism of action of Edavarone is also unknown, but its therapeutic effect of slowing the rate of progression of functional motor disability is attributed to its antioxidative properties (Cruz 2018; Abe et al. 2014). Current clinical and preclinical therapeutic development for ALS includes highly-specific antisense oligonucleotide (ASO) therapies for patients carrying *SOD1* mutation or *C9ORF72* repeat expansion and formulation of viral therapies (Ly and Miller 2018).

Disease Profile of FTD

Profound and worsening impairments in higher-order cognition, language, and social behavior comprise the predominant symptoms of FTD. FTD is named for the cerebral loci of neurodegeneration – in the frontal and temporal lobes - responsible for three main observed syndromes: behavioral variant FTD (bvFTD), semantic variant primary progressive aphasia (svPPA), and nonfluent agrammatic primary progressive aphasia (naPPA). Each syndrome has its own diagnostic criteria, with executive deficits, personality change, and apathy characterizing bvFTD (Rascovsky et al. 2011), and discrete impairments in word comprehension and agrammatism characterizing svPPA and naPPA, respectively (Gorno-Tempini et al. 2011). The bvFTD syndrome is roughly four times more prevalent than naPPA or svPPA (Hogan et al. 2016). Age at symptom onset ranges from 21-80 years, with most cases occurring between 40-64, (Coyle-Gilchrist et al. 2016) and meta-analyses estimate incidence at 2.7-4.1/100,000 (Onyike and Diehl-Schmid 2013), making FTD the 2nd most common form of dementia among individuals less than age 65. Clinical FTD syndromes are distinguished by progressive patterns of atrophy observed through structural MRI (Meeter et al. 2017). Early atrophy in the orbital and dorsolateral prefrontal cortex, anterior cingulate cortex, and insula are observed in bvFTD (Pan et al. 2012), whereas early atrophy in the left anteroinferior temporal lobe are observed in svPPA, and in the left inferior frontal cortex and insula in naPPA (Gorno-Tempini et al. 2004). All three syndromes feature progressive atrophy over disease course, with subcortical structures including the thalamus, basal ganglia,

and amygdala increasingly involved in bvFTD (Devenney et al. 2015), and increasing extent of left and right hemispheric atrophy in svPPA and naPPA (Rogalski et al. 2011; Bonner, Ash, and Grossman 2010; Grossman 2010). Around 30% of FTD patients have a family history of disease which can be largely attributed to pathogenic mutations in *C9ORF72*, *MAPT*, and *GRN* (Greaves and Rohrer 2019), although *C9ORF72* mutations are also found in <10% of sporadic cases (Majounie et al. 2012). FTD is pathologically heterogeneous, and post-mortem study shows abnormal protein inclusions in the brain tissue composed of pathologic forms of TDP-43 in ~45-50% of cases, Tau in ~45% of cases, and fused-in-sarcoma (FUS) protein in <10% of cases (Irwin, Cairns, et al. 2014). The underlying pathologic protein can only be ascertained through association with a known pathogenic mutation (e.g. TDP-43 with *C9ORF72*) or, in mutation-negative cases, upon autopsy (Irwin, Cairns, et al. 2014). Death can occur as rapidly as 2 years and as prolonged as 12 years after initial symptom onset (Coyle-Gilchrist et al. 2016). There is no FDA-approved therapy for FTD; current treatments are palliative in nature and include antidepressant and antipsychotic medications for symptom management (Boxer and Boeve 2007; Tsai and Boxer 2016). Current therapeutic development includes ASOs directed towards patients carrying *C9ORF72* repeat expansions and *MAPT* mutations, adeno-associated virus (AAV) therapies directed towards patients carrying *GRN* mutations, and monoclonal antibodies (mAb) targeting pathologic Tau (Boxer et al. 2020).

Clinical Features of ALS-FTD Spectrum Disease

While symptomatically distinct in canonical diagnoses, FTD and ALS co-occur and their considerable phenotypic overlap confers clinically-observable evidence for their existence along a continuous disease spectrum. Broad recognition of phenotypic overlap between ALS and FTD is noted in scientific and medical literature beginning in the 1980s and 1990s (Neary et al. 1990), however early case studies dating to the 1920s provide the first known descriptions of phenotypic overlap (Nitrini 2014). The earliest known report comes from France in 1921, and describes a male patient with ALS who developed impaired cognition late in disease course. Three years later, in 1924, Brazilian physicians described a 25 year-old female patient who exhibited "absolute indifference to everything around her" and whose “association of ideas was done extravagantly" – now recognized as symptoms of apathy and executive dysfunction often observed in bvFTD; they further noted that her health declined before her death one year later, leaving her ultimately bed-ridden due to severe muscular atrophy.

Contemporary population-based studies indicate 10-15% of patients with ALS meet criteria for a diagnosis of FTD and that nearly half manifest non-dementing impairment in cognition and/or behavior consistent with extra-motor frontal and temporal lobe neurodegeneration (Montuschi et al. 2015; Phukan et al. 2012; Elamin et al. 2013; Beeldman et al. 2016). Importantly, these impairments are not confounded by motoric disability, suggesting that neurological function beyond the motor system is affected in ALS. Point estimates of the proportion of the ALS patient population exhibiting behavioral

dysfunction and impairments in discrete cognitive domains have historically differed, due in part to the use of different neuropsychological screening tools. Current estimates have improved due to the use of a validated, now widely-accepted, screening instrument specifically designed to measure cognition, language, and behavior in the context of debilitating motor impairment which is inherent to ALS (Abrahams et al. 2014). Behavioral dysfunction occurs most frequently in ~40% of patients, and 20-30% of patients show executive, verbal fluency, and language impairments; impairments memory and visuospatial function are less common, affecting only 9-12% of patients (Crockford et al. 2018). Importantly, comorbid FTD and the development of extra-motor impairments in cognition, language, and/or behavior in patients with ALS are associated with more accelerated functional decline (Elamin et al. 2013), advanced disease stage, (Crockford et al. 2018) and shorter survival (Elamin, Phukan, Bede, Jordan, Byrne, Pender, and Hardiman 2011a; Hu et al. 2013), relative to motor-only ALS.

Relatively less is known about the development of ALS in patients with initial FTD. Population-based studies report that 4-9% of patients with FTD develop symptoms of ALS (Rosso, Donker Kaat, et al. 2003; Mercy et al. 2008; J. K. Johnson et al. 2005; Seelaar et al. 2007); however, few studies specify the degree of UMN and LMN involvement, and other clinical descriptors of ALS (e.g. site of symptom onset, El Escorial diagnosis) are lacking. A smaller cohort-based clinical study including 38 FTD patients reported higher percentages of FTD patients who also meet criteria for ALS (~13%) and a higher

percentage of patients with FTD who show clinical evidence of minor motor system dysfunction such as occasional fasciculations, mild wasting or weakness (~30%) (Burrell et al. 2011); these differing estimates likely reflect sample characteristics. Others have also reported similar proportions based on *post mortem* evidence of motor neuron degeneration in patients diagnosed with FTD during life (Josephs et al. 2006).

Formal diagnostic categories for the co-occurrence of ALS and FTD were not established until 2013 with the so-called 'Strong criteria' for the diagnosis of frontotemporal cognitive and behavioral syndromes in ALS (Strong et al. 2009). Revised as of 2017 to incorporate additional language and social cognition deficits (Strong et al. 2017), the Strong criteria now include four syndromes collectively considered as 'ALS frontotemporal spectrum disorder': ALS-cognitively impaired (ALSci), ALS-behaviorally impaired (ALSbi), ALS-cognitive-behavioral impairment (ALSbci), and ALS-FTD. ALSci and ALSbi are diagnosed based on discrete impairments in cognition (including verbal fluency or at least two demonstrated executive or language impairments) or behavior (including apathy, or at least two other behavioral symptoms), respectively, whereas ALSbci captures individuals who fulfill criteria for both ALSci and ALSbi. The diagnostic category of ALS-FTD is more phenotypically heterogeneous, and applies to individuals with evidence of progressive deterioration of behavior or cognition who *also* have either 1) at least three behavioral or cognitive symptoms of bvFTD, 2) at least two behavioral or cognitive symptoms of bvFTD together with loss of insight or psychotic symptoms, or 3) fulfillment of criteria for svPPA or

naPPA.

No distinct classification criteria exist for motor neuron degeneration symptoms in the context of FTD, and El Escorial classification is applied as for patients with initial ALS. The labels ‘ALS-FTD’, FTD with motor neuron disease (FTD-MND), and FTD-ALS are all used in the literature to categorize patients with initial FTD who also show symptoms of ALS; here, I will use the term FTD-ALS to denote this category of patients. This term inherently describes a heterogeneous group of patients who may have initial bvFTD, naPPA, or svPPA followed by comorbid with symptoms of ALS, and indeed bvFTD-ALS, naPPA-ALS, and svPPA-ALS patients have all been clinically observed (Vinceti et al. 2019; Coon et al. 2011), although data on relative frequency are lacking. There is evidence of motor phenotype and survival differences between FTD-ALS phenotypic subgroups, such that in one study, language-dominant FTD-ALS was associated with bulbar-onset motor symptoms and shorter survival in comparison with bvFTD-ALS (Coon et al. 2011), consistent with shorter survival from symptom onset in bulbar-onset ALS (Chiò et al. 2009).

Common Pathobiology of ALS-FTD Spectrum Disease

Patterns of neurodegeneration in ALS and FTD reflect differences in motoric, cognitive-behavioral, and blended phenotypes, but typically involve a common pathologic substrate and overlap in affected neuroanatomic regions link the two diseases in pathobiology.

ALS and FTD frequently share a common underlying proteinopathy, with

cytoplasmic inclusions predominantly composed of TDP-43, an acronym for transactive response DNA-binding protein – 43 kiloDalton, found in ~97% of ALS and ~50% of FTD cases *post mortem* (Neumann et al. 2006; Arai et al. 2006). TDP-43 is encoded by the *TARDBP* gene on chromosome 1 and is ubiquitously expressed in tissues throughout the body. In its non-pathologic state, TDP-43 is localized to the nucleus where it serves to regulate transcription, stabilize RNA, and process mRNAs for alternative splicing (Ratti and Buratti 2016). Researchers have also demonstrated a role for TDP-43 in axonal outgrowth with particular implications for motor neuron development (Fallini, Bassell, and Rossoll 2012). Pathologic TDP-43 differs from normal TDP-43 in that it is mislocalized from the nucleus to the cytoplasm, undergoing hyperphosphorylation and – in most, but not all (E. B. Lee et al. 2017), cases – ubiquitination, which encourage its affinity to aggregate into abnormal inclusions (Buratti and Baralle 2012). The exact pathomechanism of TDP-43 inclusions is debated (E. B. Lee, Lee, and Trojanowski 2011), and evidence for both loss of function and gain of function mechanisms has been presented (Hergesheimer et al. 2019).

Though its pathomechanism remains unknown, TDP-43 pathology demonstrates a key role in the neurodegenerative phenotypes across the ALS-FTD disease spectrum. TDP-43 pathology is specified into five subtypes (A, B, C, D, and E) based on ubiquitination, cortical distribution, and neuronal morphology, and each subtype corresponds to particular clinical syndromes (Mackenzie et al. 2011; Mackenzie and Neumann 2017; E. B. Lee et al. 2017). For example, Type A pathology features many neuronal cytoplasmic inclusions (NCIs) and short

dystrophic neurites (DNs), predominates in cortical layer II, and is most commonly observed in bvFTD and naPPA phenotypes (Mackenzie et al. 2011). Type B pathology features moderate NCIs, few DNs, and distribution across all cortical layers (Mackenzie et al. 2011). Type B pathology predominates in ALS and in FTD with motor neuron degeneration, leading some to propose subtype specification as an account for clinical overlap between ALS and FTD (Burrell et al. 2016).

Disease staging systems for TDP-43 pathology in ALS and bvFTD demonstrate neuroanatomic overlap in disease vulnerability and suggest the sequential spread of pathology. In a pathological staging system based on *post mortem* study of 39 bvFTD cases, TDP-43 pathology is proposed to disseminate from the orbital frontal cortex, gyrus rectus, and amygdala caudally to the motor cortex, and – most rarely - the visual cortex (Brettschneider et al. 2014). In a similar staging system in 76 ALS cases, TDP-43 pathology is proposed to disseminate sequentially from the motor cortex, brainstem motor nuclei, and spinal motor nuclei rostrally to the prefrontal and temporal cortices, with rare involvement of the hippocampus (Brettschneider et al. 2013); others have additionally shown that extra-motor pathologic burden is more severe in cases with clinical phenotypes of ALS-FTD and ALSci relative to ALS (Prudlo et al. 2016). The sequential involvement of anatomic regions itself suggests a pathologic ‘spread’ underlying the course of ALS-FTD spectrum disease, and *in vitro* and non-human studies indeed suggest evidence of cell-to-cell spread of pathologic TDP-43 aggregates (Braak et al. 2013). Isolated pathologic

aggregates spread cell-to-cell *in vitro* in a prion-like manner (Feiler et al. 2015; Nonaka et al. 2013), and introduction of human pathologic TDP-43 in a mouse model recapitulates sequential staging seen in human studies (Porta et al. 2018). Together with staging studies in humans, this research indicates similar vulnerability of the motor, frontal, and temporal cortices to TDP-43 pathology across the ALS-FTD spectrum in an anatomically contiguous manner.

Shared Genetic Architecture of ALS-FTD Spectrum Disease

In addition to overlapping clinical phenotypes and common pathobiology, FTD and ALS are further linked through a shared genetic architecture. This complex architecture features pathogenic mutations occurring in familial and sporadic forms of disease, as well as susceptibility loci that confer increased risk of ALS-FTD spectrum disease including in cases negative for pathogenic mutations.

Before discussing pathogenic mutations and risk factors in the ALS-FTD spectrum, it is necessary to clarify some key terminology. ‘Familial’ refers to the occurrence of a disease in an individual with a family history of that, or a similar, disease, and ‘sporadic’ refers to the occurrence of a disease in an individual with no known family history. The distinction between familial and sporadic disease may be subject to ascertainment bias due to differences in history-taking or applied family pedigree criteria (Turner et al. 2017). An estimated 30% of FTD cases and <10% of ALS cases occur in individuals with familial disease (Ferrari, Manzoni, and Hardy 2019), with the remainder classified as apparent sporadic. The phrases ‘familial disease’ and ‘genetic disease’ are often conflated in the

literature, as the majority of familial disease can be attributed to a known pathogenic mutation inherited in a Mendelian fashion. However, pathogenic mutations can also occur in individuals with sporadic disease (i.e. no family history of disease). The term ‘pathogenic’ is used to describe genetic mutations that are capable of causing disease, whereas susceptibility loci describe allelic variations that are associated with disease but not (or not yet) causally linked to disease incidence. These loci are typically identified through genome-wide association studies (GWAS), and in many cases consist of single base-pair changes in the genome called single nucleotide polymorphisms (SNPs).

Pathogenic mutations in the same genes are found in individuals with FTD, ALS, and blended ALS-FTD spectrum phenotypes. Interestingly, pathogenic mutations in *TARDBP* - the gene encoding TDP-43 - are rare (Van Deerlin et al. 2008; Kabashi et al. 2008), observed in an estimated <1% of familial and sporadic ALS (Turner et al. 2017) and even less frequently in patients with FTD (Benajiba et al. 2009; Caroppo et al. 2016). Hexanucleotide (GGGGCC) repeat expansions in the noncoding promoter region of *C9ORF72* on chromosome 9 are the most common genetic cause of both familial and sporadic forms of disease across the ALS-FTD spectrum (Renton et al. 2011; DeJesus-Hernandez et al. 2011). A 2012 study of 4448 patients with ALS and 1425 patients with FTD reported that 37.6% of familial ALS and 25.1% of familial FTD harbor *C9ORF72* mutations, and that 7% of sporadic ALS and 6% of sporadic FTD patients harbor *C9ORF72* mutations (Majounie et al. 2012). Translation of the hexanucleotide repeat yields five forms of dipeptide repeat proteins (DPRs)

that aggregate to form intracellular inclusions (Mori et al. 2013), and have demonstrated neuronal toxicity (Freibaum and Taylor 2017). Moreover, *C9ORF72* repeat expansions are causally associated with TDP-43 pathology (Chew et al. 2015), and aggregated DPRs both induce and are induced by intracellular aggregation of phosphorylated TDP-43 (Nonaka et al. 2018; Solomon et al. 2018), suggesting a cumulatively synergistic pathology.

Several additional - although rare - pathogenic mutations in other genes have also been causally associated with ALS, FTD, and blended ALS-FTD spectrum phenotypes. These include mutations in coding for proteins including sequestosome-1 (*SQSTM1*) (Le Ber et al. 2013), ubiquilin-2 (*UBQLN2*) (Synofzik et al. 2012), optineurin (*OPTN*) (Pottier et al. 2015), coiled-coil-helix coiled-coil-helix domain containing 10 (*CHCHD10*) (Bannwarth et al. 2014), TANK binding kinase 1 (*TBK1*) (Freischmidt et al. 2015), dynactin-1 (*DCTN1*) (Münch et al. 2005), cyclin-F (*CCNF*) (Williams et al. 2016), and TIA1 (*TIA1)* (Mackenzie et al. 2017). Interestingly, these rare mutations offer additional genetic insight into molecular mechanisms of ALS-FTD spectrum disease and implicate cellular waste disposal pathways, immune system signaling, and gene expression regulation (Ferrari, Manzoni, and Hardy 2019).

Susceptibility loci further suggest shared genetic architecture along the ALS-FTD disease spectrum. Many case-control GWAS have been separately performed in ALS (Nicolas et al. 2018; van Es et al. 2009; van Es et al. 2007; van Rheenen et al. 2016) and in FTD (Ferrari et al. 2014; Ciani et al. 2019). One GWAS from 2010 focused on ALS-FTD disease spectrum phenotypes with

confirmed TDP-43 pathology (N=515) and identified multiple SNPs near *TMEM106B* mapping to a single linkage disequilibrium block on 7p21 (Van Deerlin et al. 2010); however, this study was restricted to unrelated individuals with mutations in *GRN*. A recent repeat of this GWAS approach in 517 unrelated Caucasian patients who screened negative for *GRN* and other pathogenic mutations associated with ALS-FTD spectrum disease identified additional SNPs near *C9ORF72*, *UNC13A*, *DPP6*, and *HLA-DQA2* (Pottier et al. 2019). A joint meta-analysis of public GWAS data from 4,377 ALS patients and 435 pathology-proven FTD patients with TDP-43 also demonstrated shared genetic risk across the ALS-FTD spectrum at SNPs near *UNC13A* and *SPG8*, with *p* values for the locus near *UNC13A* increasing in significance after conditioning on *C9ORF72* (Diekstra et al. 2014). More recently, novel approaches incorporating genome-wide conjunction and conditional analyses studied the joint association between discrete phenotypes along the ALS-FTD spectrum (ALS, FTD with TDP pathology, sporadic FTD) and additional neurodegenerative phenotypes (Alzheimer disease, corticobasal degeneration, Parkinson disease, and progressive supranuclear palsy) (N=124,876), conditioning *p* values for identified loci based on shared association with two or more phenotypes (Karch et al. 2018). Over 20 loci were identified, including at known loci (e.g. near *UNC13A* and *C9ORF72*) and at novel loci (e.g. near *NSF* and *ERGIC1*). Additional approaches further suggest that susceptibility loci may contribute to the genetic architecture underlying the ALS-FTD spectrum disease in a polygenic manner, though this was demonstrated in a population of healthy individuals from the UK

Biobank study (Hagenaars et al. 2018).

Neuroanatomy of ALS-FTD Spectrum Disease

The lack of current neuroimaging biomarkers for TDP-43 pathology precludes study of its anatomic distribution *in vivo* (Feneberg et al. 2018), but MRI and DTI enable the *in vivo* study of neurodegeneration across the ALS-FTD spectrum and suggests similar anatomic overlap as seen in *post mortem* study of TDP-43 pathology. Cross-sectional studies of patients grouped by diagnosis recapitulate known disease neuroanatomy associated with ALS and FTD phenotypes (Omer et al. 2017), and demonstrate anatomic overlap in the orbitofrontal and anterior cingulate cortices, corticospinal tracts, and corpus callosum including between ALS patients without clinically-evident cognitive impairment and bvFTD patients (Crespi et al. 2018; Ferraro et al. 2018). Blended ALS-FTD disease spectrum phenotypes (e.g. ALS-FTD, ALSci, FTD-ALS) show more extensive neurodegeneration relative to discrete ALS or FTD syndromes. For example, one recent study of ALS in the context of language-variant FTD observed left precentral gyrus GM atrophy in patients with naPPA-ALS in addition to canonical naPPA-associated atrophy in the left inferior frontal gyrus, pars opercularis and triangularis, and left temporal pole (Vinceti et al. 2019). ALSci and ALS-FTD patients show increasing extramotor GM neurodegeneration in regions including the inferior, middle, and superior temporal gyri, orbitofrontal cortex, superior frontal gyrus, anterior cingulate cortex, and insula relative to frank ALS (Schuster et al. 2014; Agosta et al. 2016), and WM damage extending beyond the

corticospinal tracts and corpus callosum to include the uncinate and superior longitudinal fasciculi (Sarro et al. 2011; Agosta et al. 2016).

MRI-based disease-staging systems and longitudinal neuroimaging provide perhaps the most approximate insight into the spread of ALS-FTD spectrum disease *in vivo*. DTI data from patients with ALS and from patients bvFTD have been staged cross-sectionally and longitudinally according to severity and extent of WM neurodegeneration and confirm anatomic patterns of TDP-43 pathology staging systems (Kassubek, Müller, Del Tredici, Lulé, et al. 2018; Kassubek, Müller, Del Tredici, Hornberger, et al. 2018; Kassubek et al. 2014; Müller and Kassubek 2018). Others have staged cross-sectional multimodal imaging data with neuropsychological evaluation to demonstrate sequential cognitive involvement corresponding to GM and WM neurodegeneration in ALS, suggesting earlier-stage involvement of the middle frontal cortex relating to executive impairment (Lulé et al. 2018). Longitudinal study of GM atrophy in ALS, while inherently limited by shorter patient survival, demonstrates progressive degeneration extending from the motor cortex rostrally and inferiorly into frontotemporal regions, and to the thalamus and caudate bilaterally (Menke et al. 2014). In FTD, longitudinal study of patients grouped by clinical variant shows worsening of focal atrophy; for example svPPA show greater percent atrophy changes in the left inferior temporal lobe relative to bvFTD, whereas bvFTD show greater percent atrophy in the ventromedial prefrontal cortex relative to naPPA (Lu et al. 2013).

ENVIRONMENTAL AND GENETIC INFLUENCES ON COGNITION

Though a phenotypic spectrum, shared pathobiology and neuroanatomy, and common genetic architecture demonstrate a robust link between ALS and FTD, considerable heterogeneity between affected individuals precludes prognosis and therapeutic development. One source of heterogeneity of particular clinical importance is in cognition. Across the ALS-FTD spectrum, presence and severity of impaired cognition is consistently linked to rapid functional decline (Elamin et al. 2013), greater extent and severity of frontotemporal neurodegeneration (Agosta et al. 2016), greater caregiver burden (Caga et al. 2019), and shorter survival (Elamin, Phukan, Bede, Jordan, Byrne, Pender, and Hardiman 2011a; Hu et al. 2013). In this section, I review existing evidence for influences of environmental and genetic factors on impaired cognition, and discuss key knowledge areas that are lacking.

Environmental Influences

Environmental modifiers of disease refer to factors external to the individual that can influence facets of disease including incidence and phenotype. Numerous and diverse environmental factors have been associated incidence of ALS-FTD spectrum disease. These include the association of cigarette smoking, athletic activity, environmental toxin exposure, and military service with increased odds of ALS incidence (Sutedja et al. 2007; Veldink et al. 2005; Huisman et al. 2013; Weisskopf et al. 2005; Bozzoni et al. 2016), and traumatic brain injury with increased odds of FTD incidence in veterans (Kalkonde et al. 2012) and in

sporadic FTD (Rosso, Landweer, et al. 2003). However, in terms of cognition, identified environmental factors come from a narrower category and largely relate to cognitive engagement over the lifespan.

The discovery of the relationship between cognitive engagement and cognitive impairment comes from research on Alzheimer's disease dementia by Dr. Yaakov Stern, who coined this phenomenon 'cognitive reserve' (Stern 2002). In a seminal series of work published in the 1990s, Dr. Stern demonstrated that individuals with higher years of education or more cognitively-taxing occupations had more advanced stages of Alzheimer's disease as measured by rate of memory decline (Stern et al. 1999), temporoparietal blood perfusion seen through PET imaging (Stern et al. 1994), and survival (Stern et al. 1995). This led to the theory that cognitively-stimulating lifetime experiences like education may provide a 'reserve' that compensates for the neuropathological changes of Alzheimer's disease, thus delaying the onset of clinical manifestation. Both functional and biologic mechanisms have been proposed to underlie observations of cognitive reserve. Functional accounts posit that increased frontal executive resources cultivated through lifetime cognitive engagement allow compensation for medial temporal lobe disease. Neuroimaging studies are most often cited as supporting functional accounts of cognitive reserve, and include reports of increased frontal connectivity in highly-educated individuals associated with attenuated memory decline, brain hypometabolism, and hippocampal volume loss (Franzmeier et al. 2017; Pudas et al. 2018). Biologic accounts referred to in the literature as 'brain reserve' propose that

neuroanatomic differences might also underlie observations of cognitive reserve. In support of this, one study in healthy elderly individuals demonstrate greater frontoparietal GM volume related to higher levels of education (Solé-Padullés et al. 2009).

Evidence for cognitive reserve has been also observed in FTD, but differs in manifestation from Alzheimer's disease. Primary atrophy in the frontal and temporal lobes characterizes disease phenotypes across the ALS-FTD spectrum, inherently compromising access to proposed frontal executive resources hypothesized to facilitate functional compensation for neurodegeneration in classic accounts of cognitive reserve. Research in patients with bvFTD supports this idea, as more cognitively-demanding premorbid occupational status relates to more rapid decline in executive function but not in semantic memory, a cognitive domain that is relatively spared in patients with bvFTD (Massimo et al. 2019). Both higher occupational and educational attainment relate to lower cerebral blood flow (Borroni et al. 2009) and lower glucose metabolism (Spreng et al. 2011; Perneczky et al. 2007) in the frontal cortex in patients with bvFTD, demonstrating compromised frontal lobe activity. Furthermore, cognitive engagement is differentially associated with survival in FTD compared to Alzheimer's disease, with more cognitively-demanding premorbid occupation relating to longer survival from symptom onset in bvFTD but shorter survival from symptom onset in Alzheimer's disease (Massimo et al. 2015).

While prior research has identified cognitive engagement as an environmental contributor to cognitive impairment, several limitations necessitate

further study to understand its influence in the ALS-FTD disease spectrum. First, prior research on cognitive reserve in ALS-FTD spectrum disease have been limited to a single phenotype – bvFTD – and explicitly excluded individuals with motor symptoms. Thus, it is yet unknown whether this phenomenon extends to influence cognition in patients with other ALS-FTD spectrum phenotypes including ALS-FTD, svPPA, and naPPA. Furthermore, prior studies have not yet explored structural brain integrity, or how cognitive reserve may mediate the relationship between brain structure and cognitive impairment. These lead to the following unanswered question in environmental influence on cognition in ALS-FTD spectrum disease: how does cognitive reserve relate to structural neuroanatomy and cognitive impairment in heterogeneous phenotypes across the ALS-FTD spectrum?

Genetic Influences

Prior discussion of genetics in this introduction largely related to the incidence of disease; however, accumulating evidence suggests genetic influence on phenotypic heterogeneity in ALS-FTD spectrum disease. This includes earlier age at disease onset, more rapid symptom progression, and shorter survival in *C9ORF72* mutation carriers relative to non-carriers (Byrne et al. 2012; Suh et al. 2015; Umoh et al. 2016; Irwin, McMillan, et al. 2014; Moore et al. 2020), and in patients screening positive for SNPs near *UNC13A* (Chiò et al. 2013). Additional evidence suggests that the relationship between genetics and phenotypic heterogeneity in ALS-FTD spectrum disease extends to cognition and implicates

both pathogenic mutations and SNPs.

In the context of ALS-FTD spectrum disease, the majority of research on genotype-phenotype relationships in cognition has been focused on *C9ORF72*. First, in addition to causal association with frank ALS, frank FTD, and varied phenotypes along the ALS-FTD disease spectrum, *C9ORF72* mutations increase the likelihood that an ALS patient will develop symptoms of FTD. One population-based study in the US (N=781) reported behavioral symptoms of FTD in 14.8% of ALS patients who were carriers compared to only 1.7% of non-carriers (Umoh et al. 2016), whereas another population-based study in Ireland (N=191) reported behavioral symptoms in 50% of carriers compared to 12% non-carriers (Byrne et al. 2012). *C9ORF72* mutations are also linked to rate of cognitive decline across the ALS-FTD spectrum, with one study showing more rapid decline on a verbal fluency measure of executive function (Irwin et al. 2013) and another showing more rapid decline in visuospatial and memory function in addition to executive function (Mahoney et al. 2012). Psychiatric symptoms have also been reported in *C9ORF72* mutation carriers across the ALS-FTD disease spectrum (Snowden et al. 2012; Devenney et al. 2017). Neuroanatomic differences in *C9ORF72* carriers relative to non-carriers are also widely observed both *in vivo* and *post mortem*. A comprehensive literature review conducted in 2015 show heterogeneity of findings across published studies of neuroimaging in *C9ORF72* carriers likely due to differences in clinical phenotype and disease stage (Prado et al. 2015). However, studies accounting for these potential confounds show more extensive extramotor GM and WM degeneration particularly in frontotemporal regions in

ALS (Bede et al. 2013), which also correspond to cognitive impairment (Floeter et al. 2016). Post-mortem, *C9ORF72* mutation carriers display TDP-43 pathology in the cerebellum and hippocampus, which are rarely observed in non-carriers (Mackenzie, Frick, and Neumann 2014). Interestingly, the associations between *C9ORF72* mutation status, cognitive impairment, and neuroanatomic susceptibility appear to be epigenetically modulated: hypermethylation of the *C9ORF72* promoter shows evidence of neuroprotection in terms of cognitive decline, MRI measures of structural brain integrity, and post-mortem neuropathologic burden (Russ et al. 2015; McMillan et al. 2015).

A small, yet growing body of evidence demonstrates that genetic influence on cognition extends beyond study of known pathogenic mutations to also include susceptibility loci. These include quantitative trait loci (QTL) analyses that probe the association between a genetic locus and variation in a continuous, rather than binary (e.g. having disease vs. not having disease), disease trait. A SNP near *TMEM106B* originally identified as a susceptibility locus for TDP-43 pathology in *GRN* mutation carriers was found to relate to impaired cognition on a task of executive function in patients with ALS (Vass et al. 2011). In bvFTD patients, genotype-phenotype relationships are also observed. Risk genotype at a SNP near the *MOBP* gene was found to associate with more severe WM neurodegeneration in the corona radiata and superior and inferior longitudinal fasciculi, more severe GM neurodegeneration in the precuneus and superior temporal gyrus, and shorter survival from symptom onset in a mixed cohort of sporadic, mutation-negative bvFTD patients with underlying Tau and TDP-43

pathology (Irwin, McMillan, et al. 2014). In another study, SNPs near *MAPT*, *GRN*, *SORT1,* and *MOBP* related to distinct WM neurodegeneration in the superior longitudinal fasciculus and midbrain in sporadic, mutation-negative patients with varying ALS-FTD spectrum disease phenotypes (McMillan et al. 2014). The majority of ALS-FTD spectrum disease occurs in the absence of a family history disease or known pathogenic mutation, suggesting that the susceptibility loci mentioned here, and others yet unexplored, may influence variance in dementia for a larger proportion of ALS-FTD spectrum disease.

Here, I have discussed promising evidence for genetic influence on cognition in the ALS-FTD spectrum, yet much work in this area remains. The majority of prior work focuses on studies of individuals carrying *C9ORF72* repeat expansions, and research in mutation-negative individuals is considerably less established. Many SNPs associated with incidence of ALS-FTD spectrum diseases have been identified, yet few have been studied in regards to quantitative or qualitative trait associations with cognition. Prior efforts to characterize the quantitative disease associations with single SNPs have been limited to the study of a single clinical phenotype or to the use of a single research modality, and potential polygenic contribution to cognition in ALS-FTD spectrum disease remains relatively unexplored. These lead to the following unanswered questions: Do SNPs associated with risk of ALS-FTD spectrum disease show quantitative-trait relationships with cognition and disease neuroanatomy? Do they do so in a polygenic manner?

MULTIMODAL, NONPARAMETRIC, AND MACHINE-LEARNING APPROACHES

Thus far, I have described how ALS-FTD spectrum disease features complex overlap in clinical, neurobiologic, anatomic, and genetic characteristics. I have also reviewed prior literature in this field to identify outstanding research questions pertaining to how heterogeneity in the cognitive status of affected individuals may be shaped by environmental and genetic factors. In this section, I turn to discuss how these research questions can be addressed, focusing on multimodal, nonparametric, and machine learning approaches.

Research employing multimodal data involves the collection and analysis of data from several, rather than single, sources. Deep phenotyping of patient populations with ALS-FTD spectrum disease requires data from multiple, diverse modalities, including clinical evaluation, demographic information, neuropsychological assessment, neuroimaging acquisition, genetic screening and genome sequencing, and *post mortem* neuropathologic analysis (Toledo et al. 2014). Each modality is able to characterize a specific and different facet of the disease process. For example, neuropsychological assessments are used to characterize and quantify cognitive impairment syndromes (Abrahams et al. 2014; Strong et al. 2017), structural MRI reveals the anatomy of neurodegeneration underlying patient syndromes (Menke et al. 2017; Meeter et al. 2017), and neuropathologic and genetic study identify potential molecular causes and modifiers of disease (Neumann et al. 2006; Renton et al. 2011; DeJesus-Hernandez et al. 2011). The concurrent consideration of data from

multiple modalities allows for a characterization of disease that extends beyond a broad categorical diagnosis (i.e. clinical phenotype) to additionally describe the collection of the finest details about the condition (i.e. endophenotype) (Irwin, Cairns, et al. 2014). Multimodal approaches are critical to establish endophenotypes, for example, a patient with a clinical phenotype of 'ALS' could be endophenotyped as 'a mutation-negative patient with sporadic ALS who has impaired executive function and structural MRI evidence of extramotor frontal lobe neurodegeneration'. Endophenotypes, in turn, are critical for the development and application of precision medicine therapies designed to target specific aspects of disease (e.g. ASOs designed to target *C9ORF72* repeat expansions).

In addition to enabling deep endophenotyping, multimodal data collection provides the opportunity to evaluate the reproducibility and robustness of research findings across complementary, yet distinct, datasets. Data collection from neurodegenerative patient populations suffers limitations in sample size and is often inconsistent in protocol across research centers, thereby precluding studies of direct reproducibility. Multiple modalities of data collected on the same patient cohort or, alternatively, different modalities of data collected on independent patient cohorts, offers a partial solution to this problem by increasing the number of datasets a researcher can study. With an increased number of datasets, researchers can assess the reliability of an observed effect and evaluate whether differing sources of data converge on a common finding (Bachli et al. 2020). For example, a genetic risk factor for cognitive impairment in ALS

might be further evaluated in relation to *in vivo* frontotemporal neurodegeneration through structural neuroimaging and *post mortem* burden of pathology.

Irrespective of modality, data collected from patients often violate assumptions of statistical normality (e.g. homeoscedasticity) and thus make the use of parametric statistical methods inappropriate for their analysis (Pett 2015). Permutation-based tests are a class of nonparametric statistical methods that test the null hypothesis by calculating the probability of observing an outcome in the original data relative to random permutations of the data. Efficient permutation methods have been adopted for usage in healthcare datasets, including large and computationally-expensive neuroimaging and genetic datasets (Winkler et al. 2014; Conneely and Boehnke 2007). These methods have been previously employed to analyze both neuroimaging and genetic data from patients with ALS-FTD spectrum disease (McMillan et al. 2015; McLaughlin et al. 2017), suggesting their utility in further study of multimodal data from this patient population.

In addition to nonparametric approaches, machine-learning has been employed in the study of neurodegenerative disease (Dinov et al. 2016; Hongming Li et al. 2019), including in bvFTD (Schroeter et al. 2014; Bachli et al. 2020) and in ALS (Grollemund et al. 2019; Hothorn and Jung 2014). Machine learning uses computationally-powerful statistical techniques to make inferences and predictions from large datasets and techniques have been developed for use with single as well as multiple datasets (James et al. 2014). One machine learning technique developed for the analysis of multimodal data is sparse

canonical correlation analysis (sCCA). sCCA evaluates multivariate associations between two data sets while prioritizing sparsity by penalizing variables that contribute minimally to the statistical model (Witten, Tibshirani, and Hastie 2009; Witten and Tibshirani 2009). Importantly, variable selection from sCCA is data-driven in nature, chosen through analysis rather than by a researcher. Recently, sCCA was employed to identify SNPs associated with selective distributions of GM and WM disease in FTD patients (McMillan et al. 2014), and to identify compromised neuroanatomic networks related to executive, language, and behavioral phenotypes in patients with FTD (Avants et al. 2014). These prior studies suggest the utility of sCCA in further investigation of genotype-phenotype relationships in ALS-FTD spectrum disease. For example, this method could be used to evaluate multivariate relationships between patient genotypes (e.g. at SNPs) and performance on multiple neuropsychological measures of cognition.

The aforementioned multimodal, nonparametric, and machine learning approaches are ideally suited to the study of environmental and genetic factors influencing heterogeneous cognitive phenotypes in the context of ALS-FTD spectrum disease. Multimodal data collection enables deep endophenotyping of patients including cognitive, anatomic, and genetic profiling, and allows the evaluation of candidate environmental and genetic modifiers across multiple datasets. Nonparametric analyses of these multimodal datasets (e.g. through computationally-efficient permutation methods) is essential, as observational patient data are demonstrably heterogeneous and often do not conform to theoretical distributions of statistical normality. Additionally, sCCA, a machine-

learning approach, can be used to integrate modalities into endophenotypes by identifying relationships in data-driven subsets of variables across large datasets of differing modalities, allowing for the exploration of multivariate relationships between cognition and environmental / genetic factors. With this suitability in mind, I combine multimodal, nonparametric, and data-driven approaches in the series of research studies comprising the subsequent chapters of this thesis with the goal of elucidating environmental and genetic influences on cognition in ALS-FTD spectrum disease in a statistically-robust manner.

OVERVIEW OF DISSERTATION STUDIES

In this dissertation, I present three research studies evaluating how cognition in ALS-FTD spectrum disease is shaped by factors innate (i.e. genetic) and external (i.e. environmental) to affected individuals. I address the following specific questions:

1. How does lifetime cognitive engagement relate to cognition and disease neuroanatomy in ALS-FTD spectrum disease?
2. Do common genetic variants that confer risk for ALS-FTD spectrum disease relate to cognition and disease neuroanatomy?
3. Is there evidence of polygenic contribution via common genetic variants to cognition and disease neuroanatomy?

I address the first research question in an initial study investigating whether education and premorbid occupation level of patients with ALS-FTD spectrum disease relates to heterogeneity in cognition and neurodegeneration. I use a retrospective cohort design to study 55 patients and 90 controls with detailed demographic information, neuropsychological evaluation, and *in vivo* structural MRI from the UPenn Integrated Neurodegenerative Disease Biobank (Toledo et al. 2014). I include patients with genetic or autopsy confirmation of Tau or TDP-43 pathology in an effort to exclude dementia patients with underlying Alzheimer's disease pathology, and – in an effort to capture the phenotypic and genetic heterogeneity of ALS-FTD spectrum disease – include patients ranging in clinical phenotype and genetic mutation status. To approximate cognitive engagement across the lifespan, I define a cumulative index of cognitive reserve

based on years of completed education and ordinal scoring of premorbid occupational complexity. I identify regions of gray matter atrophy from nonparametric permutation analyses of voxel-wise gray matter probability from T1-weighted MRI in patients relative to controls, and examine the relationship between the cognitive reserve index and severity of gray matter atrophy in identified regions in patients. I further examine how cognitive performance relates to both cognitive reserve index and to gray matter regions associated with the reserve index. Given the common frontal distribution of disease in ALS-FTD spectrum patients, I hypothesize that cognitive reserve relates to less atrophic frontal cortex gray matter and preserved performance on frontally-mediated neuropsychological measures.

Next, in the second study, I investigate genetic influence on cognition and disease anatomy in ALS-FTD spectrum disease. Specifically, I test whether common genetic variation at rs12608932 (closest gene: *UNC13A*) – a locus that previously achieved genome-wide association with both ALS and FTD - further contributes to cognition, *in* vivo neurodegeneration, and *post mortem* pathologic burden. I again use a retrospective cohort design of 190 patients and 113 controls from the UPenn Integrated Neurodegenerative Disease Biobank (Toledo et al. 2014), but focus my investigation here on patients with initial onset of ALS who also have sporadic and mutation-negative forms of disease. The 190 total patients studied describe two cohorts; one with T1-weighted structural MRI (N=109), a subset of whom also had neuropsychological evaluation (N=88), and another with *post mortem* tissue samples graded for TDP-43 pathology (N=102).

I additionally study 84 controls from the publicly-available Alzheimer's Disease Neuroimaging Initiative (ADNI) with genotyping at rs12608932 and T1-weighted MRI. I identify regions of cortical thinning from nonparametric permutation analyses of voxel-wise cortical thickness measurements in patients relative to controls, and examine the relationship between rs12608932 genotype and severity of cortical thinning in identified regions in patients and ADNI controls. I continue to evaluate rs12608932 genotype and study its relation to cognitive performance and to burden of TDP-43 pathology. Based on prior associations of rs12608932 and risk for ALS and FTD, and with reduced survival in ALS, I hypothesize that rs12608932 genotype associates with greater frontotemporal disease in ALS as evident in reduced cortical thickness *in vivo*, more impaired cognitive performance, and greater burden of TDP-43 pathology *post mortem*.

Finally, in the third study, I build on prior evidence for single-allelic contribution to cognition and disease neuroanatomy from the second study to further evaluate potential polygenic contribution using a multivariate approach. I collaborate with investigators across institutions from the international Clinical Research in ALS and related disorders for Therapeutic Development (CReATe) Consortium to study an unprecedented cohort of 339 patients with longitudinal neuropsychological evaluation and genotyping at loci of common genetic variation that previously achieved genome-wide association with ALS or joint association with ALS and FTD. I use sCCA, an unsupervised machine learning approach, to identify a subset of loci that maximally contribute to cognitive heterogeneity in this cohort and derive a data-driven polygenic risk score for

impaired cognition from model estimates. I further evaluate this machine-learning derived polygenic risk score relative to anatomic disease burden in independent neuroimaging and autopsy cohorts from the UPenn Integrated Neurodegenerative Disease Biobank (Toledo et al. 2014). These include 114 ALS patients and 114 controls with T1-weighted MRI and 88 ALS patients with *post mortem* tissue samples graded for TDP-43 pathology and neuronal loss. I hypothesize that the machine learning will reveal a subset of genetic loci associated with cognitive dysfunction profiles in ALS in a polygenic manner, and that follow-up analyses in independent neuroimaging and autopsy cohorts converge to characterize quantitative traits associated with polygenic risk from identified loci.

Each study – though addressing a distinct and specific research question – shares the common goal of elucidating factors that modify cognition and disease anatomy in ALS-FTD spectrum disease. Critically, the environmental and genetic factors targeted in my research can provide novel and cost-effective biomarkers with potential for actionable use in patient prognostication and in clinical trials.

CHAPTER 2

Cognitive reserve in frontotemporal degeneration: Neuroanatomic and neuropsychological evidence

Katerina Placek, Lauren Massimo, Christopher Olm, Kylie Ternes, Kimberly Firn, Vivianna Van Deerlin, Edward B. Lee, John Q. Trojanowski, Virginia M.Y. Lee, David Irwin, Murray Grossman, and Corey T. McMillan. *Neurology*, 2016, 87:1813-1819.

Abstract

In this study, we evaluate if cognitive reserve (CR) contributes to inter-individual differences in frontal gray matter (GM) and executive impairment that underlie heterogeneity in the disease course of confirmed frontotemporal lobar degeneration (FTLD) pathology. Fifty-five patients with autopsy confirmation or a pathogenic mutation consistent with underlying tau (FTLD-Tau) or TDP-43 (FTLD-TDP) pathology and 90 demographically-comparable healthy controls were assessed with T1 MRI and neuropsychological measures (Mini Mental State Exam, letter fluency, forward digit span, Rey complex figure, and Boston Naming Test). CR was indexed using a composite measure of education and occupation. We identified reduced GM density in FTLD patients relative to controls, ran regression analyses relating reduced GM density to CR index, and correlated regions of GM associated with CR with performance on neuropsychological measures. FTLD patients exhibited reduced bilateral frontotemporal GM relative to controls, consistent with the known anatomic distribution of FTLD pathology. CR index was positively associated with letter fluency and with GM density in right dorsolateral prefrontal cortex, orbitofrontal

cortex, rostral frontal cortex, and inferior frontal gyrus. Furthermore, letter fluency correlated positively with mean GM density in frontal GM regions associated with CR. Our results indicate that executive control and verbal ability assessed by letter fluency in FTLD is mediated in part by CR and frontal GM reduction. The identification of factors influencing cognitive and anatomic heterogeneity in FTLD suggests that CR should be considered in symptom detection, prognosis, and treatment.

Introduction

Frontotemporal lobar degeneration (FTLD) is a pathologic spectrum of progressive neurodegenerative conditions affecting the frontal and temporal lobes that are associated with executive, social, and language impairments (Irwin, Cairns, et al. 2014). The disease course of FTLD is highly variable across individuals, including age at onset (J. K. Johnson et al. 2005), rate of decline (Josephs et al. 2011), and survival (Hodges et al. 2003). While some biologic mechanisms have been proposed to account for this heterogeneity (Gallagher et al. 2014; McMillan et al. 2015), environmental factors contributing to disease course remain obscure.

Cognitive reserve (CR) theories suggest environmental factors including education and occupation provide a ‘reserve’ against the clinical manifestation of neurodegenerative disease despite significant pathological burden (Stern 2009). Individuals with high CR may compensate for dementia-associated neurodegeneration by increasing recruitment of frontal executive resources to improve cognitive performance and delay symptom detection (Kemppainen et al. 2008; Bosch et al. 2010). In patients with FTLD, access to frontal executive resources is compromised early in disease course due to frontal tau or TDP-43 pathology. Prior research on CR in FTLD-spectrum disorders is largely limited to behavioral variant frontotemporal dementia (bvFTD) patients with unconfirmed FTLD pathology, and has largely focused on brain metabolism and other functional measures (Perneczky et al. 2007; Borroni et al. 2009; Spreng et al. 2011). There is a thus a need for investigation of CR in FTLD-spectrum patients

with confirmed FTLD pathology using structural measures of brain integrity.

Recent evidence from autopsy-confirmed FTLD patients suggests that higher occupational attainment is associated with longer survival from symptom onset (Massimo et al. 2015). From this perspective, FTLD patients with higher occupational attainment may detect dementia symptoms earlier than lower occupational attainment patients because of their increased frontally-mediated work demands, therefore giving the impression that they are surviving longer. This is in contrast with traditional CR accounts based on Alzheimer's disease (AD) that suggest that higher CR factors such as education and occupational attainment are associated with shorter survival.

Critically, since AD has a neuroanatomic distribution of disease distinct from FTLD, including less frontal lobe disease, it is reasonable to speculate that CR may function differently in patients with FTLD. However, to our knowledge, the anatomic and cognitive profiles associated with CR in patients with confirmed FTLD remain unknown. Given the frontal distribution of disease in FTLD, we hypothesized that CR would be positively associated with frontal cortex grey matter (GM) and with frontally-mediated neuropsychological measures.

Methods

Participants.

We report 55 patients recruited from the Penn Frontotemporal Degeneration Center at the University of Pennsylvania and clinically diagnosed by a board-certified neurologist (M.G., D.I.) using published criteria for a clinical syndrome

associated with FTLD pathology (Gorno-Tempini et al. 2011; Rascovsky et al. 2011; Armstrong et al. 2013; Strong et al. 2009). Inclusion criteria for this study required a post-mortem neuropathological diagnosis or genetic screening (see *Neuropathological Diagnosis* and *Genetic Screening*), an ante mortem T1-weighted MRI scan, neuropsychological assessment, and known occupational status and years of education. Patients' clinical syndrome included behavioral variant FTD (bvFTD; N=34), amyotrophic lateral sclerosis (ALS) with FTD (ALS-FTD, N=7), nonfluent-agrammatic primary progressive aphasia (naPPA, N=6), corticobasal syndrome (CBS, N=4), semantic-variant primary progressive aphasia (svPPA, N=1) progressive supranuclear palsy (PSP, N=2), and ALS with mild cognitive impairment (ALS-MCI, N=1). Disease duration was defined as time of symptom onset, based on caregiver report of the earliest clinical feature, until time of MRI acquisition. Disease duration can alternatively be calculated as months between first diagnosis and clinical appointment and *post hoc* analyses confirm that we see similar results when using this calculation (all $p<0.05$).

To identify regions of significant GM atrophy in FTLD (see below), we additionally recruited 90 demographically-comparable healthy controls who self-reported a negative history for neurologic or psychiatric disease, and completed an initial screening of Mini-Mental State Examination (MMSE)>27 (M. F. Folstein, Folstein, and McHugh 1975). Demographic features of FTLD patients and controls are summarized in Table 1. There were no significant differences in age, education, gender, or CR index (see below) between controls and patients (all p values >0.05).

Neuropathological Diagnosis.

Neuropathologic diagnoses of FTLD-Tau and FTLD-TDP were established according to consensus criteria (Mackenzie et al. 2010) by expert neuropathologists (J.Q.T.; E.B.L.) using immunohistochemistry with established monoclonal antibodies specific for pathogenic tau (mAb PHF-1) (Otvos et al. 1994) and TDP-43 (mAbs p409/410 or 171) (Neumann et al. 2009), as previously reported (Toledo et al. 2014). Twenty patients had a primary FTLD-spectrum neuropathologic diagnosis at autopsy including corticobasal degeneration (CBD; N=2), PSP (N=5), Pick's disease (N=3), referred to here collectively as FTLD-Tau disorders, or FTLD with TDP-43 inclusions (N=10), designated here as FTLD-TDP. Among those individuals with neuropathological confirmation of FTLD, five also had genetic mutations (see below).

Genetic Screening.

DNA was extracted from peripheral blood or brain tissue following the manufacturer's protocols (Flexigene (Qiagen) or QuickGene DNA whole blood kit (Autogen) for blood, and QIAsymphony DNA Mini Kit (Qiagen) for brain tissue). Samples were genotyped for the *C9ORF72* hexanucleotide-repeat using a modified repeat-primed polymerase-chain reaction and the *MAPT (exons 1, and 9-13), GRN* (entire coding region), and *TARDBP* genes were sequenced to identify pathogenic mutations as previously described (Toledo et al. 2014). Sequencing data was analyzed using Mutation Surveyor software (Soft Genetics, State College, PA). Genetic screening revealed that a total of 40 patients (five

also with neuropathological confirmation) had a known pathogenic mutation associated with FTLD-Tau or FTLD-TDP disorders, including mutations in *MAPT* (N=7) (Hutton et al. 1998), as well as *C9ORF72* expansions (N=20) (DeJesus-Hernandez et al. 2011; Renton et al. 2011) or mutations in the *GRN* (N=11) (Baker et al. 2006), or *TARDBP* (N=2) (Van Deerlin et al. 2008) genes, respectively.

Standard Protocol Approvals, Registrations, and Patient Consents.

All patients and controls participated in an informed consent procedure approved by an Institutional Review Board convened at the University of Pennsylvania.

Cognitive Reserve Index.

We assessed CR using a composite measure of education and occupation similar to previous reports (Borroni et al. 2009; Premi et al. 2013). Education was recorded in years and ranked with a score ranging from 1 to 4: (*1*) ≤12 years (primary or secondary education; N=13); (*2*) >12 and <16 (some post-secondary education; N=8); (*3*) 16 years (college education; N=11); and (*4*) >16 years (graduate education; N=23). Occupation was ranked on a 1-4 point scale based on US census categories: (*1*) Unskilled laborers (N=2); (*2*) Operative and service workers (N=5); (*3*) Managers, administrators, clerical and sales (N=20); (*4*) Professional and technical workers (N=28). We report a CR index as the sum of education and occupation ranks.

Neuropsychological Assessment.

Neuropsychological assessment was performed within approximately two months from date of scan (M=2.20, SE=0.53; Range=0-16). Letter fluency is a measure of executive control and verbal ability and was assessed by the number of unique words beginning with “F” a patient was able to generate in one minute (excluding proper nouns and numbers) (Tombaugh, Kozak, and Rees 1999). The MMSE is a 30-point questionnaire that evaluates global dementia severity (Crum, Anthony, Bassett, and Folstein 1993a). Forward digit span, a measure of auditory-verbal short-term memory, was assessed with repetition of increasingly longer sequences until the patient erred; the maximum number of digits on a correct trial was recorded (Wechsler 2008). Rey figure copy, a measure of visuospatial constructional ability, required patients to copy a modified Rey complex figure and was scored for accuracy on a 12-point scale (Libon et al. 2011). The Boston Naming Test, a measure of confrontational word retrieval, required patients to orally name 30 line drawings; the total number of correct responses made without aid of a stimulus cue was recorded (Kaplan, Goodglass, and Weintraub 2001). Forward digit span was unavailable for two patients, Rey copy was unavailable for five patients, and Boston Naming Test was unavailable for 11 patients.

In addition to reporting raw patient performance on neuropsychological assessments, we report the proportion of patients impaired on each task relative to published normative data of healthy controls matched to the mean age and mean education of our patients where available (Tombaugh, Kozak, and Rees

1999; Crum, Anthony, Bassett, and Folstein 1993a; Shirk et al. 2011); we report patient performance on Rey copy relative to normative data based on healthy controls recruited by the FTDC who were matched to the mean age and mean education of our patients (N=22 (50% Female); Age, years: M=61.81, SD=4.97; Education, years: M=15.73, SD=2.37). We defined patient impairment on each task as performance at or greater than 1.96 standard deviations, equivalent to $p<0.05$, below normative data from healthy controls.

We used linear regression to relate performance on neuropsychological measures to CR index including age at assessment as a nuisance covariate. We report one-tailed *p* values as we predicted higher CR index to relate to better performance on neuropsychological measures.

Neuroimaging Acquisition and Preprocessing.

High-resolution T1-weighted MPRAGE structural scans were acquired using a 3T Siemens Tim Trio scanner with an 8-channel head coil, with T=1620ms, T=3.09ms, flip angle=15°, 192x256 matrix, and $1mm^3$ voxels. T1-weighted MRI images were then preprocessed using ANTs Cortical Thickness software (Tustison et al. 2014). Briefly, each individual dataset was deformed into a standard local template space in a canonical stereotactic coordinate system. Advanced Normalization Tools (ANTs) provides a highly accurate registration routine using symmetric and topology-preserving diffeomorphic deformations to minimize bias toward the reference space for computing the mappings and to capture the large deformation necessary to aggregate images in a common

space. Then, we used N4 bias correction to minimize heterogeneity (Tustison et al. 2010), the ANTs Atropos tool to segment images into six tissue classes (cortex, white matter, CSF, subcortical grey structures, brainstem, and cerebellum) using template-based priors and to generate probability maps of each tissue. GM probability images, the sum of the cortical, subcortical, and brainstem probability images, were then transformed into Montreal Neurological Institute (MNI) space, smoothed using a 2 sigma full-width half-maximum Gaussian kernel, and downsampled to 2mm isotropic voxels.

Neuroimaging Analyses.

We used *randomise* software implemented in FSL to perform nonparametric, permutation-based statistical analyses (Winkler et al. 2014). Briefly, permutation-based statistical testing is robust to concerns regarding multiple comparisons since, rather than a traditional assessment of two sample distributions, this method assesses a true assignment of factors (e.g., group, CR index) to GM relative to many (e.g., 10,000) random assignments. We adopt *a priori* statistical thresholds consistent with similar prior reports that include FWE–correction for group comparison of GM (Ash et al. 2014), and to minimize Type II error (not observing a true regression result) we employ uncorrected *p*-values for GM regressions.

Analysis 1. First, we evaluated regions of reduced GM in FTLD patients relative to controls using a nonparametric group comparison analysis. For this analysis we constrained analysis using an explicit mask that was restricted to

include only high probability GM (>0.5). We report clusters that survive a $p<0.001$ (FWE) threshold and cluster extent of >50 adjacent voxels relative to 10,000 random permutations.

Analysis 2. Second, we conducted two regression analyses to test whether CR index is associated with GM density in FTLD patients (*Analysis 2a*) and in healthy controls (*Analysis 2b*), and restricted both analyses to a mask defined by regions of reduced GM in patients relative to controls from *Analysis 1.* This mask was used so that we could focus our interpretation of CR in the context of GM regions affected by FTLD. Otherwise, it would be difficult to interpret how regions of GM that are not different from controls contribute to cognitive function in domains impaired in FTLD syndromes. Refer to Table 4 for whole-brain analyses that do not include an explicit mask of reduced GM density.

We report clusters that survive a $p<0.05$ (uncorrected) threshold and cluster extent threshold of >50 adjacent voxels relative to 10,000 random permutations. *Analysis 2a* (FTLD patients) included disease duration and age at MRI as nuisance covariates, and *Analysis 2b* (healthy controls) included age at MRI as a nuisance covariate in an effort to control for factors associated with individual differences in GM but not specifically associated with CR.

Analysis 3. In a third and final set of analyses, we used Pearson correlations to examine the relationship between regions associated with CR in FTLD patients and performance on neuropsychological measures. For each patient, we extracted the mean GM density in each region identified as associated with CR index from *Analysis 2a*. We then correlated mean GM

density with performance on each neuropsychological test and report Bonferroni-corrected *p*-values.

Results

Neuropsychological assessment.

Patients demonstrated most impairment on letter fluency (70.91% impaired) consistent with compromised frontal resources relative to other domains including attention on forward digit span (18.87% impaired), visuospatial function on Rey copy (56% impaired), non-specific global cognitive impairment on MMSE (58.18% impaired), and language deficits on the Boston Naming Test (36.36% impaired) (Table 1). Regression analyses indicated that higher CR index was associated with better performance on letter fluency (95% CI=-0.015, 1.38; t=1.96, p=0.028), but not MMSE, forward digit span, Rey copy, or Boston Naming Test (all p>0.05).

Neuroimaging.

FTLD patients had reduced GM density relative to controls throughout bilateral frontal and temporal lobes, consistent with the known pattern of GM disease associated with FTLD pathology (Analysis 1, Table 2, Figure 1A).

By restricting analysis to regions where patients exhibited reduced GM density relative to controls, we next found that CR index in patients was positively associated with GM density in right frontal cortex, including rostral, orbital, inferior, and dorsolateral prefrontal regions (Analysis 2a, Table 2, Figure 1B).

Thus, a patient with a higher CR index exhibited higher frontal GM density in these diseased regions in comparison to a patient with a lower CR index. We found no inverse association between CR index and GM density in any region of reduced GM density relative to controls (not shown).

To examine the specificity of the relationship between CR index and GM density in FTLD, we performed a comparable analysis in controls restricted to the same GM regions as the regression analysis in patients. We found that CR index in controls was positively associated with GM density in the left inferior frontal gyrus only (Analysis 2b, Table 2, Figure 1C).

In patients, frontal GM density related to higher CR index was also positively associated with performance on letter fluency ($p<0.05$), but not MMSE, forward digit span, Rey copy, or Boston Naming Test (all $p>0.05$) (Analysis 3, Table 3, Figure 2). This finding was anatomically specific such that regions of frontal GM density not related to CR were not associated with performance on letter fluency, or MMSE, forward digit span, Rey copy, or the Boston Naming Test (all $p>0.05$) (not shown).

Post hoc Analyses.

While our primary analyses use a CR index, we performed *post hoc* analyses to evaluate whether education and occupational attainment alone are similarly related to neuroimaging results (Table 5, Figure 3). These *post hoc* analyses largely converge with our CR index analyses: when considered separately, educational and occupational category are positively associated with GM density

in right frontal cortical regions similar to results obtained when using the CR index. Educational category also positively relates to GM density in the right caudate and left premotor cortex, and occupational category also positively relates to regions in bilateral temporal cortices and in the left frontal cortex.

To evaluate whether right frontal GM density associated with CR is partially driven by sources of heterogeneity in our cohort we also performed *post hoc* logistic regression analyses evaluating neuropathological, genetic, and clinical subgroups. First, we observed that mean GM density in right rostral, orbital, inferior, and dorsolateral prefrontal regions were not significant predictors of tau versus TDP-43 pathological subgroups (all factors $p>0.10$). Second, we observed that each of these four GM regions also were not significant predictors of genetic mutation or sporadic forms of disease (all factors $p>0.10$). Last, we evaluated whether the four GM regions associated with CR were significant predictors of bvFTD clinical phenotype versus other phenotypes, since bvFTD is the predominant phenotype in our cohort. This analysis revealed that one region, right dorsolateral prefrontal cortex, ($p=0.048$) was more reduced in bvFTD relative to other phenotypes, however all remaining regions did not differ (all $p>0.05$). Together, these findings are suggestive that these sources of heterogeneity are not likely confounding our observed findings related to CR in FTLD.

Discussion

Our results suggest that CR contributes to inter-individual differences in reduced

frontal GM density and executive impairment that underlie heterogeneity in the disease course of FTLD. In an analysis restricted to frontal and temporal lobe regions of reduced GM density relative to controls, patients with higher CR exhibited higher GM density in right frontal cortical regions compared to patients with lower CR. Moreover, we demonstrate that regions related to CR in FTLD appear to be specific: CR in control participants was only related to higher GM density in the left IFG. In FTLD patients, higher frontal GM density in right frontal cortical regions was associated with superior performance on letter fluency, a neuropsychological measure of executive control and verbal ability indicative of frontal lobe integrity. These findings are consistent with a reserve model linking preserved frontal anatomic integrity and superior strategic processing to the prolonged survival of patients with FTLD who have higher education and occupational attainment (Massimo et al. 2015).

Our findings are consistent with other evidence suggesting that CR is an environmental factor influencing the spectrum of disease in FTLD. Prior neuroimaging studies of behavioral bvFTD patients with unconfirmed FTLD pathology indicate CR may counteract the onset of dementia (Borroni et al. 2009; Perneczky et al. 2007; Spreng et al. 2011), while a recent survival analysis in patients with autopsy-confirmed FTLD pathology by our group indicates that higher occupational attainment is associated with prolonged survival from symptom onset (Massimo et al. 2015). These studies suggest that, despite the commonality of frontotemporal-predominant neurodegeneration, patients with FTLD appear to exhibit heterogeneous disease course that is determined in part

by environmental factors related to CR. It is imperative to identify and understand potential environmental contributors to the rate of decline in FTLD, as these may serve as prognostic markers and eventual therapeutic targets.

Our analysis of CR in control participants demonstrates specificity of higher GM density in right frontal cortex associated with CR in FTLD patients. Several other studies have investigated CR in healthy controls, demonstrating a positive relationship between CR and structural brain integrity. For example, one study demonstrates that in healthy older controls, higher years of education are positively associated with GM volume in the superior temporal gyrus, insula, and anterior cingulate cortex (Arenaza-Urquijo et al. 2013). While we similarly found a positive association between CR index and GM density in the left IFG in our healthy control group, this region did not overlap with any region of higher GM density associated with higher CR index in our patient group. We interpret this to suggest that our findings of higher GM density associated with higher CR in the right rostral and orbital frontal cortex, dorsolateral prefrontal cortex, and inferior frontal gyrus are specific to FTLD patients. Future work is necessary to evaluate the potential role of left IFG in CR in healthy controls, but limited neuropsychological data in the current control cohort precludes our ability to evaluate the behavioral consequences of this association.

To our knowledge, no prior studies have examined the association between CR and heterogeneity in neuroanatomical structure and cognitive function in patients with a clinical FTLD syndrome due to confirmed FTLD-Tau or FTLD-TDP pathology. Some studies report that patients with clinically diagnosed

FTD who have higher education and occupation exhibit evidence of greater frontal disease as measured by regional cerebral glucose utilization and regional cerebral blood flow (Borroni et al. 2009; Perneczky et al. 2007; Spreng et al. 2011). These studies have been interpreted to suggest that CR confers compensatory benefit such that individuals with FTD who have higher CR can better withstand accumulating frontotemporal pathology than individuals with lower CR, and therefore delay symptom presentation. This resembles findings reported in Alzheimer's disease (Kemppainen et al. 2008; Bosch et al. 2010). However, differences in patient population and study design necessitate cautious interpretation of these results. For example, as many as 16.7% of patients with FTD-related syndromes have AD neuropathology (Forman et al. 2006). Furthermore, the relationship between higher CR and greater frontotemporal pathology is difficult to interpret in this work in the absence of neuropsychological data. For example, it is unclear if for the same severity of frontotemporal pathology, FTD patients with higher CR demonstrate better cognitive or behavioral function than FTD patients with lower CR. Future research is needed to provide a more detailed assessment of how structural GM, functional imaging, and neuropsychological measures interact in the context of CR.

Several mechanisms may underlie the association between higher CR index and higher right frontal cortex GM density and superior letter fluency in FTLD patients. One possible mechanism is inter-individual differences in genetic predisposition. For example, genetic studies in healthy twins suggest that there is a genetic contribution to normal variation in human cognitive function and brain

morphology, including frontal cortex GM volume (Pol et al. 2006). Another possibility is that higher frontal GM and superior cognitive function in FTLD patients with higher CR may be a function of earlier stages of disease. For example, patients with higher education and occupational attainment who are more dependent on frontally-mediated executive functions may be more sensitive to the emergence of cognitive difficulty, leading to clinical symptom detection at an earlier stage of disease. Either of these proposed mechanisms could result in patients with higher CR exhibiting relatively higher frontal GM and superior letter fluency compared to patients with lower CR. Longitudinal structural and functional neuroimaging and neuropsychological assessment in controls and FTLD patients are necessary to evaluate individual differences in rates of disease progression underlying heterogeneity in FTLD disease course related to education and occupation as proxies of CR.

Our findings contribute to a growing body of evidence for factors thought to influence heterogeneity in FTLD disease course, and suggest the consideration of both biological and environmental factors. Others suggest that risk alleles in single nucleotide polymorphisms, including rs1768208 in myelin oligodendrocyte basic protein (*MOBP*) gene and rs1990622 in the *TMEM106B* gene (McMillan et al. 2014; Gallagher et al. 2014), are associated with greater pathology and earlier age of onset and death in FTLD. Moreover, recent evidence indicates that epigenetic factors like *C9ORF72* promoter hypermethylation are associated with reduced neuronal loss and reduced GM atrophy in frontal cortex (McMillan et al. 2015). While genetic factors may

contribute to heterogeneity in the disease course of FTLD, our exploratory analyses suggest that genetic status is not a confounder of the current observations. Future research should investigate interactions between environmental factors, like CR, and biological factors, like genetics and epigenetics, on clinical heterogeneity in FTLD.

Several potential caveats should be considered in the current study. We assessed CR using a composite index of education and occupation. While our observation that education and occupation independently also relate to right frontal GM, we also observed that each of these measures has some distinct frontal associations with GM. Future work evaluating the difference between these measures would be valuable. Moreover, future research should also consider other environmental factors implicated in CR such as midlife leisure activities (Scarmeas et al. 2001). Our patient cohort self-reported predominant white race and most received a college education; thus, more racially and educationally-diverse samples are needed in future studies of CR in patients with FTLD. Another caveat to consider is that the current study cohort included several different clinical phenotypes with a majority of our sample being comprised of bvFTD and the remaining consisting of primary progressive aphasias and movement disorders (e.g., CBS, PSP). While future research is necessary to stratify by clinical phenotype, our *post hoc* analyses suggested that only one GM region, right dorsolateral prefrontal cortex, was related to bvFTD. Importantly, regions implicated in CR that are most likely to be shared across clinical phenotypes, such as right inferior frontal gyrus, did not show differences

in GM density. Other sources of heterogeneity in our patient cohort including presence/absence of a genetic mutation and FTLD-Tau/FTLD-TDP pathology also did not appear to contribute to GM differences in regions related to CR, though future studies must address these distinct groups.

With these caveats in mind, we conclude that CR is an environmental factor contributing to heterogeneity in executive control and verbal ability of patients with known FTLD pathology mediated by neuroanatomic structure. These findings stimulate investigation into additional environmental contributors to disease course, and suggest their importance in prognostic considerations and treatment trials in patients with FTLD.

Table 1. Mean (standard deviation) of frontotemporal lobar degeneration patient and control demographics and neuropsychological evaluation.

	FTLD	Controls
N (F)	55 (20)	90 (40)
Age, years	61.2 (8.07)	60.3 (8.65)
Education, years	16.1 (3.11)	15.4 (2.49)
Education, N per rank:		
1	13	14
2	8	28
3	11	27
4	23	21
Occupation, N per rank		
1	2	-
2	5	13
3	20	30
4	28	47
CR Index	6.14(1.79)	5.98 (1.55)
MMSE (max=30)	23.36 (6.85)	-
Proportion impaired (%)	*32/55 (58.18)*	
Letter Fluency (words/min)	6.04 (4.72)	-
Proportion impaired (%)	*39/55 (70.91)*	
Forward Digit Span (# repeated)	5.17 (1.71)	-
Proportion impaired (%)	*10/53 (18.87)*	
Rey copy (max=12)	9.48 (3.56)	-
Proportion impaired (%)	*28/50 (56)*	
Boston Naming Test (max=30)	20.5 (8.08)	
Proportion impaired (%)	*16/44 (36.36)*	

Abbreviation: MMSE=Mini-Mental State Examination

Table 2. Results of a group comparison analysis showing regions of reduced gray matter (GM) density in frontotemporal lobar degeneration (FTLD) patients relative to healthy controls (*Analysis 1*), and results of regression analyses in patients (*Analysis 2a*) and controls (*Analysis 2b*) showing a positive relationship between GM density and cognitive reserve (CR) in regions from *Analysis 1*.

Neuroanatomic region (BA)	**L/R**	**MNI Coordinates**			***p* value**	**Voxels**
Analysis 1. Reduced GM density in FTLD relative to controls:		x	y	z		
Occipital Temporal Cortex (37)	R	36	0	-46	0.001	8250
Inferior Temporal Cortex (20)	L	-34	0	-46	0.001	6966
Orbital Frontal Cortex (11/25)	L	-8	24	-26	0.001	838
Thalamus/Hypothalamus	R	2	-10	-14	0.001	586
Hippocampus	R	32	-10	-24	0.001	180
Parietal Cortex (40)	L	-62	-28	20	0.001	142
Hippocampus	L	-22	-10	-26	0.001	119
Superior Temporal Cortex (22)	R	66	-30	18	0.001	75
Parietal Cortex (40)	R	62	-28	40	0.001	72
Angular Gyrus (39)	R	64	-44	26	0.001	54
Analysis 2a. Higher GM density associated with higher CR in FTLD:						
Rostral Frontal Cortex (10)	R	32	50	20	0.001	133
Orbital Frontal Cortex (47)	R	50	28	-4	0.003	123
Inferior Frontal Gyrus (44/45)	R	52	12	22	0.01	64
Dorsolateral Prefrontal Cortex (8/9)	R	44	6	40	0.005	59
Analysis 2b. Higher GM density associated with higher CR in controls:						
Inferior Frontal Gyrus (44/45)	L	-42	18	6	0.003	60

Abbreviations: BA = Brodmann area; L/R = Left/Right; MNI = Montreal Neurological Institute

Table 3. In FTLD patients, mean GM density in regions positively associated with CR from *Analysis 2a* are also positively correlated with performance on letter fluency, but not on MMSE, forward digit span, Rey copy, or the Boston Naming Test (*Analysis 3*).

	Letter Fluency N=55	**MMSE** N=55	**Forward Digit Span** N=53	**Rey Copy** N=50	**Boston Naming Test** N=44
R Rostral Frontal Cortex	*0.45, *p*=0.02	0.31, *p*=0.4	0.082, *p*>0.99	0.16, *p*>0.99	0.12, *p*>0.99
R Orbital Frontal Cortex	0.39, *p*=0.06	0.29, *p*=0.58	0.098, *p*>0.99	0.21, *p*>0.99	0.21, *p*>0.99
R Inferior Frontal Gyrus	**0.51, *p*<0.001	0.28, *p*=0.8	0.14, *p*>0.99	0.39, *p*=0.1	0.39, *p*>0.99
R Dorsolateral Prefrontal Cortex	*0.40, *p*=0.04	0.23, *p* > 0.99	0.24, *p*>0.99	0.34, *p*=0.32	0.34, *p*>0.99

Note. Bonferroni corrected *$p<0.05$; **$p<0.001$

Table 4. Results of two whole-brain regression analyses examining CR index relative to whole-brain GM density in FTLD patients and in controls. We report clusters that survive a $p<0.05$ (uncorrected) threshold and cluster extent threshold of >50 adjacent voxels relative to 10,000 random permutations. Regression analysis in patients included disease duration and age at MRI as nuisance covariates, and regression analysis in healthy controls included age at MRI as a nuisance covariate.

Neuroanatomic region (BA)	**L / R**	**MNI Coordinates**			***p* value**	**Voxels**
Higher GM density associated with higher CR in FTLD:		x	y	z		
Dorsolateral Prefrontal Cortex (9)	R	34	36	38	0.001	479
Rostral Frontal Cortex (10)	R	36	42	10	0.001	455
Visual Association Cortex (18)	L	-30	-90	-10	0.001	339
Fusiform Gyrus (37)	R	60	-40	-22	0.001	296
Visual Association Cortex (19)	R	36	-88	-14	0.001	234
Orbital Frontal Cortex (47)	R	50	28	-4	0.003	195
Premotor Cortex (6)	L	-26	0	64	0.001	115
Premotor Cortex (6)	R	58	2	44	0.003	110
Supramarginal Gyrus (40)	L	-54	-40	40	0.001	106
Premotor Cortex (6)	L	-10	-106	2	0.001	90
Premotor Cortex (6)	R	28	6	56	0.001	64
Supramarginal Gyrus (40)	R	56	-40	36	0.004	61
Middle Temporal Gyrus (21)	L	-62	-24	-16	0.002	60
Cerebellum	L	-34	-86	26	0.002	59
Rostral Frontal Cortex (10)	L	-44	46	22	0.004	59
Middle Temporal Gyrus (21)	L	-64	-38	-8	0.003	54
Supramarginal Gyrus (40)	R	50	-48	44	0.004	52
Higher GM density associated with higher CR in controls:						
Posterior Cingulate Cortex (31)	L	-8	-38	-44	0.001	392
Anterior Temporal Cortex (38)	R	46	22	-30	0.001	183
Inferior Frontal Gyrus (45)	L	-56	22	12	0.001	117
Visual Association Cortex (18)	L	-4	-96	-14	0.001	107
Visual Association Cortex (18)	L	-4	-96	14	0.004	74
Primary Visual Cortex (17)	R	8	-82	8	0.005	61
Orbital Frontal Cortex (11)	R	12	34	-26	0.003	56
Primary Sensory Cortex (1)	R	64	-18	34	0.001	52

Abbreviations: BA = Brodmann area; L/R = Left/Right; MNI = Montreal Neurological Institute

Table 5. Results of two regression analyses showing a positive relationship between GM density and education and occupation in regions from *Analysis 1* in FTLD patients. We report clusters that survive a $p<0.05$ (uncorrected) threshold and cluster extent threshold of >50 adjacent voxels relative to 10,000 random permutations. Regression analysis in patients included disease duration and age at MRI as nuisance covariates.

Neuroanatomic region (BA)	**L/R**	**MNI Coordinates**			***p* value**	**Voxels**
Higher GM density associated with higher education in FTLD:		x	y	z		
Inferior Frontal Gyrus (44)	R	58	10	22	0.002	316
Rostral Frontal Cortex (10)	R	42	44	10	0.001	194
Orbital Frontal Cortex (47)	R	50	34	-6	0.002	146
Caudate (48)	R	12	8	10	0.005	119
Premotor Cortex (6)	L	-50	0	50	0.006	66
Higher GM density associated with higher occupation in FTLD:						
Orbital Frontal Cortex (47)	R	44	30	-14	0.001	1310
Orbital Frontal Cortex (47)	L	-40	30	-16	0.001	508
Orbital Frontal Cortex (11)	R	8	36	-24	0.001	473
Middle Temporal Gyrus (21)	R	58	-8	-16	0.002	448
Inferior Frontal Gyrus (44)	R	58	10	20	0.001	436
Rostral Frontal Cortex (10)	L	-36	56	6	0.002	262
Anterior Temporal Cortex (38)	R	30	8	-40	0.004	135
Anterior Temporal Cortex (38)	L	-32	18	-36	0.007	87
Inferior Frontal Gyrus (44)	L	-62	-18	-22	0.003	74
Dorsolateral Prefrontal Cortex (46)	L	-40	30	22	0.005	65
Orbital Frontal Cortex (11)	L	-8	40	-24	0.007	52

Abbreviations: BA = Brodmann area; L/R = Left/Right; MNI = Montreal Neurological Institute

Figure 1. (*Analysis 1*) Results of a nonparametric t-test showing regions of reduced grey matter (GM) density in Frontotemporal Lobar Degeneration patients (N=55) relative to demographically-comparable controls (N=90). (*Analysis 2a*) Results of a regression analysis in FTLD patients (N=55) restricted to regions of reduced GM density from *Analysis 1* (white regions) demonstrating that GM density in the right dorsolateral prefrontal cortex, rostral frontal cortex, orbital frontal cortex, and inferior frontal gyrus is positively associated with Cognitive Reserve (CR) index. (*Analysis 2b*) Results of a regression analysis in healthy controls (N=90) demonstrating that GM density in the left inferior frontal gyrus is positively associated with CR index. Color bar represents 1—*p*-value with yellow representing highest significance.

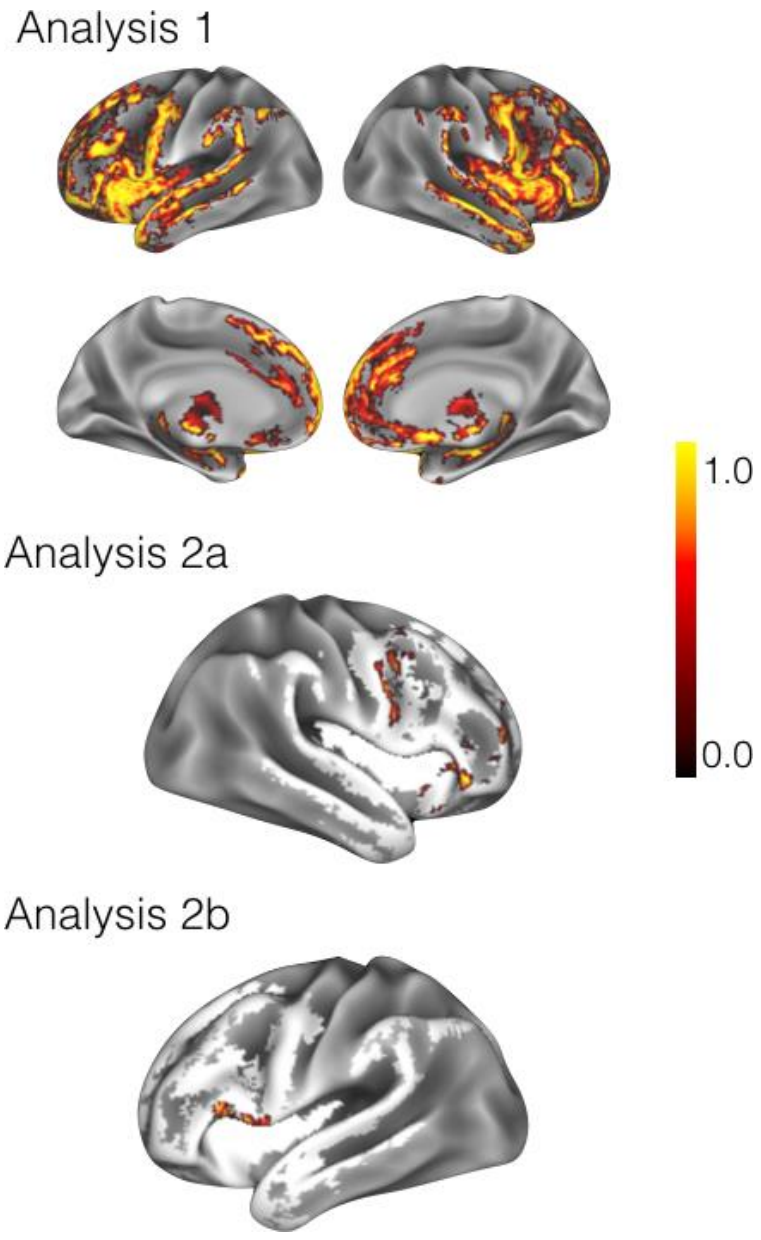


Figure 2. Mean GM density in the right rostral frontal cortex, inferior frontal gyrus, orbital frontal cortex, and dorsolateral prefrontal cortex positively associated with CR from *Analysis 2a* are also positively associated with performance on letter fluency in FTLD patients (N=55).

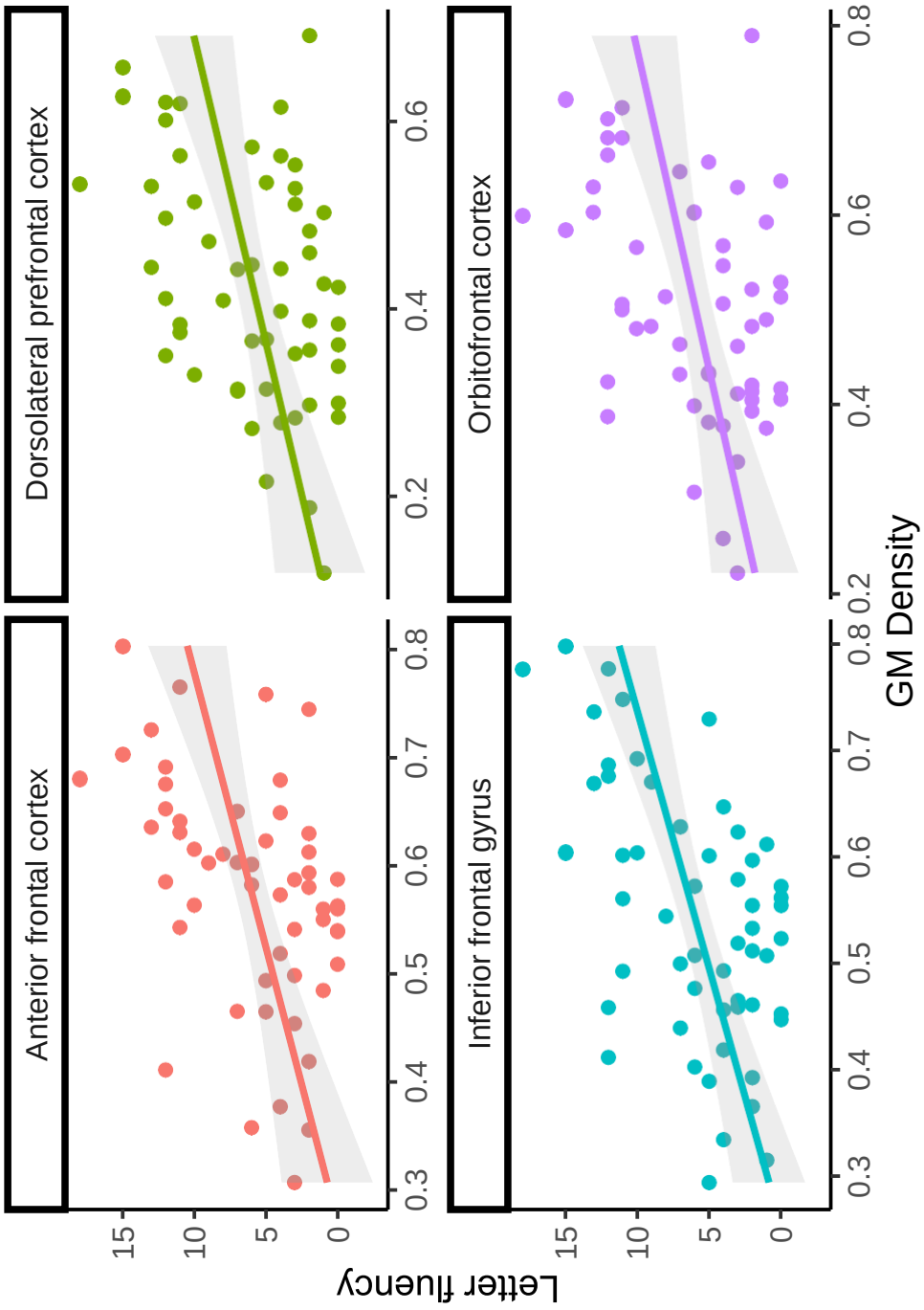
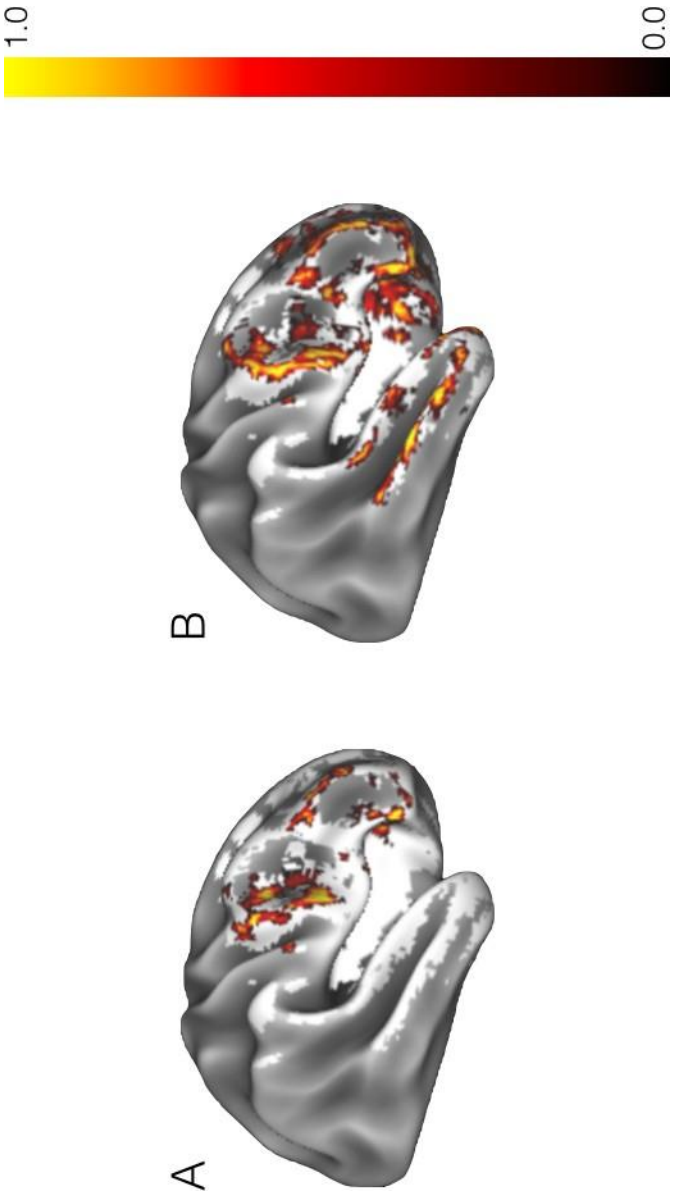


Figure 3. Results of a regression analysis in FTLD patients (N=55) restricted to regions of reduced GM density from *Analysis 1* (pictured in white) demonstrating that when considered separately, educational category (A) and occupational category (B) each are significantly positively associated with right frontal cortex GM density, largely converging with our results using Cognitive Reserve (CR) index as a combined metric of educational and occupational category (*Post hoc Analyses*). Color bar represents 1—*p*-value with yellow representing highest significance.



CHAPTER 3

UNC13A polymorphism contributes to frontotemporal disease in sporadic amyotrophic lateral sclerosis

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Abstract

The majority (90-95%) of amyotrophic lateral sclerosis (ALS) is sporadic, and ~50% of patients develop symptoms of FTD associated with shorter survival. The genetic polymorphism rs12608932 in *UNC13A* confers increased risk of sporadic ALS and sporadic frontotemporal degeneration (FTD), and modifies survival in ALS. Here, we evaluate whether rs12608932 is also associated with frontotemporal disease in sporadic ALS. We identified reduced cortical thickness in sporadic ALS with T1-MRI (N=109) relative to controls (N=113), and observed that minor allele (C) carriers exhibited greater reduction of cortical thickness in dorsal prefrontal, ventromedial prefrontal, anterior temporal, and middle temporal cortices and worse performance on a frontal-lobe mediated cognitive test (reverse digit span). Association between rs12608932 and cortical thickness was not observed in an independent control cohort from the Alzheimer's Disease Neuroimaging Initiative (N=84). In sporadic ALS with autopsy data (N=102), minor allele homozygotes exhibited greater burden of pTDP-43 pathology in the middle frontal, middle temporal, and motor cortices. Our findings demonstrate converging evidence that rs12608932 may modify frontotemporal disease in

sporadic ALS, and suggest that rs12608932 may function as a prognostic indicator and could be used to define patient endophenotypes in clinical trials.

Introduction

Amyotrophic lateral sclerosis (ALS) is a multi-system disorder primarily characterized by progressive degeneration of the upper and lower motor neurons (UMN, LMN) and affected individuals typically survive 2-5 years from symptom onset (Chiò et al. 2009). An estimated ~50% of individuals with ALS also develop impairments in at least one cognitive domain including executive function, social behavior, or language indicative of extramotor neurodegeneration in the frontal and temporal lobes, and about 10% of these individuals develop multi-domain impairments consistent with frank frontotemporal degeneration (FTD) (Montuschi et al. 2015; Ringholz et al. 2005). While ALS and FTD commonly feature tar DNA-binding protein-43kda (TDP-43) pathology (Neumann et al. 2006) and can both be caused by pathogenic *C9ORF72* repeat expansions (Renton et al. 2011; DeJesus-Hernandez et al. 2011), the mechanisms influencing the risk of progression from ALS to develop cognitive impairment and FTD have been under-evaluated. The presence of FTD is consistently associated with shorter survival in ALS patients (Govaarts et al. 2016; Elamin, Phukan, Bede, Jordan, Byrne, Pender, and Hardiman 2011b), and therefore it is critical to identify risk factors for frontotemporal disease in ALS.

The vast majority of ALS is considered “sporadic”, with only a small proportion of approximately 5-10% of ALS patients featuring a familial history or

autosomal dominant source of disease (e.g. *C9ORF72* expansions) (Taylor, Brown, and Cleveland 2016). Therefore, it is important to consider sources of common genetic variation that may influence risk of disease – including FTD - in ALS. Case-control genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with increased odds of having ALS (van Rheenen et al. 2016) or FTD (Van Deerlin et al. 2010). More recently, two loci, including rs3849942 in the *C9ORF72* gene and rs4239633 in the *UNC13A* gene demonstrate shared genetic overlap between ALS and FTD (Karch et al. 2018). The observed rs3849942 SNP is a marker of the *C9ORF72* expansion haplotype (Jones et al. 2013) with no additional genetic influence reported. However, loci in *UNC13A* including rs12608932, that is in high linkage disequilibrium (LD) with rs4239633 (D'=0.83), are associated with increased risk of sporadic ALS (van Es et al. 2009) and sporadic FTD with underlying TDP-43 pathology (Diekstra et al. 2014). Clinical studies have further suggested that the minor allele (C) of rs12608932 (MAF=0.43) is associated with shorter survival in sporadic ALS, which has been demonstrated in multiple populations and under both additive and recessive minor allele models (Diekstra et al. 2012; Vidal-Taboada et al. 2015; Chiò et al. 2013).

In this report we perform a multimodal evaluation of rs12608932 to further investigate evidence for shared risk between ALS and FTD. Specifically, we test the hypothesis that the disease-associated allele (C) in rs12608932 is associated with frontotemporal cortical disease in sporadic ALS, including reduced cortical thickness, impaired cognitive performance, and increased vulnerability to TDP-43

pathology.

Methods

We evaluated 190 sporadic ALS cases distributed across a neuroimaging, neuropathological, and/or neuropsychological cohort, described in detail below.

Neuroimaging cohort.

We retrospectively evaluated 109 sporadic ALS patients recruited for research between 2004 and 2017 from the Penn Comprehensive ALS Clinic and Penn Frontotemporal Degeneration Center at the University of Pennsylvania Perelman School of Medicine. All patients were diagnosed with ALS by a board-certified neurologist (L.E., L.M., M.G., D.I) using the revised El Escorial criteria (Brooks et al. 2000), including possible, probable, and definite ALS. All patients were also assessed for frontotemporal dysfunction using established criteria (Strong et al. 2009) and those patients enrolled in research prior to 2009 were retrospectively evaluated through a chart review; in total 26 patients were diagnosed with ALS-FTD and 11 patients were diagnosed with ALS-cognitive impairment (ALSci).

To identify regions of significant cortical thinning in ALS, we recruited 113 demographically-comparable healthy controls who self-reported a negative history for neurologic or psychiatric disease and scored >27 on the MMSE. There were no statistically significant differences in age, education, or sex between controls and ALS (all p values > .05). We assessed participant race and ethnicity via self-report. While population diversity is known to influence allele frequency across individuals, rs12608932 has a relatively equal minor allele frequency

across populations (e.g., European 0.35, Africans 0.33, American 0.30) and post-hoc analyses limited to the majority race and ethnicity of our patient population (e.g. white non-Latino) remain significant or approach significance. Demographic features of ALS and controls are summarized in Table 6.

All participants participated in an informed consent procedure approved by an Institutional Review Board convened at the University of Pennsylvania.

Autopsy cohort.

We evaluated neuropathological data from 102 sporadic ALS autopsy cases who were diagnosed by a board-certified neuropathologist (JQT, EBL) with ALS due to TDP-43 pathology using immunohistochemistry (Lippa et al. 2009; Neumann et al. 2006) and published criteria (Mackenzie et al. 2011). This cohort included 21 patients from the ALS neuroimaging cohort. Autopsy cases were identified from the Integrated Neurodegenerative Disease Database (Toledo et al. 2014) after excluding cases with a family history of neurodegenerative disease or a known genetic mutation associated with ALS (see *Genetic Screening*). Within this autopsy cohort we rated the extent of phosphorylated TDP-43 (pTDP-43) intraneuronal inclusions (dots, wisps, skeins) for each sampled region on a semi-quantitative ordinal scale: 0 = none/rare, 1 = mild, 2 = moderate, 3 = severe/numerous (Toledo et al. 2014). All neuropathological ratings were performed by an expert neuropathologist (JQT, EBL) blinded to genotype. Demographic features of ALS autopsy cases are summarized in Table 7.

Alzheimer's disease neuroimaging initiative (ADNI) cohort.

To evaluate the disease specificity of any observed neuroanatomic and genetic associations we additionally performed cortical thickness analyses in 84 amyloid-negative (florbetapir SUVR <1.11) (Landau et al. 2012), cognitively-normal healthy controls from the publicly-available Alzheimer's Disease Neuroimaging Initiative (ADNI) database.

Genetic screening.

DNA was extracted from peripheral blood or frozen brain tissue following the manufacturer's protocols (Flexigene (Qiagen) or QuickGene DNA whole blood kit (Autogen) for blood, and QIAsymphony DNA Mini Kit (Qiagen) for brain tissue).

All patients were screened for *C9ORF72* hexanucleotide repeat expansions using a modified repeat-primed polymerase-chain reaction (PCR) as previously described (Suh et al. 2015), and we excluded any patient with >=30 hexanucleotide repeats. Of the remaining individuals, we evaluated family history using a three-generation pedigree history as previously reported (Wood et al. 2013). For cases with a family history of the same disease we sequenced 45 genes previously associated with neurodegenerative disease (Toledo et al. 2014), including four genes known to be associated with ALS (e.g. *SOD1* (D. R. Rosen 1993), *TARDBP* (Kabashi et al. 2008), *FUS* (Kwiatkowski et al. 2009; Vance et al. 2009), and *VCP* (J. O. Johnson et al. 2010)). Sequencing was performed using a custom-targeted next-generation sequencing panel (MiND-

Seq) (Toledo et al. 2014), and analyzed using Mutation Surveyor software (Soft Genetics, State College, PA). We excluded any individuals identified as having a pathogenic mutation.

SNP genotyping.

All neuroimaging and neuropathology cases were genotyped using peripheral or brain DNA, extracted as above, for rs12608932 using a custom-designed Pan Neurodegenerative Disease-oriented Risk Allele (PANDoRA) panel designed to genotype 92 common and risk allele variants identified in association and other studies as modifying risk of disease or a phenotype for several neurodegenerative diseases, including ALS as well as FTD, Parkinson's disease, and Alzheimer's disease (Toledo et al. 2014). While our analyses focus on a single genotype, rs12608932, this approach provides a cost-effective manner to genotyping that can be used for future comparative studies as previously reported by our center (McMillan et al. 2018; McMillan et al. 2014). Briefly, the 92 SNP Type assays were designed by D3 Assay Design tool (Fluidigm). Allele-specific PCR was performed using the 96.96 Dynamic Array integrated fluidic circuits (Fluidigm) and genotyping was carried out using the BioMark HD system (Fluidigm) according to the manufacturer's protocol. The genotype call data was collected and analyzed using BioMark Genotyping Analysis software.

All ADNI healthy control data was genotyped using the Illumina 660K, as previously described (Del-Aguila et al. 2018). As part of routine quality control steps, single-nucleotide polymorphisms (SNPs) with minor allele frequency < 1%,

call rates < 98%, Hardy–Weinberg equilibrium p-values >10^{-6} and individuals with > 2% missing genotypes were removed before imputation. The dataset was imputed, separately, using SHAPEIT/IMPUTE2 with the 1000 Genomes Project as the reference panel. All genotypes with dosage levels <0.9 for all three possible genotypes or with information scores <0.3 were excluded. Variants out of Hardy Weinberg Equilibrium (HWE) ($p<1x10^{-6}$) or with a genotyping rate below 95% were also omitted from the analyses. Population structure was inferred by principal component (PC) analysis using PLINK v.1.9. PLINK v1.9 was also used to find duplicate and related individuals who were eliminated from the analysis.

Clinical evaluations.

Detailed clinical evaluations were available for a subset of 88 (79%) sporadic ALS patients in the neuroimaging cohort. These patients were clinically assessed within approximately two months of neuroimaging acquisition date (M =1.62, SD = 2.63) for motor function using the Revised ALS Functional Rating Scale (ALSFRS-R) (Cedarbaum, Stambler, Malta, Fuller, Hilt, Thurmond, and Nakanishi 1999a) and cognitive function using the Forward and Reverse Digit Span, the Visual-Verbal Test (VVT), letter fluency, and the Mini-Mental State Exam (MMSE). Forward digit span, a measure of auditory-verbal short-term memory, and reverse digit span, a measure of auditory-verbal working memory, were assessed in an untimed manner with repetition of increasingly longer sequences until the patient erred; the maximum number of digits on a correct trial was recorded (Wechsler 1945). Our group has previously demonstrated that the

VVT is a brief, untimed measure of cognitive flexibility with minimal motor demands appropriate for use in an ALS patient population (Evans et al. 2015). Participants first identify a feature shared by three of four simple geometric designs, and are next challenged to identify a different feature shared by another combination of three of the four same geometric designs; a discrepancy between the number correct on the first and second identifications of 10 trials is considered a sign of reduced mental flexibility. Letter fluency is a measure of executive control and verbal ability (Abrahams et al. 2000), and was assessed by the number of unique words beginning with “F” a patient was able to generate in one minute (excluding proper nouns and numbers); patients did not complete letter fluency if bulbar upper or lower motor symptoms were present upon exam. The MMSE is a 30-point questionnaire that evaluates global dementia severity (Crum, Anthony, Bassett, and Folstein 1993b); we calculated the percentage correct on MMSE as some patients were not able to complete the entire test due to motor disability.

In addition to reporting raw patient performance on neuropsychological assessments, we report the percent of patients impaired on each task relative to published normative data of healthy controls matched to the mean age and mean education of our patients where available (Tombaugh, Kozak, and Rees 1999; Weintraub et al. 2009). We report patient performance on the Visual Verbal Test relative to normative data based on healthy controls recruited by the Penn Frontotemporal Degeneration Center who were matched to the mean age and mean education of our patients (N=31 (17 Female); Age, years: M=60.58,

SD=12.80; Education, years: M=15.29, SD=2.52). We defined patient impairment on each task as performance at or greater than 1.96 standard deviations, equivalent to $p<0.05$, below normative data from healthy controls.

Neuroimaging acquisition & processing.

High-resolution T1-weighted MPRAGE structural scans were acquired for neuroimaging participants using a 3T Siemens Tim Trio scanner with an 8-channel head coil, with T=1620ms, T=3.09ms, flip angle=15°, 192x256 matrix, and 1mm^3 voxels. T1-weighted MRI images were then preprocessed using ANTs Cortical Thickness software (Tustison et al. 2014). Each individual dataset was deformed into a standard local template space in a canonical stereotactic coordinate system. Advanced Normalization Tools (ANTs) provides a highly accurate registration routine using symmetric and topology-preserving diffeomorphic deformations to minimize bias toward the reference space and to capture the deformation necessary to aggregate images in a common space. Then, we used N4 bias correction to minimize heterogeneity (Tustison et al. 2010) and the ANTs Atropos tool to segment images into six tissue classes (cortex, white matter, CSF, subcortical grey structures, brainstem, and cerebellum) using template-based priors and to generate probability maps of each tissue. Voxel-wise cortical thickness was measured in millimeters (mm) from the pial surface and then transformed into Montreal Neurological Institute (MNI) space, smoothed using a 2 sigma full-width half-maximum Gaussian kernel, and downsampled to 2mm isotropic voxels.

Neuroimaging analyses.

We used *randomise* software from FSL to perform nonparametric, permutation-based statistical analyses of cortical thickness images from our ALS and control neuroimaging cohorts and the ADNI control cohort. Permutation-based statistical testing is robust to concerns regarding multiple comparisons since, rather than a traditional assessment of two sample distributions, this method assesses a true assignment of factors (e.g., genotype) to cortical thickness relative to many (e.g., 10,000) random assignments (Winkler et al. 2014).

First, we identified regions of reduced cortical thickness in ALS relative to healthy controls, constraining analysis using an explicit mask restricted to high probability cortex (>.4). We report clusters that survive $p<.005$ threshold-free cluster enhancement (TFCE) (Smith and Nichols 2009) corrected for family-wise error (FWE) relative to 10,000 random permutations.

Next, we evaluated whether rs12608932 genotype relates to magnitude of reduced cortical thickness in ALS, covarying for disease duration, age, and sex in an effort to control for factors associated with reduced cortical thickness but not specifically associated with SNP genotype. We restricted this analysis to a mask defined by regions of reduced cortical thickness in ALS relative to controls so that we could focus our interpretation in the context of cortical degeneration affected by ALS. We report clusters that survive $p<.01$ (uncorrected) threshold and cluster extent threshold of >20 adjacent voxels relative to 10,000 random permutations; we employ an uncorrected threshold to minimize the chance of

Type II error (not observing a true result).

We performed comparable analyses to evaluate SNP genotype relative to cortical thickness in the ADNI control cohort and adopted the same statistical thresholds as described above.

Statistical analyses.

All additional statistical analyses were performed using R. For assessment of ordinal neuropathology data we performed ordinal logistic regression using the *MASS* package in R to investigate whether burden of TDP-43 pathology differed according to genotype at rs12608932, covarying for age, sex, and disease duration at death. For clinical comparisons, we used multiple linear regression to evaluate the association between genotype at rs12608932 and performance on forward and reverse digit span, the MMSE, the VVT, and letter fluency; we included age, sex, disease duration, ALSFRS-R total score, and cognitive diagnosis (i.e. diagnosis of ALS-FTD, ALSci) as covariates in each analysis.

Results

Reduced cortical thickness in ALS associated with the rs12608932 minor allele.

A group comparison of ALS patients relative to healthy controls revealed reduced cortical thickness in the bilateral frontal and temporal lobes, consistent with the pattern of cortical degeneration associated with ALS-FTD spectrum disorders (Table 8, Figure 4).

To evaluate disease-specific genotype and neuroanatomic associations

we restricted our subsequent analyses to regions of reduced cortical thickness observed in ALS. Evaluation of rs12608932 under a minor allele additive model revealed reduced cortical thickness associated with the minor allele (C) in the right anterior temporal lobe, bilateral ventromedial prefrontal cortex, left middle temporal gyrus, and left dorsal prefrontal cortex (Table 8, Figure 4A). Evaluation of rs12608932 under a minor allele recessive model revealed reduced cortical thickness associated with the minor allele (C) in regions including the dorsal and dorsolateral prefrontal, ventromedial prefrontal, anterior temporal, and middle temporal cortices (Table 8, Figure 4B).

Investigation of the inverse association between rs12608932 genotype (i.e. number of major non-risk allele “A”) and cortical thickness yielded no statistically significant findings for either additive or recessive models (not shown), suggesting that the rs12608932 minor allele C is specifically associated with reduced cortical thickness in these regions.

To examine the disease specificity of the relationship between rs12608932 genotype and reduced cortical thickness in ALS, we performed comparable analyses in the ADNI control cohort restricted to the same regions of reduced cortical thickness observed in ALS. We observed no relationship between cortical thickness and genotype at rs12608932 under minor allele additive or recessive models (not shown).

Working memory performance associated with rs12608932.

Given the observed association between rs12608932 and frontal and temporal

cortices, we evaluated each clinical assessment to investigate potential clinical consequences of the observed neuroanatomic and genetic associations.

We first investigated whether ALSFRS-R relates to rs12608932 genotype. Under a minor allele additive model, minor allele heterozygotes (AC) (β=3.41, *p*=.04) but not minor allele homozygotes at rs12608932 (CC) (β=1.97, *p*=.35) demonstrated higher ALSFRS-R total score compared to major allele homozygotes (AA); disease duration (β=-.037, *p*=.1), age (β=.059, *p*=.38), sex (β=1.53, *p*=.33), and cognitive diagnosis (ALSci: β=-2.34, *p*=.37; ALS-FTD: β=-0.88, *p*=.62) were not found to significantly affect ALSFRS-R Total in this model. However, under a minor allele recessive model, we did not observe a significant difference in ALSFRS-R Total score between minor allele heterozygotes (AC) and minor allele homozygotes (CC) at rs12608932 (β=0.34, *p*=.87) compared to major allele homozygotes (AA). To account for the observed difference in ALSFRS-R under a minor allele additive model, we covaried for ALSFRS-R Total score in all subsequent regressions.

Under a minor allele additive model, we observed a trend whereby minor allele homozygotes at rs12608932 (CC) (β=-.81, *p*=.086) performed worse than major allele homozygotes (AA) on reverse digit span after accounting for cognitive diagnosis (ALSci: β=-1.73, *p*=.0014; ALS-FTD: β=-2.09, *p*<.00001); heterozygous genotype (AC) (β=.32, *p*=.38), disease duration (β=-.0022, *p*=.64), ALSFRS-R total score (β=-.012, *p*=.60), and age (β=-.0021, *p*=.89) did not relate to reverse digit span performance in this model.

Under a minor allele recessive model, minor allele homozygotes at rs12608932 (CC) (β=-0.96, p=.029) performed significantly worse than major allele homozygotes (AA) and heterozygotes (AC) on reverse digit span after accounting for cognitive diagnosis (ALSci: β=-1.69, p=.0017; ALS-FTD: β=-2.08, *p*<.00001); disease duration (β=-.0018, *p*=.69), ALSFRS-R total score (β=-.0013, *p*=.95), age (β=-.0013, *p*=.76), and sex (β=.55, *p*=.11), did not significantly relate to reverse digit span performance in this model.

Performance on other neuropsychological tests was not found to associate with rs12608932 genotype, including forward digit span (CC: β=-.03, *p*=.93; CA: β=.40, *p*=.20), letter fluency (CC: β=.26, *p*=.85; CA: β=1.61 *p*=.15), MMSE (CC: β=.036, *p*=.28; CA: β=.006, *p*=.82), and the VVT (CC: β=-.22, *p*=.67; CA: β=-.40, *p*=.32). We observed similar results under minor allele recessive models: forward digit span (CC: β=-.22, *p*=.57), letter fluency (CC: β=-.39, *p*=.76), MMSE (CC: β=.034, *p*=.26), and the VVT (CC: β=.12, *p*=.80).

pTDP-43 pathologic burden associated with rs12608932.

To evaluate converging evidence for our observed genetic and neuroanatomic associations, we assessed ordinal neuropathologist ratings of pTDP-43 pathologic burden in the middle frontal, temporal, and motor cortices, which were associated with rs12608932 in the above neuroimaging analyses (Figure 5).

Minor allele homozygotes (CC) were 8.26 times (95% CI: 2.01, 38.64; *p* = .0043) more likely and heterozygotes (AC) were 5.53 times (95% CI: 1.78, 21.21;

p = .0057) more likely to have higher TDP-43 burden in the middle frontal cortex relative to major allele homozygotes (AA). Minor allele homozygotes (CC) were 4.40 times (95% CI: 1.25, 16.23; *p* = .022) more likely to have higher TDP-43 burden in the temporal cortex and – reaching marginal significance - 3.04 times (95% CI: 0.98, 9.72; *p* = .056) more likely to have higher TDP-43 burden in the motor cortex relative to major allele homozygotes (AA); minor allele heterozygotes (AC) were not more likely to have higher TDP-43 burden relative to major allele homozygotes in either region (both *p* values > .1).

We repeated our analysis of TDP-43 pathologic burden in the middle frontal, temporal, and motor cortices in our autopsy cohort after excluding 21 individuals in the autopsy cohort who were also in the neuroimaging cohort. Similar to our findings in the entire autopsy cohort, minor allele homozygotes (CC) were 28.35 times (95% CI: 4.72, 249.31; *p* = .00064) more likely and heterozygotes (AC) were 11.49 times (95% CI: 2.78, 78.76; *p* = .0027) more likely to have higher TDP-43 burden in the middle frontal cortex relative to major allele homozygotes (AA). Minor allele homozygotes (CC) were 11.60 times (95% CI: 2.23, 72.49; *p* = .005) more likely to have higher TDP-43 burden in the temporal cortex relative to major allele homozygotes (AA); minor allele heterozygotes (AC) were not more likely to have higher TDP-43 burden relative to major allele homozygotes (*p* > .9). We did not observe any statistically significant differences in TDP-43 pathologic burden in the motor cortex relative to rs12608932 in this smaller cohort.

Discussion

In this study, we evaluated whether rs12608932 genotype in *UNC13A* was associated with frontotemporal disease in sporadic ALS using a novel multimodal approach integrating genetic, neuroimaging, clinical, and neuropathology data. Our results indicate that sporadic ALS patients who are carriers of the rs12608932 minor allele (C) show reduced cortical thickness in regions including the dorsal and ventromedial prefrontal cortex, anterior and middle temporal cortex, and premotor cortex, and that minor allele homozygotes (CC) demonstrate worse performance on reverse digit span, a frontal-lobe mediated cognitive test. We did not observe a relationship between rs12608932 genotype and cortical thickness in the amyloid-negative ADNI healthy control cohort, suggesting that the association between rs12608932 and reduced cortical thickness is specific to ALS. Furthermore, in our sporadic ALS autopsy cohort, we demonstrate that carriers of the rs12608932 minor allele have increased odds of pTDP-43 pathologic burden in the middle frontal cortex, middle temporal cortex, and motor cortex, consistent with our neuroimaging findings. To our knowledge, our study provides novel evidence that the minor allele of rs12608932 in *UNC13A* is associated with *in vivo* frontotemporal cortical atrophy, impaired cognitive performance, and greater burden of pTDP-43 pathological inclusions in sporadic ALS.

rs12608932 was first identified through a two-stage GWAS as a susceptibility locus for sporadic ALS with a combined $P = 2.53 \times 10(-14)$ (van Es et al. 2009). rs12608932 maps to a haplotype block within the boundaries of

gene *UNC13A*, which regulates presynaptic vesicle priming and glutamate release at neuromuscular synapses, and mice lacking the *UNC13A* homolog have arrested synaptic vesicle maturation and disrupted glutamatergic transmission (Augustin et al. 1999). Subsequent population-based study indicated the minor allele (C) at rs12608932 as a risk factor for shorter survival in sporadic ALS under both additive and recessive models (Diekstra et al. 2012; Chiò et al. 2013; Vidal-Taboada et al. 2015), and as a modifier of physical symptom progression on the ALSFRS-R (Vidal-Taboada et al. 2015). In addition to risk and progression of sporadic ALS, rs12608932 was also identified to serve as a risk locus for sporadic FTLD-TDP (Diekstra et al. 2014), suggesting rs12608932 as a potential link between ALS and FTLD-TDP. More recently, an additional SNP in *UNC13A* in high LD with rs12608932 (rs4239633; D'=0.83) was identified as demonstrating selective genetic overlap between ALS and FTD in GWAS meta-analysis (Karch et al. 2018).

Our findings corroborate rs12608932 as a genetic link between ALS and FTD with TDP-43 pathology, and specifically demonstrate that the minor allele is associated with reduced cortical thickness, worse working memory performance, and greater burden of TDP-43 pathology in the frontal and temporal lobes. Importantly, we use continuous disease traits from multiple modalities (i.e. structural imaging, cognitive testing, neuropathology data) to present converging evidence that rs12608932 confers increased risk of frontotemporal disease in sporadic ALS and relates to patient cognitive performance. This approach offers an advance over discovery GWAS that compare only categorical clinical

designations (e.g. ALS vs. healthy controls), and allows detailed phenotypic characterization associated with rs12608932 genotype.

Furthermore, the observed relationship between rs12608932 and both frontotemporal cortical thinning and burden of TDP-43 pathology are consistent with the disease anatomy of ALS-FTD spectrum disorders. Structural MRI studies have previously demonstrated progressive frontotemporal gray matter degeneration over disease course (Menke et al. 2014; Verstraete et al. 2014; Kwan et al. 2013; Verstraete et al. 2012; Keil et al. 2012; Senda et al. 2011; Müller et al. 2016), and that degree of frontotemporal cortical thinning relates to cognitive-behavioral phenotype (Agosta et al. 2016; Schuster et al. 2014). Additionally, staging of neuropathological burden suggests stereotyped propagation of TDP-43 from motor regions (brainstem, spinal cord) to frontal and temporal neocortex over the course of disease (Brettschneider et al. 2013; Braak et al. 2013). Our finding of greater TDP-43 pathologic burden associated with the rs12608932 in frontal and temporal neocortex may thus be interpreted to reflect more progressive disease propagation in sporadic ALS patients who are minor allele carriers. This finding could also be related to TDP-43 pathologic subtype (Mackenzie et al. 2011), and future work is necessary to 1) evaluate the influence of TDP-43 pathologic subtype on the anatomic distribution and phenotypic presentation (e.g. comorbid symptoms of frontotemporal disease) of disease, and 2) investigate how rs12608932 genotype relates to TDP-43 pathologic subtype. With this in mind, rs12608932 genotype may potentially be used prognostically to evaluate risk of frontotemporal cortical disease in ALS, which has been

previously associated with reduced survival (Govaarts et al. 2016; Elamin, Phukan, Bede, Jordan, Byrne, Pender, and Hardiman 2011b).

The observed relationships between rs12608932, reduced cortical thickness in the dorsal and ventromedial prefrontal cortices, and worse performance on reverse digit span are congruent with the neuroanatomy of working memory. Indeed, functional activation of the dorsal and ventromedial prefrontal cortices relates to memory load function and information retrieval on tasks of working memory, respectively (Rypma and D'Esposito 1999).

We also observed a relationship between rs12608932 and reduced cortical thickness in the right orbital frontal cortex, right anterior temporal lobe, and left middle temporal gyrus. Our group has previously shown that reduced cortical thickness in the orbital frontal cortex is associated with behavioral disinhibition and apathy in patients with FTD (Massimo et al. 2009), while others have shown that cortical thinning in the right anterior temporal lobe and left middle temporal gyrus are associated with impaired semantic knowledge and theory of mind in patients with FTD (Irish, Hodges, and Piguet 2014; Rohrer et al. 2009). Impairments in behavior and language are common to both ALS and FTD (Beeldman et al. 2018), and prospective studies are necessary to evaluate potential rs12608932 associations relative to language and behavioral function using more comprehensive neuropsychological batteries like the Edinburgh Cognitive Assessment Scale (ECAS) (Abrahams et al. 2014), which were not available in this retrospective study.

Our findings add to an increasing body of evidence in support of a

clinicopathologic continuum between ALS and FTD with underlying TDP-43 pathology, and specifically suggest that genetic polymorphisms may relate to the phenotypic presentation of frontotemporal disease in sporadic ALS. This is consistent with prior work from our group demonstrating that TDP-43 pathology-associated SNPs relate to selective neuroanatomic distribution of cortical atrophy and white matter degeneration in patients with sporadic forms of FTD (McMillan et al. 2014), modify disease onset and survival in FTD with *C9ORF72* repeat expansions ("TMEM106B is a Genetic Modifier of Frontotemporal Lobar Degeneration with C9ORF72 Hexanucleotide Repeat Expansions (I5-1.008)," 2014) (Gallagher et al. 2014), and confer risk for impaired executive function in ALS (Vass et al. 2011).

The identification of genetic polymorphisms associated with disease phenotypes holds important implications for both basic science research and translational application. Our observed association between rs12608932 and frontotemporal disease in sporadic ALS motivates further investigation into potential mechanisms of disease vulnerability associated with genetic polymorphisms. While the function of gene *UNC13A* has previously been characterized (Augustin et al. 1999), future work is necessary to determine the extent to which rs12608932 genotype provides an actual disease modifier or is an association related to matching downstream transcription sites or other long-range interactions (Haiquan Li et al. 2016). In regards to translational application, our findings may contribute to patient stratification for clinical trials. Prior research has demonstrated baseline differences in patients stratified for SNP genotype

and indicate a contribution of genetic polymorphisms in dose-response to treatment (D. Wang et al. 2011), leading to the incorporation of genetic polymorphisms in the design and analysis of clinical trials (Zhang et al. 2017).

Several caveats should be considered in the present study. Our data were limited according to strict inclusion criteria to investigate the clinical, pathologic, and regional anatomical differences in sporadic ALS patients relative to rs12608932 genotype. While we establish multiple sources of converging evidence for rs12608932 genotype relating to frontal disease, replication of the present findings in a large independent cohort using a prospective design is necessary. Furthermore, additional research is required to determine the extent to which pTDP-43 pathological burden directly or indirectly relates to reductions in cortical thickness. In this study, our evaluation of cognitive performance was retrospective and limited to measures broadly assessing executive function and global cognition. Future studies using revised diagnostic criteria for frontotemporal dysfunction in ALS (Strong et al. 2017) and specialized assessment of cognitive function in patients with ALS, such as the Edinburgh Cognitive and Behavioral ALS Screen (Abrahams et al. 2014), are necessary to also assess impairments in other domains including language and behavior. Here, we focus on genetic contributions to frontotemporal disease in sporadic ALS. However, environmental factors such as those associated with cognitive reserve have been demonstrated to influence frontotemporal disease neuroanatomy in FTD (Massimo et al. 2018; Massimo et al. 2015; Placek et al. 2016), also when considered in addition to genetic polymorphisms (Premi et al.

2017). Additional study is necessary to examine frontotemporal disease in sporadic ALS relative to both genetic polymorphisms and environmental factors associated with cognitive reserve.

With these caveats in mind, our research demonstrates converging clinical, neuroimaging, and pathologic evidence supporting the hypothesis that the common genetic polymorphism rs12608932 contributes to frontotemporal disease phenotype in sporadic ALS. These findings stimulate investigation into additional genetic contributors to the nature of disease in sporadic ALS, and suggest their importance in prognostic consideration and treatment trials in patients with ALS.

Table 6. Demographic, clinical, and genetic information for neuroimaging and ADNI cohorts.

	ALS	Controls	ADNI Controls
N (F)	109 (44)	113 (52)	84
Age, Y	59.54 (10.96)	61.54 (8.73)	75.44 (6.40)
Education, Y	15.26 (2.99)	15.35 (2.43)	-
Race, N			-
White	100	90	
Black	6	22	
Multi-racial	1	1	
Other	1	-	
Unknown	1	-	
Ethnicity, N			-
Latino	2	1	
Non-Latino	105	111	
Other	1	-	
Unknown	1	1	
Disease duration, M	38.67 (34.25)	-	-
ALSFRS-R	33.81 (7.39)	-	-
Forward digit span, % impaired	6.53 (1.45), 4.54	-	-
Reverse digit span, % impaired	4.21 (1.70), 13.63	-	-
Letter fluency, % impaired	11.29 (5.41), 23.86	-	-
Visual Verbal Test, % impaired	7.8 (4.13), 9.09	-	-
MMSE, % correct, % impaired	91.82 (14.11), 25.00	-	-
rs12608932 genotypes, N			
AA	43	-	41
AC	46	-	34
CC	20	-	9

Abbreviations: Alzheimer's Disease Neuroimaging Initiative – ADNI; ALS Functional Rating Scale-Revised - ALSFRS-R; Mini-Mental State Exam – MMSE

Table 7. Demographic, clinical, and genetic information for autopsy cohort.

	ALS (entire cohort)	ALS (no neuroimaging)
N (F)	102 (40)	81 (29)
Age at Death, Y	65.01 (10.83)	66.21 (10.99)
Race, N		
White	71	
Black	3	
Multi-racial	-	
Other	1	
Unknown	27	
Ethnicity, N		
Latino	2	
Non-Latino	72	
Other	-	
Unknown	28	
Education, Y	15.18 (3.48)	14.76 (3.44)
Disease duration at Death, Y	4.83(4.98)	4.87(5.42)
rs12608932 genotypes, N		
AA	38	30
AC	47	39
CC	17	12

Table 8. Regions of reduced cortical thickness for ALS relative to controls and associated with rs12608932 genotype in ALS.

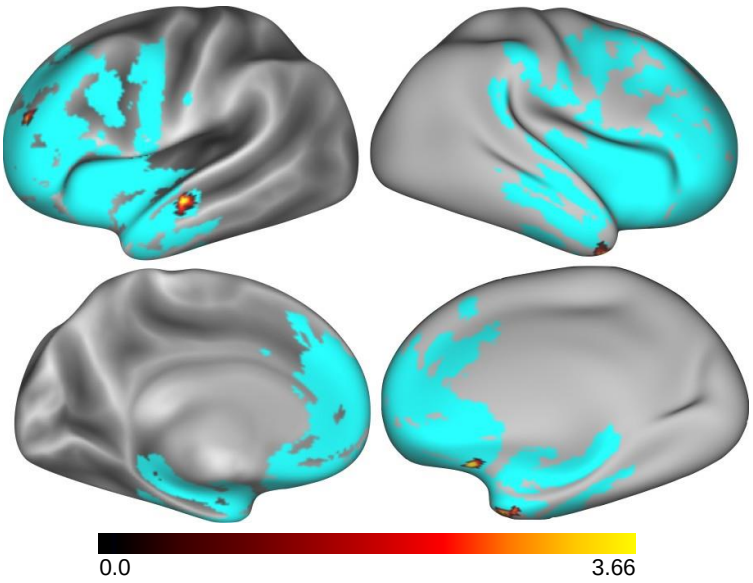
Neuroanatomic region (BA)	L / R	MNI Coordinates x	y	z	*T* statistic	*p* value	Voxels
Reduced cortical thickness in ALS relative to controls[1]:							**25627**
Insular Cortex (13)	R	40	22	0	6.72	0.0006	
Inferior Frontal Gyrus (45)	L	-32	28	2	6.18	0.0001	
Dorsolateral Prefrontal Cortex (9)	-	0	46	18	6.12	0.0001	
Inferior Frontal Gyrus (45)	R	34	22	8	5.96	0.0007	
Ventromedial Orbital Frontal Cortex (11)	R	12	14	-20	5.91	0.0001	
Ventromedial Orbital Frontal Cortex (11)	L	-12	14	-18	5.79	0.0001	
Middle Temporal Gyrus (21)	R	64	-32	10	3.54	0.004	
Reduced cortical thickness associated with rs12608932 minor allele in ALS: Additive model							
Anterior Temporal Lobe (38)	R	34	10	-40	3.08	0.002	56
Ventromedial Prefrontal Cortex (11)	L	-20	26	-24	3.21	0.001	27
Middle Temporal Gyrus (22)	L	-50	-10	-12	2.88	0.003	24
Dorsal Prefrontal Cortex (10)	L	-28	44	28	3.56	0.001	23
Ventromedial Prefrontal Cortex (11)	R	16	16	-24	3.66	0.001	21
Reduced cortical thickness associated with rs12608932 minor allele in ALS: Recessive model							
Anterior Temporal Lobe (20)	R	30	10	-44	3.50	0.001	238
Middle Temporal Gyrus (21)	L	-50	-10	-14	4.04	0.001	83
Anterior Temporal Lobe (20)	L	-56	-8	-28	3.56	0.001	62
Premotor Cortex (6)	L	-50	2	42	3.90	0.001	52
Dorsolateral Prefrontal Cortex (9)	R	32	48	34	3.23	0.001	44
Primary Motor Cortex (4)	R	48	-12	40	3.07	0.001	39
Superior Temporal Cortex (22)	R	66	-36	10	3.30	0.001	35
Orbital Prefrontal Cortex (11)	R	8	34	-8	2.81	0.001	31
Orbital Prefrontal Cortex (11)	L	-20	30	-24	3.23	0.001	31
Ventromedial Prefrontal Cortex (47)	R	30	22	4	3.45	0.001	30
Hippocampus (54)	R	30	-38	2	2.56	0.003	30
Insula (13)	R	36	-12	2	2.88	0.001	29
Anterior Premotor Cortex (8)	R	36	28	48	4.00	0.001	25
Dorsal Prefrontal Cortex (10)	L	-34	52	16	3.16	0.001	25
Middle Temporal Gyrus (21)	R	50	8	-20	2.97	0.003	25

Abbreviations: BA = Brodmann area; L/R = Left/Right; MNI = Montreal Neurological Institute

Note.[1] Cortical regions identified from peak voxel coordinates in an effort to describe sub-peaks within a larger, contiguous cluster.

Figure 4. Reduced cortical thickness associated with rs12608932 genotype. Analyses were restricted to regions of reduced cortical thickness identified in sporadic ALS (N=109) relative to healthy controls (N= 113) (light blue regions in A and B). A) Patients with sporadic ALS who are carriers of the minor allele (C) exhibited greater reduction of cortical thickness in dorsal prefrontal, ventromedial prefrontal, anterior temporal, and middle temporal cortices (regions indicated in red-yellow heatmap). B) Patients with sporadic ALS who are homozygous (CC) or heterozygous (AC) for the minor allele exhibited greater reduction of cortical thickness in the frontal and temporal cortices (regions indicated in red-yellow heatmap). Heatmaps indicate the associated T-statistic for each voxel, with yellow representing the highest value.

A



B

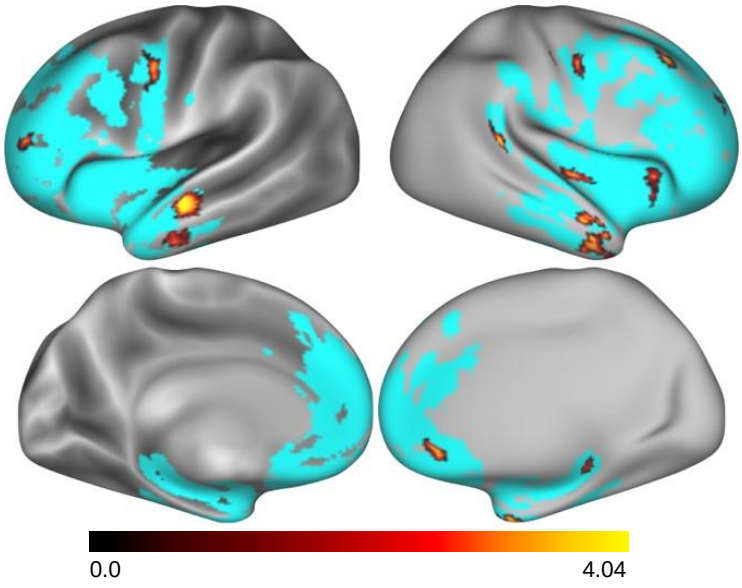
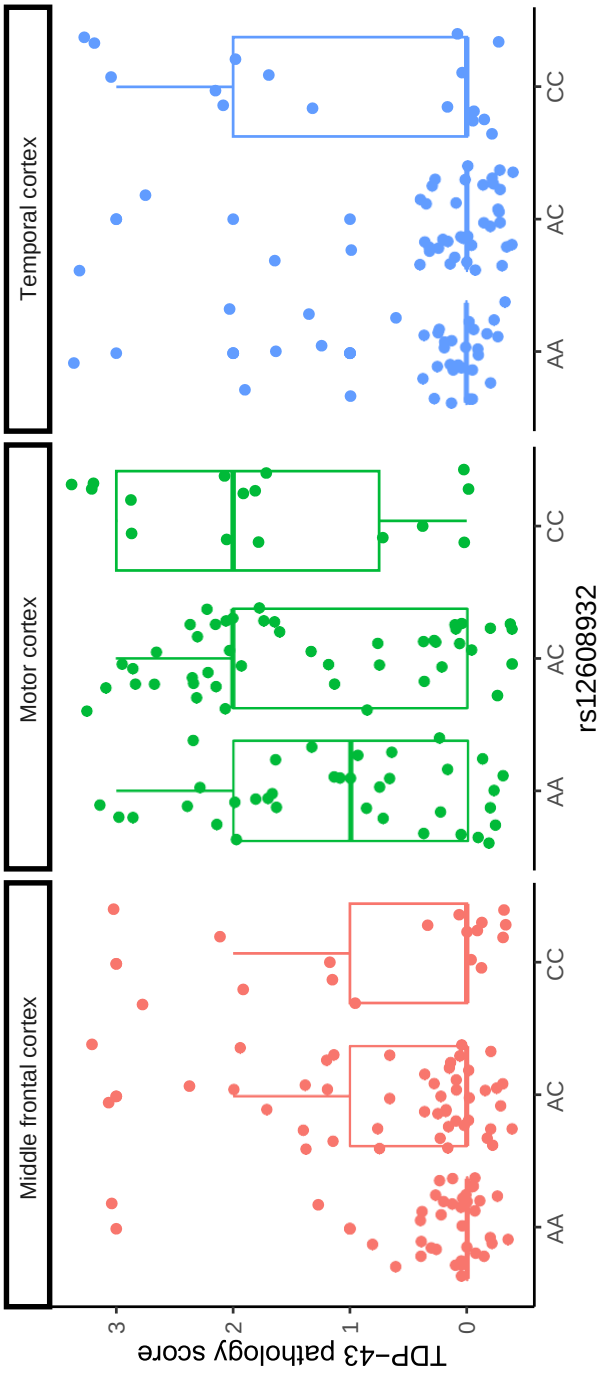


Figure 5. Greater burden of pTDP-43 pathology associated with rs12608932 genotype. Box and whisker plots of pTDP-43 pathologic burden in our sporadic ALS autopsy cohort (N=102) show that minor allele homozygotes (CC) and heterozygotes (AC) feature greater burden of pathology in the middle frontal cortex. Minor allele homozygotes but not minor allele heterozygotes also feature greater burden of pathology in the superior/middle temporal cortex and motor cortex.



CHAPTER 4

Machine learning suggests polygenic contribution to cognitive dysfunction in amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a multi-system disease characterized primarily by progressive muscle weakness. Cognitive/behavioral dysfunction is observed in as many as 50% of patients, however factors influencing risk for cognitive dysfunction remain elusive. Using sparse canonical correlation analysis (sCCA), an unsupervised machine-learning technique, we observed that single nucleotide polymorphisms collectively associate with baseline cognitive performance in 327 ALS patients from the multicenter Clinical Research in ALS and Related Disorders for Therapeutic Development (CReATe) consortium. We demonstrate that a polygenic risk score derived using sCCA relates to longitudinal cognitive decline in the same cohort, and also to *in vivo* cortical thinning in the orbital frontal cortex, anterior cingulate cortex, lateral temporal cortex, premotor cortex, and hippocampus (N=114); as well as *post mortem* motor cortical neuronal loss (N=88) in independent ALS cohorts from the University of Pennsylvania Integrated Neurodegenerative Disease Biobank. Our

findings suggest that common genetic polymorphisms may exert a polygenic contribution to the risk of cortical disease vulnerability and cognitive dysfunction in ALS.

Introduction

As many as half of patients with amyotrophic lateral sclerosis (ALS) manifest progressive decline in cognition consistent with extra-motor frontal and temporal lobe neurodegeneration, including 14% also diagnosed with frontotemporal dementia (FTD) (Montuschi et al. 2015; Beeldman et al. 2016). Comorbid cognitive dysfunction is a marker of poorer prognosis in this fatal disease and confers risk for more rapid functional decline, shorter survival, and greater caregiver burden (Elamin et al. 2013; Crockford et al. 2018; Hu et al. 2013; Caga et al. 2019). While linkage analysis and genome-wide association studies (GWAS) have identified rare causal mutations (DeJesus-Hernandez et al. 2011; Renton et al. 2011; Van Deerlin et al. 2008; Freischmidt et al. 2015) and common risk loci (van Rheenen et al. 2016; Nicolas et al. 2018; van Es et al. 2009; Diekstra et al. 2014; Karch et al. 2018) linking ALS and FTD, whether and how identified variants relate to phenotypic heterogeneity, including in cognition, remain largely unexplored.

The genetic landscape of ALS is largely characterized by ‘apparently sporadic’ disease occurring in 90% of patients with neither a known family disease history nor an identifiable pathogenic mutation (Turner et al. 2017). Population-based studies estimate that only 5-10% of non-familial and 40-50% of

familial ALS cases can be attributed to known pathogenic mutations (Umoh et al. 2016) (e.g. *C9ORF72* (Renton et al. 2011; DeJesus-Hernandez et al. 2011), *NEK1* (Kenna et al. 2016), *SOD1* (D. R. Rosen et al. 1993)), but GWAS have revealed many loci of common genetic variation that confer risk for ALS and FTD. Indeed, recent evidence, supports a polygenic contribution to disease risk from common genetic variants (McLaughlin et al. 2017; Ciga et al. 2019). These include the largest ALS GWAS to-date which newly identified risk variants in the *KIF5A* gene (Nicolas et al. 2018), and genome-wide conjunction and conditional false discovery rate (FDR) analyses demonstrating shared genetic contributions between ALS and FTD from common single nucleotide polymorphisms (SNPs) at known and novel loci (Karch et al. 2018).

An accumulating body of research suggests that SNPs associated with risk of ALS and FTD demonstrate quantitative trait modification of patient phenotype. For example, a SNP in identified as a risk locus for ALS and FTD was found to contribute to cognitive decline, *in vivo* cortical degeneration in the prefrontal and temporal cortices, and *post mortem* pathologic burden of hyperphosphorylated TAR-DNA binding protein [43 kDa] (TDP-43) in the middle frontal, temporal, and motor cortices (Placek et al. 2019). Another SNP identified as a risk locus for FTD with underlying TDP-43 pathology was additionally associated with cognition in patients with ALS (Vass et al. 2011). Others have recently demonstrated shared polygenic risk between ALS and other traits (e.g. smoking, education) and diseases (e.g. schizophrenia) (Ciga et al. 2019; McLaughlin et al. 2017; Hagenaars et al. 2018), suggesting that a single variant

is unlikely to fully account for observed disease phenotype modification. Presently there are no published studies evaluating polygenic contribution to cognitive dysfunction in ALS.

Here we employed an unsupervised machine-learning approach, sparse canonical correlation analysis (sCCA), to identify and evaluate a potential polygenic contribution to cognitive dysfunction in ALS. Traditional approaches for constructing polygenic scores identify variants associated with disease risk through GWAS in a univariate manner, and then compute the sum of alleles at each identified variant, weighted by their effect sizes. In this study, we used data-driven sCCA to identify polygenic associations with a continuous phenotype of cognitive performance in ALS. This method employs sparsity to select maximally-contributing variants and assigns corresponding weights based on model contribution with minimal *a priori* assumptions. We used sCCA to derive a polygenic risk score for cognitive dysfunction in a large longitudinal cohort of cognitively characterized patients with ALS or a related disorder participating in the Phenotype-Genotype-Biomarker (PGB) study of the Clinical Research in ALS and Related Disorders for Therapeutic Development (CReATe) consortium. We then evaluated independent neuroimaging and autopsy cohorts of patients with ALS from the University of Pennsylvania Integrated Neurodegenerative Disease Biobank (UPenn Biobank) (Toledo et al. 2014) to evaluate whether polygenic risk for cognitive dysfunction also relates to *in vivo* cortical neurodegeneration and *ex vivo* cortical neuronal loss and TDP-43 pathology. We focused our investigation on SNPs achieving genome-wide significance in the largest published ALS

GWAS (Nicolas et al. 2018), and SNPs identified as shared risk loci for both ALS and FTD (Karch et al. 2018). We hypothesized that a sparse multivariate approach would reveal a subset of genetic loci associated with cognitive dysfunction profiles in ALS in a polygenic manner, and that follow-up analyses in independent neuroimaging and autopsy cohorts would converge to characterize quantitative traits associated with polygenic risk from identified loci.

Results

Heterogeneity of baseline cognitive and motor phenotype in ALS patients. Smaller-scale studies have shown that ALS patients have impairments in executive, verbal fluency, and language domains, but with relative sparing of memory and visuospatial function (Crockford et al. 2018). The Edinburgh Cognitive and Behavioral ALS Screen (ECAS) was developed to measure cognitive function minimally confounded by motor disability and includes an "ALS-Specific" score that captures impairments in language, executive function, and verbal fluency domains that are frequently observed in ALS patients, and an "ALS-Non-Specific" score that captures less frequently observed impairments in memory and visuospatial function, in addition to overall performance (ECAS Total score) (Abrahams et al. 2014). To measure the extent of heterogeneity in cognitive dysfunction, we evaluated 327 patients with ALS or a related disorder (e.g., ALS-FTD, primary lateral sclerosis (PLS), progressive muscular atrophy (PMA)) participating in the PGB study of the CReATe consortium (NCT02327845) (Table 9). We used linear mixed-effects (LME) to model

variability between individuals in baseline performance and rate of decline on the ECAS (Total Score, ALS-Specific Score, ALS-Non-Specific Score, and scores for each individual cognitive domain), and on clinical measures of physical disability on the ALS Functional Rating Scale – Revised (ALSFRS-R), and clinician ratings of upper motor neuron (UMN) and lower motor neuron (LMN) symptom severity; each model included covariate adjustment for potential confounders including age, education, bulbar onset, and disease duration. We confirmed that cognitive and motor performance at baseline are heterogeneous across individuals (Figure 6A), and correlation analyses suggested that this is independent of disability in physical function or clinical burden of UMN/LMN signs (all $R<0.2$; Figure 6B). Together this establishes the heterogeneity of baseline and longitudinal cognitive and motor phenotypes within the PGB cohort.

Multivariate analyses indicate polygenic contributions to baseline cognitive performance.

To identify potential polygenic contributions to cognitive impairment in ALS we employed sCCA (Witten, Tibshirani, and Hastie 2009), an unsupervised machine-learning approach enabling identification of multivariate relationships between a dataset of one modality (e.g. genetic variables including allele dosage of SNPs) and another modality (e.g. clinical measures of motor and cognitive function). Traditional CCA identifies a linear combination of all variables that maximize the correlation between datasets, resulting in an association of variables from one dataset (e.g., SNPs) and variables from another dataset (e.g.,

clinical scores) (Witten, Tibshirani, and Hastie 2009). The “sparse” component of sCCA additionally incorporates an L1 penalty that shrinks the absolute value of the magnitude of coefficients to yield sparse models (i.e. models with fewer variables) such that some coefficients are zero, and the variables associated with them are effectively eliminated from the model. As a result, variables that contribute little variance to the model are dropped and instead of a linear combination of all model variables, we are able to identify a data-driven subset of variables from one dataset that relate to a subset of variables from another dataset. Unstandardized regression coefficients resulting from sCCA serve as canonical weights indicating the direction and strength of the relationships between selected variables.

We evaluated an allele-dosage dataset comprised of 33 SNPs identified as shared risk loci for both ALS and FTD (Karch et al. 2018), and 12 SNPs identified as risk loci for ALS from the largest published case-control GWAS (Nicolas et al. 2018), with the latter chosen to include loci associated with ALS but not specifically with FTD (Figure 6C; Table 11). We included the first two principle components from a PCA and binary variables for sex, *C9ORF72* repeat expansion status, and other mutation status (e.g. *SOD1*) in this dataset to account for inter-individual genetic differences in population structure, sex, and mutation status. We then used sCCA to examine the association between this genetic dataset and a dataset comprised of adjusted baseline clinical performance on functional motor and cognitive measures extracted from LME models.

After optimizing model sparsity parameters (Figure 7), we ran sCCA 10,000 times and employed random bootstrapped subsamples of 75% of participants in each iteration (Figure 8). We then calculated the median canonical correlation, the median canonical weight for each genetic variable, and the proportion of times each clinical feature was chosen out of 10,000 iterations as a percentage. We report percentages rather than median canonical weight for clinical features because the optimized L1 parameter for the clinical dataset was the most stringent (i.e. 0.1), thus resulting in only one variable from the clinical dataset being chosen in each of the 10,000 iterations.

To assess model performance under the null hypothesis (no association between genetic factors and clinical phenotypes), we similarly ran 10,000 bootstrap sCCAs using the same L1 and subsampling parameters and in each model iteration we randomly permuted each dataset 100 times. We also examined the proportion of times each variable was selected by this null model (i.e. achieving a non-zero canonical weight), and defined a *p* value for the true, unpermuted model by calculating the probability of observing a canonical correlation greater than or equal to the median canonical correlation under sCCA modeling of the true data relative to the canonical correlations observed under null sCCA modeling of randomly permuted data.

We observed that a subset of 29 genetic variables were correlated with a single clinical feature, achieving a median canonical correlation between the two datasets of R=0.35 (95% Confidence Interval: 0.23, 0.42); p=0.019) (Figure 9, Figure 10). Over the 10,000 iterations, the most frequently selected clinical

variable was the ECAS ALS-Specific score (percentage of times selected: 37%), followed by the ECAS Total (29%), Executive Function (17%), Language (9.5%), Verbal Fluency (2.3%), ALS-Specific (2.2%), Memory (2%), and Visuospatial (0.34%) scores. The ALSFRS-R and UMN and LMN assessments were each selected in less than 0.05% of the model iterations. By contrast, performance of sCCA modeling under the null hypothesis demonstrated that each clinical variable was selected in a largely equal proportion of iterations (all variables ranging 5.9% to 9.4%), demonstrating that the true sCCA modeling selected cognitive and not motor features beyond what would be expected by chance (Figure 11A).

Of the 29 selected genetic variables, the 12 most highly weighted were rs1768208 and rs9820623 (*MOBP*), rs7224296 (*NSF*), rs538622 (*ERGIC1*), rs10143310 (*ATXN3*), rs6603044 (*BTBD1*), rs4239633 (*UNC13A*), rs2068667 (*NFASC*), rs10488631 (*TNPO3*), rs11185393 (*AMY1A*), rs3828599 (*GPX3*), and sex. Twenty-seven of the 29 genetic variables selected were SNPs, and 85% of model-selected SNPs (23/27) were shared risk loci for ALS and FTD (Karch et al. 2018). Modeling under the null revealed that each genetic variable achieved a largely equal median weight, and thus there were no stronger model contributions from any subset of genetic variables (Figure 11B). The association of genetic variants most frequently with the ECAS ALS-Specific score suggests polygenic contribution to impairment in domains of cognition frequently impaired in patients with ALS (e.g. language, verbal fluency, and executive function) that are also the most impaired domains of cognition observed in FTD.

Polygenic score captures baseline cognition as well as longitudinal rate of cognitive decline, but not motor decline.

Next we investigated potential polygenic contributions to rate of decline in motor and cognitive performance in the CReATe PGB cohort. Investigation of baseline performance may only capture differences in cognitive impairment at a single (somewhat arbitrary) point in time, but not differences in the trajectory of cognitive decline over time.

To evaluate association with longitudinal decline, we first calculated a weighted polygenic score (wPGS) by computing a weighted sum of allele dosage for each individual multiplied by the median canonical weights for each genetic variant resulting from the prior sCCA. Spearman rank-order correlations between the wPGS and adjusted baseline estimates of the 4 clinical features selected in 10% or more of the 10,000 iterations (e.g. ECAS ALS-Specific, Total, Executive Function, and Language scores) resulted in correlation values similar to the canonical correlation observed from sCCA (e.g. for ECAS ALS-Specific: $rs(329)$=-0.34, p=2.4×10^{-10}) (Figure 12A), suggesting construct validity.

We then conducted Spearman's rank order correlations between the wPGS and adjusted rate of decline on each measure of cognitive and motor performance using a Bonferroni family-wise error correction. To obtain adjusted rates of decline, we extracted individual slope estimates from prior LME (see above) for the 277 individuals (85%) with 2 or more observations on the ECAS, ALSFRS-R, and UMN and LMN assessments. We observed significant negative

relationships between the wPGS and rate of decline on ECAS ALS-Specific ($rs(277)=-0.21$, $p=5.3\times10^{-3}$), ALS-NonSpecific ($rs(277)=-0.19$, $p=0.016$), and Total scores ($rs(277)=-0.26$, $p=8.1\times10^{-5}$) (Figure 12B), but not on the ALSFRS-R or UMN and LMN scores (all p >0.9). These findings suggest polygenic contribution to rate of cognitive – but not motor – decline from the SNPs associated with risk of ALS or joint risk of ALS and FTD that were included in this analysis.

Polygenic score associates with cortical thinning in the UPenn Biobank.

Cognitive dysfunction in ALS, including performance on the ECAS, has previously been attributed to sequential disease progression rostrally and caudally from the motor cortex (Lulé et al. 2018; Agosta et al. 2016; Müller and Kassubek 2018) and advancing disease stage (Crockford et al. 2018). To evaluate the neuroanatomic basis for polygenic contribution to cognitive performance in patients with ALS, we applied the wPGS score derived in the CReATe PGB Cohort to an independent cohort of patients with ALS from the UPenn Biobank. We used voxel-wise *in vivo* measures of reduced cortical thickness (in mm^3) in ALS patients. Cross-sectional measurements of cortical thickness were derived from T1-weighted magnetic resonance imaging (MRI) in 114 patients with ALS and 114 age, sex, and education-matched healthy controls who were recruited for research from UPenn (Table 10A). Nonparametric modeling using 10,000 random permutations revealed extensive reduction of cortical thickness bilaterally in the frontal and temporal cortices of patients with

ALS relative to healthy controls (Table 12, Figure 13A).

After identifying regions of reduced cortical thickness in patients with ALS, we investigated whether the wPGS derived from sCCA modeling in the CReATe PGB cohort contributed to magnitude of reduced cortical thickness in the independent UPenn Biobank neuroimaging cohort. Nonparametric modeling using 10,000 random permutations with adjustments for potential confounds in age, disease duration, and scanning acquisition revealed that a higher wPGS (i.e. greater risk) associated with reduced cortical thickness in the orbital prefrontal cortex, anterior cingulate cortex, premotor cortex, lateral temporal cortex, and hippocampus (Figure 13B, Table 12). Identified frontal and temporal lobe cortical regions are known to support the domains of cognitive dysfunction characterized by the ECAS (Lulé et al. 2018). These findings provide a potential neuroanatomical basis for observed polygenic relationships between the wPGS and baseline cognitive performance and rate of decline, and are consistent with prior associations of cortical neurodegeneration with cognitive dysfunction in patients with ALS (Agosta et al. 2016).

Polygenic score associates with neocortical neuronal loss in the UPenn Biobank. To complement these *in vivo* neuroanatomical data, we also explored whether polygenic risk for cognitive dysfunction associated with *post-mortem* anatomical distribution of neuronal loss and TDP-43 pathology. We assessed the magnitude of neuronal loss and TDP-43 pathological inclusions on an ordinal scale in tissue sampled from the middle frontal, cingulate, motor, and superior / middle temporal

cortices in 88 autopsy cases with confirmed ALS due to underlying TDP-43 pathology (Table 10B). We conducted ordinal logistic regression with covariate adjustment for age at death and disease duration and found that ALS cases with higher wPGS were 2.05 times more likely (95% CI: 1.05, 4.10; p=0.0043) to have greater neuronal loss in the motor cortex relative to ALS cases with a lower wPGS (Figure 13C); older age at death and longer disease duration were not found to influence likelihood of greater neuronal loss (both p>0.05). We observed no statistically significant associations between the wPGS and neuronal loss in any other region, or between the wPGS and TDP-43 pathology in any other region (all p values >.1). These findings suggest that polygenic risk for cognitive dysfunction is associated with the neuroanatomic distribution of neuronal loss in ALS cases with end-stage disease.

Discussion

In this study, we evaluated polygenic contributions to cognitive dysfunction in patients with ALS by employing machine learning. We identified polygenic risk for cognitive dysfunction from genetic variables associated with risk of ALS and FTD, which we further investigated through quantitative-trait evaluations of two independent ALS cohorts with *in vivo* neuroimaging and *post-mortem* neuropathology data. Our results indicate a polygenic contribution to the presence and rate of decline of cognitive dysfunction in domains specifically impaired in ALS. Converging evidence from independent cohorts further demonstrates the generalizability of polygenic contribution to biologically-

plausible associations including reduced *in vivo* cortical thickness and *post-mortem* cortical neurodegeneration, including in the prefrontal and temporal cortices. These findings contribute novel evidence in support of polygenic contribution to cognitive dysfunction in ALS, quantitative anatomic characterization of identified polygenic risk associated with cognitive dysfunction, and further detailed phenotypic evidence for genetic overlap between ALS and FTD. Below, we highlight clinical, biological, and methodological implications for our observations.

Our findings add to an increasing body of evidence for genetic contribution to phenotypic variability in ALS, and support the idea that polygenic variation accounts for, at least a portion, of variability in cognitive dysfunction and cortical disease burden in ALS. While cognitive impairment has been more frequently linked to genetic mutations causally associated with ALS, such as *C9ORF72* repeat expansions (Byrne et al. 2012), studies examining individual variants have implicated SNPs as risk factors for ALS and/or FTD (Placek et al. 2019; Vass et al. 2011). However, mounting evidence suggests that there are polygenic, rather than single allele, modifiers of disease risk and phenotype in ALS and related neurodegenerative diseases (Hagenaars et al. 2018; McLaughlin et al. 2017; Ciga et al. 2019). Our observation of 27 SNPs collectively associated most frequently with the ECAS ALS-Specific score, a combined measure of executive, language, and verbal fluency domains most commonly affected in ALS, is consistent with the idea of polygenic contribution to phenotypic variability in ALS. Notably, our observed polygenic association in the CReATe PGB Cohort appears

specific to cognitive variability: we demonstrate relative independence of cognitive performance and motor disease severity (i.e. UMN or LMN symptom assessments, functional performance on the ALSFRS-R) and observe no evidence for polygenic association with motor disease severity. This suggests that, in this study, polygenic risk for cognitive dysfunction does not appear to be confounded by motor disease severity.

Of the 27 identified SNPs, the majority (85%) are shared risk loci for ALS and FTD (Karch et al. 2018). SNPs in or near the *MOBP, NSF, ATXN3, ERGIC1,* and *UNC13A* genes were among those with the strongest model contributions (i.e. with the highest canonical weights). Relative to random permutations, the frequency of the selection of these ALS and FTD loci outweighed their selection by chance and outweighed the selection of ALS-only risk genotypes, emphasizing the relative contribution of polygenic overlap between ALS and FTD. Furthermore, these results suggest a conceptual distinction between genetic risk for disease and genetic risk for phenotypic differences within a disease. Two of these loci in or near the *MOBP* gene (rs9820623, rs1768208) were amongst the most heavily weighted. Our group has previously shown that SNPs mapped to *MOBP*, including rs1768208, relate to regional neurodegeneration in patients with sporadic forms of FTD and shorter survival in FTD patients with underlying tau or TDP-43 pathology (Irwin, McMillan, et al. 2014; McMillan et al. 2014). Our group has also demonstrated an additive dose-response relationship between the minor allele of rs12608932 (D') in *UNC13A* and *in vivo* cortical thinning in the dorsal prefrontal cortex, greater burden of

TDP-43 pathology in the middle frontal and motor cortices at autopsy, and worse performance on a measure of working memory associated with executive function (Placek et al. 2019). rs538622 near *ERGIC1*, originally identified through conditional FDR as a shared risk locus for ALS and FTD, has also been previously demonstrated to contribute to quantitative trait modification in ALS by relating to reduced expression of the protein BNIP1 in ALS patient motor neurons (Karch et al. 2018). Other top-weighted variants near *NSF* and *ATXN3* indicate potential biological plausibility: rs10143310 is found near *ATXN3* which encodes a de-ubiquitinating enzyme, and polyglutamine expansions in *ATXN3* cause spinocerebellar ataxia – type 3 (Burnett, Li, and Pittman 2003). rs7224296 near *NSF* tags the *MAPT* H1 haplotype (Yokoyama et al. 2017) and is associated with increased risk for FTD syndromes including progressive supranuclear palsy and corticobasal degeneration (Ferrari et al. 2017), as well as Alzheimer's and Parkinson's diseases (Desikan et al. 2015).

While the mechanism of polygenic contribution to cognitive dysfunction in ALS requires further investigation, we speculate based on our findings that identified SNPs may contribute to neuroanatomic disease burden. A weighted polygenic risk score derived from the observed multivariate genotype-phenotype correlation in the CReATe PGB cohort showed robust relationships in independent validation cohorts to both *in vivo* cortical thinning and *post-mortem* cortical neuronal loss. Anatomically, these findings were largely consistent with prior *in vivo* structural imaging studies of neurodegeneration associated with cognitive dysfunction and with *ex vivo* investigations of cortical thinning in ALS

(Lulé et al. 2018; Agosta et al. 2016; Prudlo et al. 2016). Thus, in addition to indicating polygenic contribution to cognitive dysfunction in ALS, our findings suggest a possible mechanism of observed findings via disease pathophysiology. Beyond the potential biological mechanism of identifying polygenic contributions to ALS disease heterogeneity, we additionally suggest that sCCA may provide a tool for defining polygenic factors of disease risk. While sCCA has been widely applied to imaging-genetic studies (Parkhomenko, Tritchler, and Beyene 2009), we are unaware of prior applications using sCCA to define and polygenic score based on rich clinical phenotypic and biomarker data. Traditional approaches to the generation of polygenic scores include using data from established, typically case-control GWAS, but practical considerations involve the selection of how many variants to include in a model and how to define the weights of an appropriate statistical model (Sugrue and Desikan 2019). Critically, rather than an arbitrary selection of variants and their weights, the sparsity parameter of sCCA facilitates an unsupervised, data-driven method to select the number of variants to include in the model and the canonical correlation provides data-driven weights to define the statistical model. The positive or negative direction of model-derived weights is potentially biologically informative, and could reflect ‘risk’ (i.e. positive weight) or ‘protective’ (i.e. a negative weight) effects. Further investigation is necessary to clarify the relationships between model-selected SNPs and model-derived canonical weight from both biological (e.g., some SNPs and/or genes may contribute more strongly to risk factors) and mathematical (e.g. weights may be constrained by minor allele frequency)

perspectives. Nonetheless, sCCA may provide an optimal method for future studies of polygenic variation and direct research efforts towards model-selected variants.

Several limitations should be considered in the present study. Here, we focus our analysis on a hypothesized relatively small set of SNPs selected *a priori* from previous large-scale GWAS based on genome-wide association with ALS (Nicolas et al. 2018) or shared risk between ALS and FTD (Karch et al. 2018). Other genetic variants not included in the present study may also contribute to cognitive dysfunction in ALS and related disorders, and future genome-wide or more broad genotype selection strategies (e.g., targeted pathways) are necessary to elucidate discovery of novel genetic contributions to cognition that have not been identified through prior case-control studies. However, since our focused SNP selection targets previously validated genotypes from GWAS studies, these larger scale studies necessitate further validation in independent cohorts, many of which are lacking the rich phenotype data needed to identify cognitive dysfunction. We derived a weighted polygenic score from bootstrap sCCA modeling to further investigate polygenic associations with longitudinal clinical and cognitive performance, and to investigate polygenic associations with *in vivo* and *post-mortem* disease neuroanatomy in independent ALS cohorts from the UPenn Biobank. While we define our polygenic score from sCCA using adjusted estimates of baseline cognitive and motor performance, future work using longitudinal data as the starting point to define polygenic associations may further elucidate genetic risk

for cognitive dysfunction in ALS. However, our finding that polygenic risk associated with baseline cognitive dysfunction also relates to longitudinal cognitive decline in the PGB cohort and relevant disease anatomy in independent cohorts suggests its relevance to longitudinal cognitive phenotypes in ALS. Previous critique of polygenic scores suggest calculation based on GWAS-defined odds ratios for univariate risk loci or undue influence by population variance limit their use in clinical and prognostic settings (Wald and Old 2019). To avoid these potential confounds, our computation of a weighted polygenic risk score is based on model-selected parameters derived from an analysis including all genetic variants and covariates for genetic mutation status and sex in an effort to account for multivariate genetic relationships. We also included the first two principal components in our model from a PCA conducted in the PGB CReATe cohort in an effort to account for differences in population heterogeneity (Price et al. 2006).

Our analyses focused on the investigation of genetic contribution to cognitive dysfunction in ALS, yet it is well established that behavioral impairment is also part of the ALS spectrum disease (Lillo et al. 2010). Further research is necessary to investigate polygenic risk for behavioral dysfunction in ALS, and whether loci included in our calculated polygenic score confer risk for both cognitive and behavioral dysfunction. While this study demonstrates converging, multimodal evidence for polygenic risk in independent neuroimaging and autopsy cohorts, replication in additional, large cohorts that allow for robust cross-validation is warranted, however, alternative datasets that contain detailed

genetic and cognitive phenotyping for ALS are currently lacking, and the CReATe PGB cohort represents the largest of its kind. Future research investigating additional, large-scale patient cohorts with genetic and phenotypic data is necessary.

With these limitations in mind, our research demonstrates converging clinical, neuroimaging, and pathologic evidence for polygenic contribution to cognitive dysfunction and cortical neurodegeneration in ALS. These findings should stimulate further investigation into polygenic risk for cognitive disease vulnerability in ALS and suggest their importance in prognostic consideration and treatment trials. More broadly, this work provides insight into genetic contribution to heterogeneous phenotypes in neurodegenerative disease and supports evidence for polygenic architecture in these conditions.

Methods

Participants: CReATe Consortium

Participants consisted of 339 individuals clinically diagnosed by a board-certified neurologist with a sporadic or familial form of amyotrophic lateral sclerosis (ALS), amyotrophic lateral sclerosis with frontotemporal dementia (ALS-FTD), progressive muscular atrophy (PMA), or primary lateral sclerosis (PLS) who were enrolled and evaluated through the CReATe Consortium's Phenotype-Genotype-Biomarker (PGB) study. All participants provided written informed consent. The PGB study is registered on clinicaltrials.gov (NCT02327845) and the University of Miami IRB (the central IRB for the CReATe Consortium) approved the study.

This study entails participant blood DNA samples available for genetic screening and longitudinal evaluation at regularly-scheduled visits (ALS, ALS-FTD, and PMA: 0 (baseline), 3, 6, 12, and 18 months; PLS: 0 (baseline), 6, 12, 18, and 24 months). Participants were evaluated at each visit using the ALSFRS-R (Cedarbaum, Stambler, Malta, Fuller, Hilt, Thurmond, and Nakanishi 1999b) and alternate versions of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (Abrahams et al. 2014). Upper motor neuron (UMN) and lower motor neuron (LMN) burden scores were calculated from a detailed elemental neuromuscular examination by summing within and across each spinal region resulting in a score ranging from 0 (none) to 10 (worst). Site (e.g. limb, bulbar) and date of motor symptom onset were recorded for each participant. We excluded nine individuals with missing or incomplete data that precluded subsequent analysis and, in an effort to avoid confounds associated with clear outliers, three individuals with extreme values at baseline on the ECAS Visuospatial Score (i.e. >5 standard deviations from group mean), resulting in a total of 327 participants. Of the nine excluded individuals with missing or incomplete data, one had no genotyping data available, one had no information for UMN burden, and seven had no information for date of motor symptom onset.

Genotyping: CReATe Consortium

Peripheral blood mononuclear cell DNA was extracted using the QIAamp DNA Blood Mini Kit Qiagen #51106 and quantified using the Quant-iT dsDNA Assay Kit (Life Technologies cat#Q33130). The DNA integrity was verified by agarose

gel electrophoresis (E-Gel, Life Technologies, cat#G8008-01). Unique samples were barcoded and whole genome sequencing (WGS) was performed at the HudsonAlpha Institute for Biotechnology Genomic Services Laboratory (Huntsville, Alabama) (HA) using Illumina HiSeq X10 sequencers to generate approximately 360 million paired-end reads, each 150 base pairs (bp) in length. Peripheral DNA was extracted from participant blood samples and screened for known pathogenic mutations associated with ALS and related diseases.

Screening included repeat-primed PCR for *C9ORF72* repeat expansions and WGS curated and validated via Sanger sequencing for pathogenic mutations associated with ALS and/or FTD in *ANG, CHCHD10, CHMP2B, FUS, GRN, hnRNPA1, hnRNPA2B1, MAPT, MATR3, OPTN, PFN1, SETX, SOD1, SPG11, SQSTM1, TARDBP, TBK1, TUBA4A, UBQLN2, VCP*. The PGB study also includes patients with hereditary spastic paraplegia (HSP) that were excluded in the current analysis, but we additionally screened individuals for pathogenic mutations in 67 additional genes associated with HSP and seven genes associated with distal hereditary motor neuropathy.

Whole genome sequencing (WGS) data were generated using paired-end 150 bp reads aligned to the GRCh38 human reference using the Burrows-Wheeler Aligner (BWA-ALN v0.7.12)(Heng Li and Durbin 2010) and processed using the Genome Analysis Toolkit (GATK) best-practices workflow implemented in GATK v3.4.0 (McKenna et al. 2010). Variants for individual samples were called with HaplotypeCaller, producing individual variant call format files (gVCFs) that we combined using a joint genotyping step to produce a multi-sample VCF

(pVCF). Variant filtration was performed using Variant Quality Score Recalibration (VQSR), which assigns a score to each variant and a pass/fail label and evaluated this in the context of hard filtering thresholds (Minimum Genotype Quality (GQ)≥ 20, minimum mean depth value (DP)≥ 10). Variant annotation was performed using Variant Effect Predictor (VEP) (Hunt et al. 2018) and in-house pipelines including non-coding variant allele frequencies from Genome Aggregation Database (gnomAD).(Karczewski et al. 2019) In-house scripts were used to identify false positives resulting from paralogous mapping or/and gaps in the current human genome assembly. VCFs were further decomposed prior to analyses using the Decompose function of Vt (Tan, Abecasis, and Kang 2015).

To control for population substructure, we additionally derived the first two principal components scores for each in the CReATe PBG cohort using principal components analysis (PCA) as implemented in Eigenstrat software (Price et al. 2006).

From the WGS data we extracted 45 hypothesized variants from WGS that previously achieved genome-wide significance for association with ALS (Nicolas et al. 2018) or joint association with ALS and FTD via GWAS (Karch et al. 2018). Proxy loci were genotyped (linkage disequilibrium (LD) $R^2 > 0.80$) when genetic data were not available for previously-published loci. One locus, rs12973192, was common to both references, and another locus (rs2425220 (Karch et al. 2018)) was excluded from analysis due to high level of missingness across samples; no LD proxy was identified. We then used Plink software (Purcell et al. 2007) to recode participant genotypes according to additive genetic

models (e.g. 0 = no minor allele copies, 1 = one minor allele copy, 2 = two minor allele copies), since the dominant or recessive nature of the loci included in this study remains unknown.

Linear Mixed-Effects Modeling of the ECAS and clinical measures

We conducted linear mixed-effects modeling of performance on the ECAS, ALSFRS-R, and UMN and LMN scores using the *nlme* package in R. Each model was fit using maximum likelihood. In addition to the ECAS Total Score, we analyzed Executive Function, Language, Verbal Fluency, Memory, and Visuospatial scores and summary scores (ALS-Specific score, ALS-Non-Specific score) each as dependent variables to analyze patient performance in separate cognitive domains and in clinically-grouped cognitive domains. Fixed effects included age at baseline visit (in years), lag between age of symptom onset and age at baseline visit (in years), college education (yes / no), bulbar onset (yes / no) and visit time-point (in months), and we included individual by visit time-point as random effects. This allowed us to obtain adjusted estimates of baseline performance (i.e. intercept) and rate of decline (i.e. slope) per individual, having regressed out potential confounding variables as fixed effects.

We conducted Spearman's rank-order correlations between baseline performance and rate of decline using a Bonferroni family-wise error correction for multiple comparisons.

Sparse Canonical Correlation Analysis

We next conducted sparse canonical correlation analysis (sCCA) to select a parsimonious linear combination of variables that maximize the correlation between two multivariate datasets using the *PMA* package in R (Witten, Tibshirani, and Hastie 2009). The first dataset comprised scaled intercepts from each clinical variable per participant (i.e. adjusted baseline performance on the ALSFRS-R, UMN and LMN assessments, and ECAS). The second comprised minor allele counts per individual for each of the 45 SNPs (e.g. 0 = no minor allele copies, 1 = one minor allele copy, 2 = two minor allele copies), binary variables for sex (0 = Female, 1 = Male), *C9ORF72* repeat expansion status (0 = noncarrier, 1 = carrier), and other mutation status (0 = noncarrier, 1 = carrier) and, in an effort to account for potential population differences in population variance we also included the raw estimates for the first two principle components per participant derived from a PCA conducted in the CReATe PGB cohort, which has previously been demonstrated to account for the majority of population structure (Price et al. 2006).

We assumed standard (e.g. unordered) organization of each dataset, and selected regularization parameters for the sCCA analysis using a grid search of 100 combinations of L1 values between 0 (most sparse) and 1 (least sparse) in increments of 0.1. We selected the combination of L1 values yielding the highest canonical correlation of the first variate for subsequent analysis, as similarly reported (Xia et al. 2018).

Using these L1 parameters, we ran 10,000 bootstrap sCCAs and on each iteration employed randomly-generated subsamples comprising 75% of the PGB

cohort. From this, we calculated the median canonical correlation for sCCA and the median canonical weights for each variable. We utilize the median in these estimates rather than the maximum or mean value in an effort to avoid bias from outliers and to increase the reliability and reproducibility of model estimates.

We next investigated model performance under a null hypothesis (i.e. no association between clinical and genetic datasets) by using randomly-permuted data. Using the same L1 parameters, we again ran 10,000 bootstrap sCCAs and on each iteration employed randomly-generated subsamples of 75% of participants; however, on each iteration we randomly permuted each dataset 100 times using the *randomizeMatrix* function from the *picante* package in R. We calculated a *p* value by reporting the probability of observing a canonical correlation greater than or equal to the median canonical correlation under sCCA modeling of the true data relative to the canonical correlations observed under null sCCA modeling of randomly permuted data. We also examined the proportion (i.e. out of 10,000) of times each variable was selected by the model (i.e. achieving a non-zero canonical weight) under true and null modeling.

Polygenic Score

To evaluate the applicability of our sCCA model, we used the output of the model to calculate a weighted polygenic score (wPGS) for each individual by computing a weighted sum of allele dosage across all genotypes for each individual. Weights were derived for each genetic variable by using the median canonical weight over the 10,000 bootstrap sCCAs.

To investigate construct validity, we conducted Spearman's rank-order correlations between the wPGS and adjusted estimates of baseline performance (i.e. LME-derived intercepts) on the most frequently selected clinical measure(s) selected from sCCA.

To investigate longitudinal performance associated with the wPGS, we conducted Spearman's rank-order correlations between the wPGS and adjusted rates of decline (i.e. LME-derived slopes) on all clinical measures using a Bonferroni familywise error correction for multiple comparisons. We restricted this analysis to CReATe participants with data at 2 or more timepoints (N=277 out of 327 participants), or 84.7% of the participant cohort.

Participants: UPenn Biobank neuroimaging cohort

We retrospectively evaluated 114 patients with ALS and 114 healthy controls matched for age, sex, and education from the UPenn Biobank who were recruited for research between 2006 and 2019 from the Penn Comprehensive ALS Clinic and Penn Frontotemporal Degeneration Center (Table 10) (Toledo et al. 2014). Inclusion criteria for ALS patients consisted of complete genotyping at analyzed SNPs, white non-Latino racial and ethnic background (population diversity is known to influence allele frequencies across individuals), disease duration from symptom onset < 2.5 standard deviations from respective group means (to avoid confounds associated with clear outliers), and T1-weighted MRI. All patients were diagnosed with ALS by a board-certified neurologist (L.E., L.M., M.G., D.I) using revised El Escorial criteria (Brooks et al. 2000) and assessed for

ALS frontotemporal spectrum disorder using established criteria (Strong et al. 2017); those patients enrolled in research prior to 2017 were retrospectively evaluated through chart review. All ALS patients and healthy controls participated in an informed consent procedure approved by an Institutional Review Board convened at UPenn.

Participants: UPenn Biobank autopsy cohort

We evaluated brain tissue samples from 88 ALS autopsy cases identified from the UPenn Biobank (Toledo et al. 2014) who were diagnosed by a board-certified neuropathologist (J.Q.T., E.B.L.) with ALS due to TDP-43 pathology using immunohistochemistry (Neumann et al. 2006) and published criteria (Mackenzie et al. 2011); this cohort included 21 patients from the ALS neuroimaging cohort. Inclusion criteria consisted of complete genotyping at analyzed SNPs, white non-Latino racial and ethnic background (population diversity is known to influence allele frequencies across individuals), disease duration from symptom onset < 2.5 standard deviations from respective group means (to avoid confounds associated with clear outliers), and brain tissue samples from the middle frontal, motor, cingulate, and superior / temporal cortices, and the cornu ammonis 1 (CA1) / subiculum of the hippocampus for analysis of neuronal loss and TDP-43 pathology. Nine individuals were missing neuronal loss or TDP-43 pathology data for at least 1 sampled region (Table 13).

Genetic Screening and SNP Genotyping: UPenn Biobank

DNA was extracted from peripheral blood or frozen brain tissue following the manufacturer's protocols (Flexigene (Qiagen) or QuickGene DNA whole blood kit (Autogen) for blood, and QIAsymphony DNA Mini Kit (Qiagen) for brain tissue). All patients were screened for *C9ORF72* hexanucleotide repeat expansions using a modified repeat-primed polymerase-chain reaction (PCR) as previously described (Suh et al. 2015). Of the remaining individuals, we evaluated family history using a 3-generation pedigree history as previously reported (Wood et al. 2013). For cases with a family history of the same disease we sequenced 45 genes previously associated with neurodegenerative disease, including genes known to be associated with ALS (e.g. *SOD1* (D. R. Rosen et al. 1993), *TBK1* (Freischmidt et al. 2015)). Sequencing was performed using a custom-targeted next-generation sequencing panel (MiND-Seq) (Toledo et al. 2014) and analyzed using Mutation Surveyor software (Soft Genetics, State College, PA).

For analyses of UPenn Biobank samples, we genotyped peripheral or brain cerebellum DNA of each case using the Illumina Infinium Global Screening Array through the Children's Hospital of Philadelphia (CHOP) Center for Applied Genomics Core according to manufacturer's specifications. PLINK (Purcell et al. 2007) was then used to remove variants with <95% call rate, Hardy-Weinberg equilibrium (HWE) p-value < 10^{-6} and individuals with >5% missing genotypes. Using the remaining genotypes from samples passing quality control, we performed genome-wide imputation of allele dosages with the Haplotype Reference Consortium reference panel r1.1 (McCarthy et al. 2016) on the Michigan Imputation Server (Das et al. 2016) to predict genotypes at

ungenotyped genomic positions, applying strict pre-phasing, pre-imputation filtering, and variant position and strand alignment control.

Neuroimaging Processing and Analyses

High-resolution T1-weighted MPRAGE structural scans were acquired for neuroimaging participants using a 3T Siemens Tim Trio scanner with an 8-channel head coil, with T=1620ms, T=3.09ms, flip angle=15°, 192x256 matrix, and $1mm^3$ voxels. T1-weighted MRI images were then preprocessed using Advanced Normalization Tools (ANTs) software (Tustison et al. 2014). Each individual dataset was deformed into a standard local template space in a canonical stereotactic coordinate system. ANTs provide a highly accurate registration routine using symmetric and topology-preserving diffeomorphic deformations to minimize bias toward the reference space and to capture the deformation necessary to aggregate images in a common space. Then, we used N4 bias correction to minimize heterogeneity (Tustison et al. 2010) and the ANTs Atropos tool to segment images into six tissue classes (cortex, white matter, cerebrospinal fluid, subcortical grey structures, brainstem, and cerebellum) using template-based priors and to generate probability maps of each tissue. Voxel-wise cortical thickness was measured in millimeters (mm^3) from the pial surface and then transformed into Montreal Neurological Institute (MNI) space, smoothed using a 3 sigma full-width half-maximum Gaussian kernel, and downsampled to 2mm isotropic voxels.

We used *randomise* software from FSL to perform nonparametric,

permutation-based statistical analyses of cortical thickness images from our neuroimaging cohort. Permutation-based statistical testing is robust to concerns regarding multiple comparisons since, rather than a traditional assessment of two sample distributions, this method assesses a true assignment of factors (e.g. wPGS) to cortical thickness relative to many (e.g., 10,000) random assignments (Winkler et al. 2014).

We first identified reduced cortical thickness in ALS patients relative to healthy controls. We constrained analysis using an explicit mask restricted to high probability cortex (>0.4) and report clusters that survive $p<0.05$ threshold-free cluster enhancement (TFCE) (Smith and Nichols 2009) corrected for family-wise error relative to 10,000 random permutations.

We next evaluated whether wPGS relates to magnitude of reduced cortical thickness, covarying for age, disease duration, and scanner acquisition in an effort to control for factors associated with reduced cortical thickness but not specifically associated with polygenic risk. We constrained analysis to an explicit mask including regions of reduced cortical thickness identified relative to healthy controls (see above). We report clusters that survive uncorrected $p<0.01$ with a cluster extent threshold of 10 voxels relative to 10,000 random permutations; we employ an uncorrected threshold to minimize the chance of Type II error (not observing a true result).

Neuropathology Processing and Analyses

Extent of neuronal loss and of phosphorylated TDP-43 intraneuronal inclusions

(dots, wisps, skeins) in sampled regions from the middle frontal, cingulate, motor, and superior / middle temporal cortices, and the CA1 / subiculum of the hippocampus were assessed on a semi-quantitative ordinal scale: 0=none/rare, 1=mild, 2=moderate, 3=severe/numerous. All neuropathological ratings were performed by an expert neuropathologist (J.Q.T., E.B.L.) blinded to patient genotype. We conducted ordinal logistic regression using the *MASS* package in *R* to investigate whether extent of neuronal loss rated using Hematoxylin and eosin (H&E) and burden of TDP-43 pathology rated using mAbs p409/410 or 171 (Lippa et al. 2009; Neumann et al. 2009) immunohistochemistry differed according to wPGS, covarying for age and disease duration.

Table 9: Demographics of ALS patients from the Phenotype-Genotype Biomarker study of the CReATe Consortium.

	ALS	ALS-FTD	PLS	PMA
N	279	13	22	13
Sex, Male (%)	163 (58.4)	11 (84.6)	11 (50.0)	8 (61.5)
Number of Visits, Mean (SD)	3.09 (1.37)	3.00 (1.15)	2.86 (1.28)	3.38 (1.45)
Age at Symptom Onset, Mean (SD)	56.32 (12.56)	64.00 (9.11)	49.68 (7.39)	48.08 (15.31)
Symptom Onset to Baseline (years), Mean (SD)	3.59 (4.98)	3.62 (2.63)	8.45 (6.12)	7.77 (7.17)
Site of Symptom Onset, N (%)				
Bulbar	45 (17.1)	4 (33.3)	5 (22.7)	-
Bulbar & Limb	7 (2.7)	-	3 (13.6)	-
Bulbar & Other	7 (2.7)	1 (8.3)	-	-
Limb	175 (66.5)	3 (25)	13 (59.1)	11 (84.6)
Limb & Other	22 (8.4)	-	1 (4.5)	1 (7.7)
Other	7 (2.7)	4 (33.3)	-	1 (7.7)
College Education or greater, N (%)	196 (71.3)	9 (69.2)	20 (90.9)	10 (76.9)
Mutation Carrier, N (%)	34 (12.2)	3 (20.0)	0 (0.0)	0 (0.0)
C9ORF72	22 (7.9)	3 (20.0)	-	-
C9ORF72 and UBQLN2	1 (0.4)	-	-	-
SOD1	8 (2.9)	-	-	-
SQSTM1	1 (0.4)	-	-	-
TARDBP	1 (0.4)	-	-	-
TBK1	1 (0.4)	-	-	-
Baseline ALSFRS-R (0-48), Mean (SD)	35.00 (7.09)	35.00 (5.99)	36.50 (5.95)	33.62 (7.83)
UMN Score (0-10), Mean (SD)	2.70 (1.68)	2.45 (2.00)	4.54 (1.33)	0.87 (0.73)
LMN Score (0-10), Mean (SD)	2.54 (1.48)	2.81 (1.76)	0.59 (0.96)	4.84 (1.93)
ECAS, Mean (SD)				
ALS Specific (0-100)	80.94 (10.85)	52.62 (12.07)	87.95 (7.47)	81.62 (11.61)
Language (0-28)	25.85 (2.66)	21.38 (3.93)	26.82 (1.97)	26.62 (1.26)
Verbal Fluency (0- 24)	16.62 (5.11)	7.83 (5.36)	26.82 (1.97)	16.77 (4.36)
Executive (0-48)	38.47 (5.94)	24.00(10.51)	26.82 (1.97)	38.23 (7.50)
ALS Non-Specific (0-36)	28.04 (3.78)	19.69 (8.30)	29.73 (2.76)	27.62 (6.31)
Memory (0-24)	16.45 (3.54)	9.46 (7.15)	17.95 (2.84)	15.69 (6.20)
Visuospatial (0- 12)	11.59 (0.79)	11.08 (1.24)	11.77 (0.43)	11.92 (0.28)
ECAS Total (0-136)	108.97 (13.02)	72.31 (18.53)	117.68 (9.12)	109.23 (16.47)

Abbreviations: CReATe = Clinical Research in ALS and Related Disorders for Therapeutic Development; PLS = Primary lateral sclerosis, PMA = Progressive muscular atrophy; ALSFRS-R = Revised ALS Functional Rating Scale; UMN = upper motor neuron; LMN = lower motor neuron; ECAS = Edinburgh Cognitive and Behavioral ALS Screen; SD = standard deviation

Table 10. Demographics of independent neuroimaging and autopsy ALS and control cohorts from UPenn.

A. Neuroimaging Cohort

	ALS	**Healthy Control**
N (Male)	114 (64)	114 (64)
Age at MRI in Years, M (SD)	59.34 (10.92)	61.87 (12.18)
Education in Years, M (SD)	15.09 (2.98)	15.87 (2.47)
Disease Duration in Years, M (SD)	3.02 (2.52)	-
Mutation Carrier, N (%)		
C9ORF72	14 (12.28)	-
SOD1	1 (0.87)	-
VCP	1(0.87)	-
Site of Symptom Onset, N (%)		
Bulbar	26 (22.81)	-
Limb	79 (69.3)	-
Cognitive	9 (7.89)	-
ALSFRS-R, M (SD)	33.23 (7.32)	-

B. Autopsy Cohort

N (Male)	88 (49)
Age at Death Years, M (SD)	63.72 (10.24)
Disease Duration at Death in Years, M (SD)	4.24 (3.41)
Mutation Carrier, N (%)	
C9ORF72	15 (17.04)
Site of Symptom Onset, N (%)	
Bulbar	23
Limb	60
Cognitive	3
Respiratory	1
Unknown	1

Abbreviations: ALSFRS-R = ALS Functional Rating Scale – Revised; M = Mean, SD = standard deviation

Table 11: List of genetic variants analyzed.

Marker Name	Nearest Gene	Chr	1000 Genome GMAF	GRCh38 Position	Proxy Marker	Proxy HG19 Position
rs2068667	*NFASC*	1	0.208	chr1:204948552	rs11240317	chr1:204920322
rs11185393	*AMY1A*	1	0.368	chr1:104209379	rs67205957	chr1:104752258
rs515342	*ASB1*	2	0.214	chr2:238458655	rs508986	chr2:239337691
rs9820623	*MOBP*	3	0.406	chr3:39452367	rs6765697	chr3:39493239
rs13079368	*MOBP*	3	0.275	chr3:39471060	rs1464047	chr3:39526874
rs1768208	*MOBP*	3	0.323	chr3:39481512	rs616147	chr3:39534481
rs10463311	*TNIP1*	5	0.431	chr5:151031274	-	-
rs3828599	*GPX3*	5	0.417	chr5:151022235	rs4958872	chr5:150402334
rs538622	*ERGIC1*	5	0.32	chr5:172920676	rs2446192	chr5:172352369
rs17111695	*NAF1*	5	0.183	chr5:151052885	rs12518386	chr5:150438085
rs757651	*REEP2*	5	0.016	chr5:138455779	rs149312547	chr5:137792021
rs10488631	*TNPO3*	7	0.059	chr7:128954129	rs12539741	chr7:128596805
rs17070492	*LOC101927815*	8	0.208	chr8:2563763	-	-
rs7813314	*BC045738*	8	0.2	chr8:2558274	rs6996532	chr8:2417678
rs10869188	*C9ORF72*	9	0.49	chr9:72614090	rs7032232	chr9:75229116
rs870901	*AK097706*	9	0.133	chr9:107086201	rs60743641	chr9:109854824
rs10511816	*MOBKL2B*	9	0.206	chr9:27468463	rs12551344	chr9:27466817
rs3849943	*C9ORF72*	9	0.183	chr9:27543384	-	-
rs3849942	*C9ORF72*	9	0.183	chr9:27543283	-	-
rs13302855	*C9ORF72*	9	0.086	chr9:27595997	rs34460171	chr9:27594491
rs3849943	*C9ORF72*	9	0.183	chr9:27543384	-	-
rs732389	*AK294518*	10	0.205	chr10:78584745	rs7071538	chr10:80338173
rs7118388	*CAT*	11	0.454	chr11:34432600	rs1962369	chr11:34456941
rs12803540	*CAT*	11	0.138	chr11:34471200	rs17881488	chr11:34492443
rs117027576	*KIF5A*	12	0.00913	chr12:56922819	-	-
rs113247976	*KIF5A*	12	0.007	chr12:57581917	-	-
rs142321490	*KIF5A*	12	0.006	chr12:58282349	-	-
rs74654358	*TBK1*	12	0.012	chr12:64488187	-	-
rs118082508	*KIF5A*	12	0.005	chr12:5692503	-	-
rs116900480	*KIF5A*	12	0.006	chr12:58262322	-	-
rs1578303	*HTR2A*	13	0.204	chr13:47389011	rs144877054	chr13:47962781
rs10492593	*PCDH9*	13	0.121	chr13:66919985	rs73208976	chr13:67486924
rs17446243	*TTL/TEL*	13	0.116	chr13:40174794	rs78375967	chr13:40751567

rs10139154	*SCFD1*	14	0.428	chr14:30678292	-	-
rs10143310	*ATXN3*	14	0.339	chr14:92074037	-	-
rs12886280	*NUBPL*	14	0.412	chr14:31829453	rs35875023	chr14:32298974
rs6603044	*BTBD1*	15	0.332	chr15:83015059	rs12904695	chr15:83700365
rs9901522	*PMP22*	17	0.18	chr17:14770617	-	-
rs739439	*KIAA0524*	17	0.105	chr17:28396803	rs35714695	chr17:26719788
rs2240601	*MSI2*	17	0.192	chr17:57673751	rs16942143	chr17:55748611
rs2285642	*GGNBP2*	17	0.407	chr17:36556904	rs10707226	chr17:34916453
rs7224296	*NSF*	17	0.472	chr17:46722680	rs9912530	chr17:44836302
rs12973192	*UNC13A*	19	0.278	chr19:17642430	-	-
rs12608932	*UNC13A*	19	0.43	chr19:17641880	rs12973192	chr19:17753239
rs4239633	*UNC13A*	19	0.28	chr19:17631660	rs71162163	chr19:17744075
rs75087725	*C21orf72*	21	0.003	chr21:44333234	-	-

Abbreviations: CReATe = Clinical Research in ALS and Related Disorders for Therapeutic Development; GMAF = global minor allele frequency; Chr = chromosome; GRCh38 = Genome Reference Consortium Human Build 38; HG19 = Human Genome Project 19

Table 12: Peak voxel coordinates for regions of reduced cortical thickness for ALS relative to healthy controls and associated with increased weighted polygenic score in patients with sporadic ALS from the independent validation cohort from UPenn.

Neuroanatomic region (BA)	L/R	MNI Coordinates x	y	z	*T* statistic	*p* value	Voxels
Reduced cortical thickness in ALS relative to healthy controls[1]:							
						<.001	42994
Anterior cingulate cortex (32)	L	-2	48	10	7.2		
Dorsolateral prefrontal cortex (9)	L	-2	48	18	7.08		
Anterior premotor cortex (8)	L	-2	30	36	6.76		
Orbitofrontal cortex (11)	R	8	26	-26	6.71		
Insula (13)	R	40	16	-12	6.46		
Insula (13)	R	36	22	4	6.37		
Anterior prefrontal cortex (10)	R	26	58	0	6.32		
Insula (13)	R	42	2	0	6.26		
Dorsolateral prefrontal cortex (9)	R	2	48	18	6.22		
Anterior cingulate cortex (32)	R	2	30	22	6		
Reduced cortical thickness associated with wPGS in ALS:							
Lateral temporal cortex (21)	L	-66	-46	-8	3.01	0.003	34
Premotor cortex (6)	R	36	-14	70	3.05	0.001	23
Premotor cortex (6)	L	-14	-8	76	3	0.002	21
Orbital prefrontal cortex (47)	R	34	42	-8	2.67	0.005	18
Lateral temporal cortex (21)	L	-66	-44	6	2.54	0.002	13
Anterior cingulate cortex (32)	R	14	40	0	2.59	0.004	13
Hippocampus (54)	L	-24	-30	-8	2.74	0.004	10

Abbreviations: BA = Brodmann area; L/R = Left/Right; MNI = Montreal Neurological Institute

Note.[1] Cortical regions identified from peak voxel coordinates in an effort to describe sub-peaks within a larger, contiguous cluster.

Table 13: Number of UPenn ALS autopsy cases for each neuropathological measurement in each sampled neuroanatomical region.

Region	Neuropathological Measurement	N
Middle frontal cortex	Neuronal loss	87
Middle frontal cortex	TDP-43	87
Cingulate cortex	Neuronal loss	88
Cingulate cortex	TDP-43	87
Motor cortex	Neuronal loss	84
Motor cortex	TDP-43	86
Superior / middle temporal cortex	Neuronal loss	87
Superior / middle temporal cortex	TDP-43	84
CA1 / subiculum (hippocampus)	Neuronal loss	88
CA1 / subiculum (hippocampus)	TDP-43	85

Abbreviations: CA1 = cornu ammonis 1; TDP-43 = TAR DNA binding protein - 43 kiloDaltons

Figure 6. Clinical and genetic heterogeneity in ALS patients from the CReATe Phenotype-Genotype Biomarker study. A) Standard deviation from mean baseline performance and rate of decline on each clinical variable for each participant. B) Correlation matrix between baseline performance and rate of decline on each clinical measure across all participants. C) Categorical coding for each genetic variable for each participant (*see next page*).

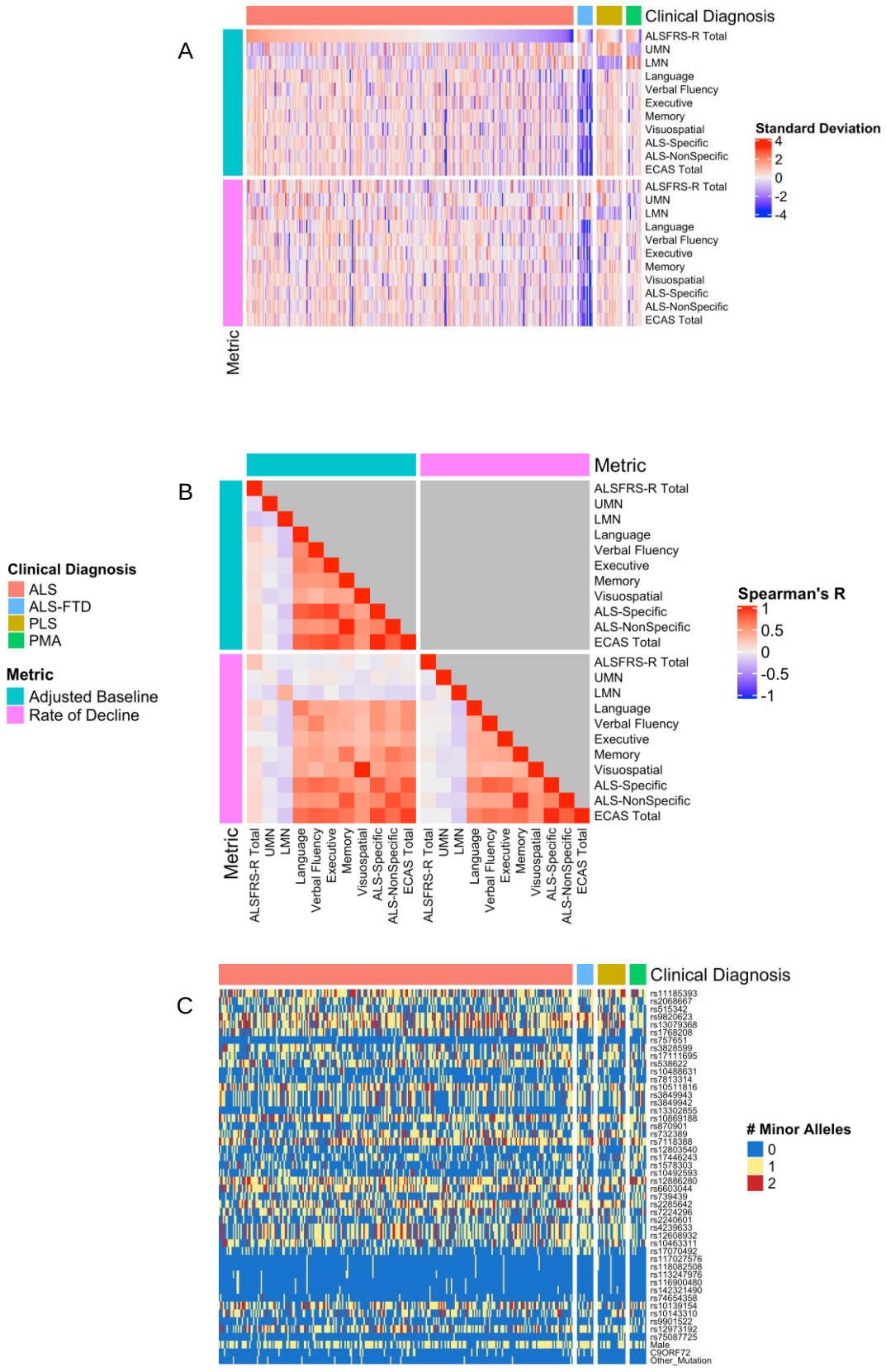

A
Clinical Diagnosis
ALSFRS-R Total
UMN
LMN
Language
Verbal Fluency
Executive
Memory
Visuospatial
ALS-Specific
ALS-NonSpecific
ECAS Total
Standard Deviation
4
2
0
-2
-4
Metric
B
Metric
Spearman's R
1
0.5
0
-0.5
-1
Clinical Diagnosis
ALS
ALS-FTD
PLS
PMA
Metric
Adjusted Baseline
Rate of Decline
C
Clinical Diagnosis
Minor Alleles
0
1
2
Male
C9ORF72
Other_Mutation

Figure 7. Gridsearch for sparse canonical correlation analysis parameters. Each column indicates 1 of 100 unique combinations of L1 parameters (ranging 0.1 to 1) applied to clinical and genetic datasets, and each row lists a variable entered into the sCCA. The heatmap denotes the canonical weight strength between clinical variables and genetic variables within each column; warmer colors indicate positive weights and cooler colors indicate negative weights.

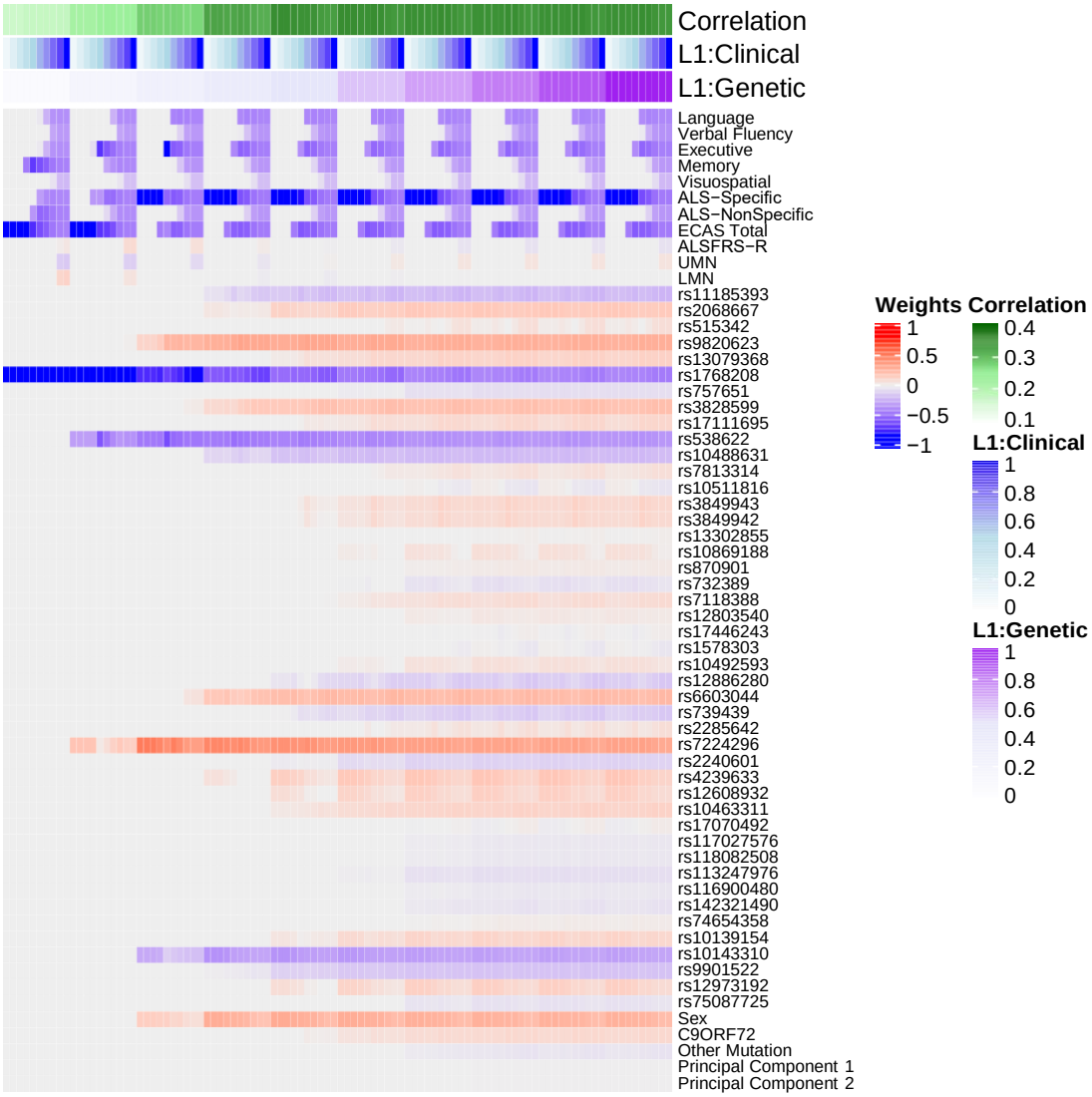


Figure 8. 10,000 iterations of sparse canonical correlation analysis modeling using bootstrapped subsampling. Each column indicates 1 of 10,000 iterations from randomly-bootstrapped subsamples of 75% of participants in the CReATe PGB cohort, and each row lists a variable entered into the sCCA. The heatmap denotes the canonical weight strength for each clinical variables and genetic variables within each column; warmer colors indicate positive weights and cooler colors indicate negative weights.

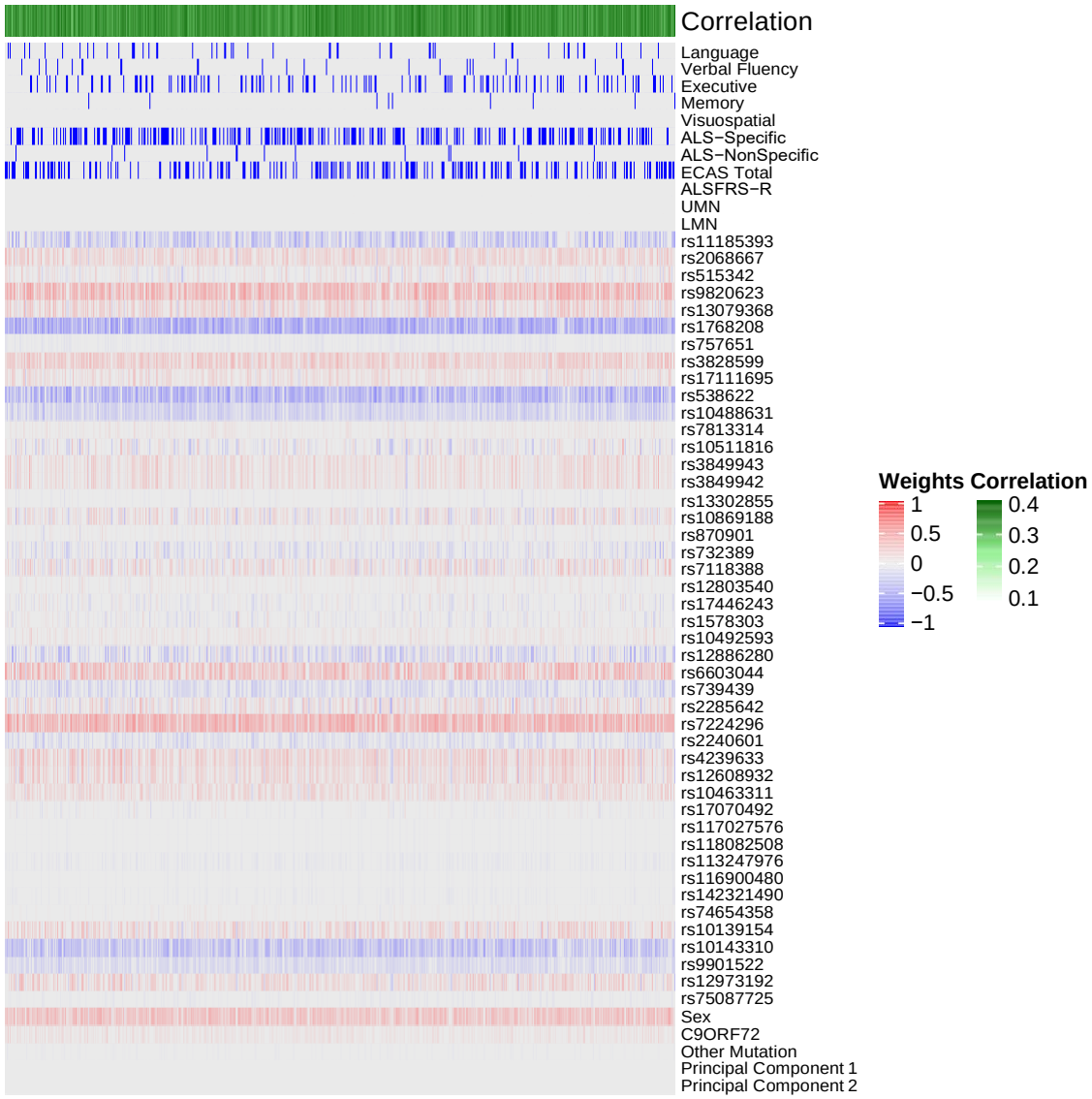


Figure 9. *p* value calculation for sparse canonical correlation analysis modeling. Histogram showing the frequency of canonical correlations achieved for sCCA modeling under the null hypothesis across 10,000 randomly bootstrapped sCCAs of randomly permuted data. The vertical turquoise line demonstrates the median canonical correlation achieved under sCCA modeling of the original, unpermuted data, and the *p* value is calculated as the proportion of times the median canonical correlation was achieved for sCCA modeling of randomly permuted data.

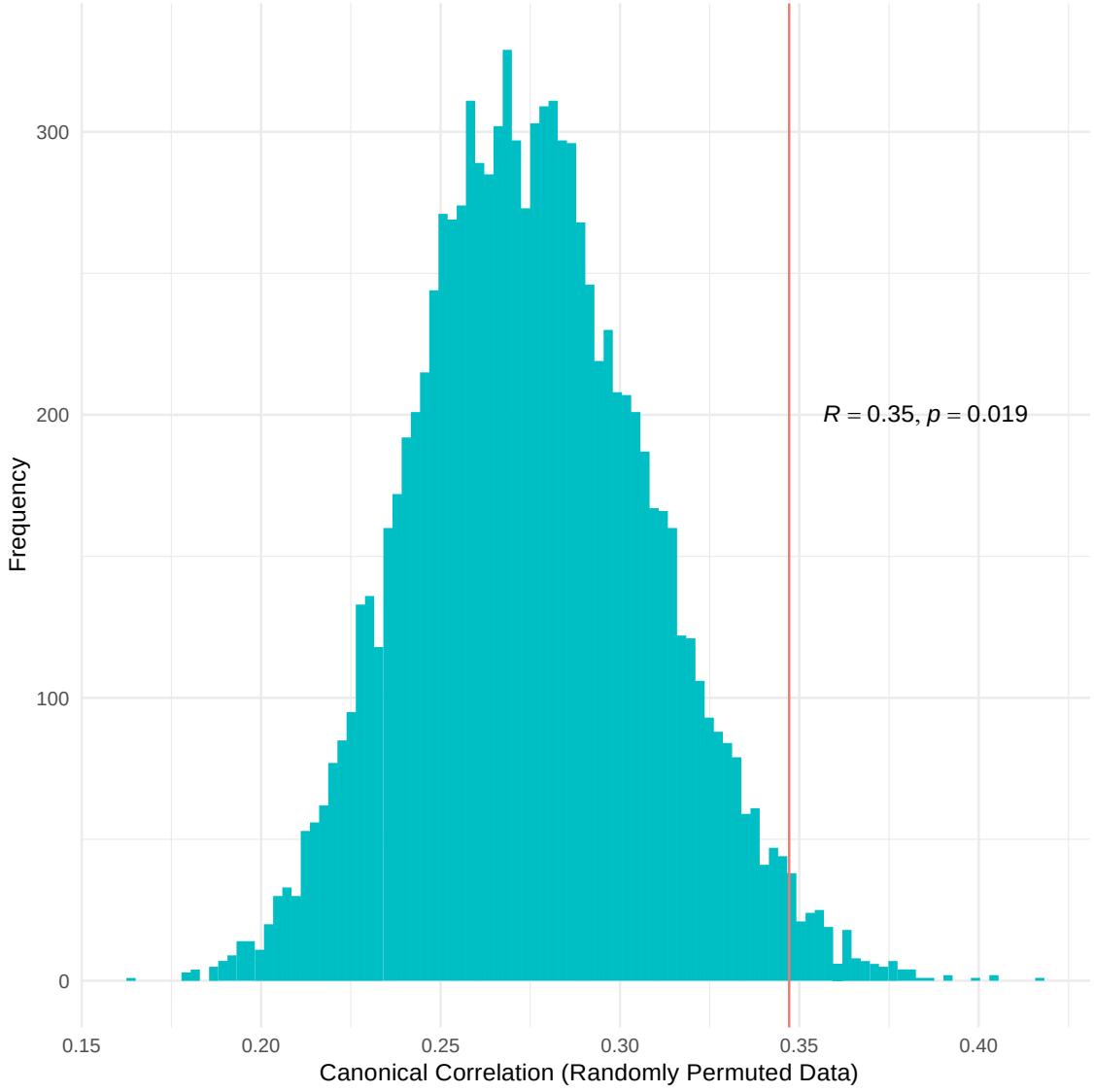


Figure 10. Sparse polygenic relationship between clinical and genetic variation in ALS patients from the CReATe Phenotype-Genotype Biomarker study**.** sCCA selected Edinburgh Cognitive and Behavioral ALS Screen (ECAS) subscores and 29 genetic variables which resulted in the maximal correlation between clinical and genetic variates in the CReATe PGB cohort. The color key denotes which variables were selected for inclusion in the model: Green = selected, Gray = not selected. The heatmap denotes the median canonical weight associated with each relationship between each selected SNP and the ECAS Score, with warmer colors indicative of positive weights and cooler colors indicative of negative weights.

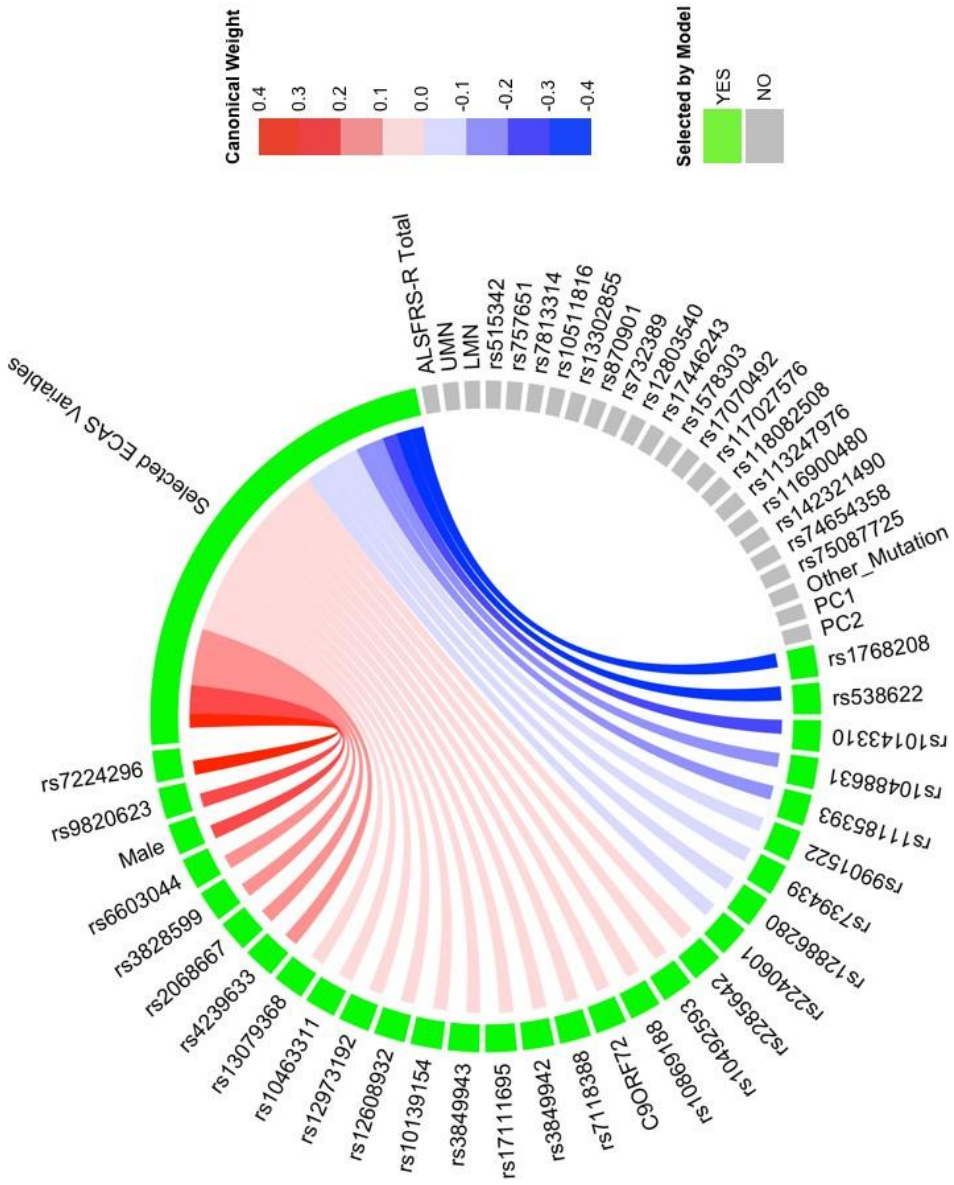


Figure 11. Variables selected sparse canonical correlation analysis modeling using original, unpermuted data and, under the null hypothesis, randomly-permuted data. A) Bar graphs demonstrating the proportion of times out of 10,000 randomly-bootstrapped sCCAs that each of the 11 clinical variables were selected by sCCA using randomly permuted (turquoise) and original (coral) data. B) Bar graphs demonstrating the number of times out of 10,000 randomly-bootstrapped sCCAs that each of the 45 investigated SNPs were selected by sCCA using randomly permuted (turquoise) and original (coral) data. SNPs are organized according to prior genome-wide association with ALS or joint association with ALS and FTD.

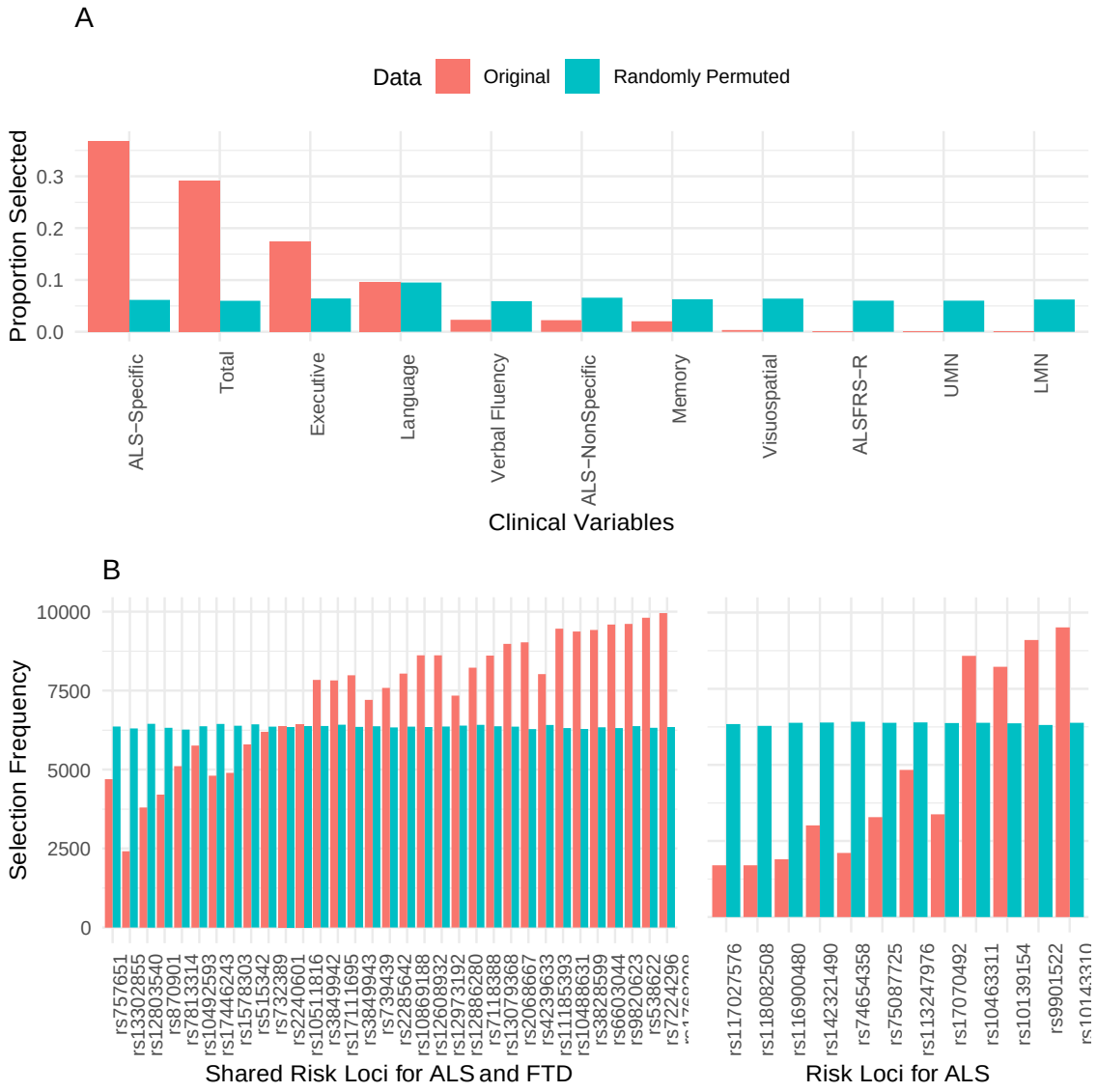


Figure 12. Correlation between weighted polygenic risk score and cognitive performance in ALS patients from the CReATe Phenotype-Genotype Biomarker study**.** Weighted polygenic risk score correlates with A) adjusted baseline performance on the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) ALS-Specific, Total, Executive Function, and Language scores, and B) rate of decline on the ALS-Specific, ALS-NonSpecific, and Total scores.

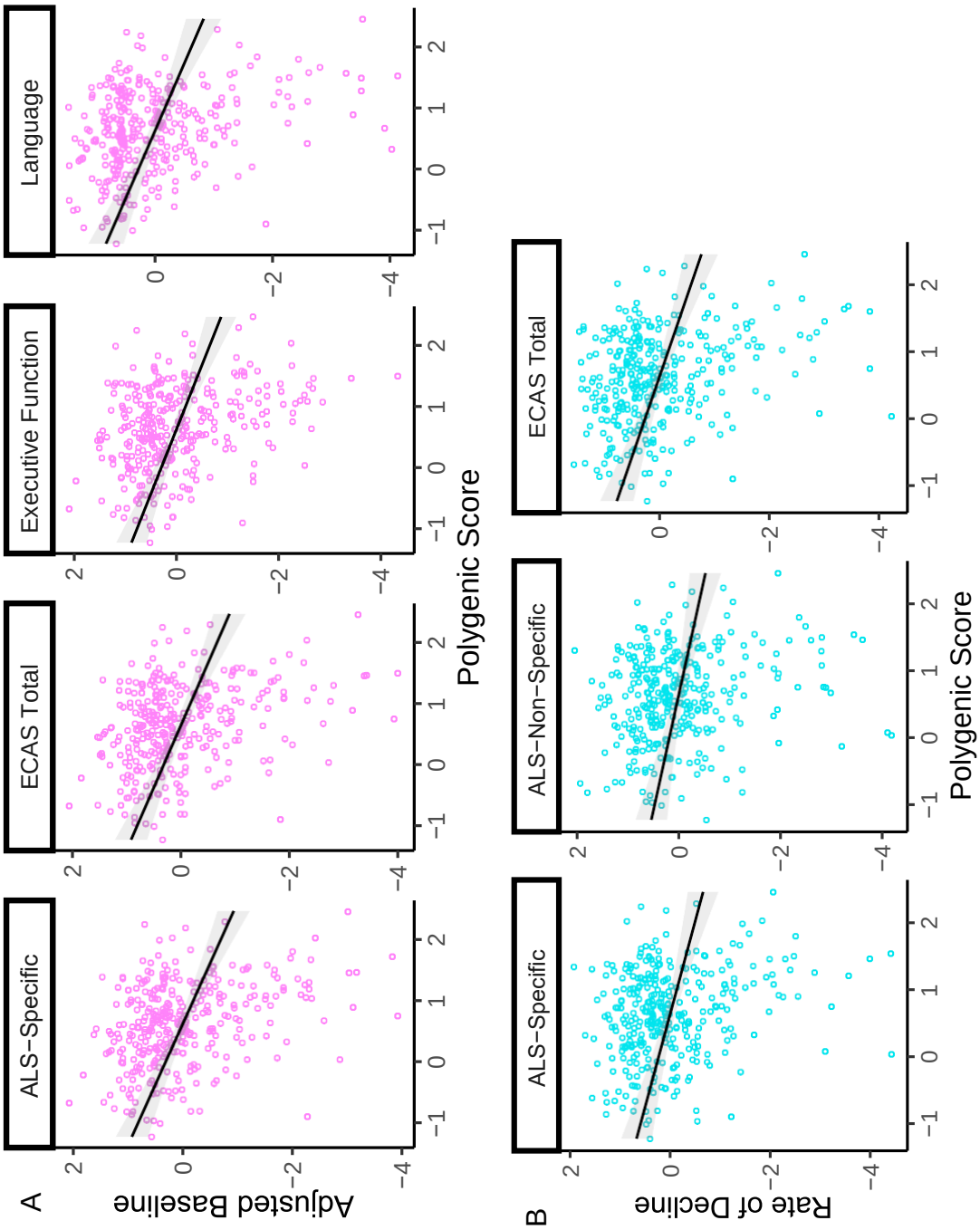
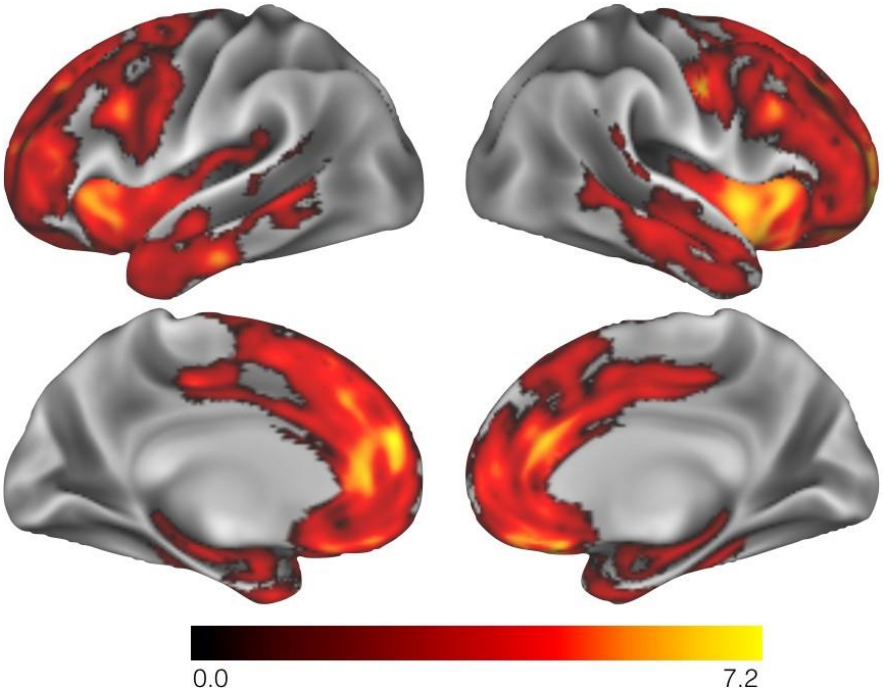
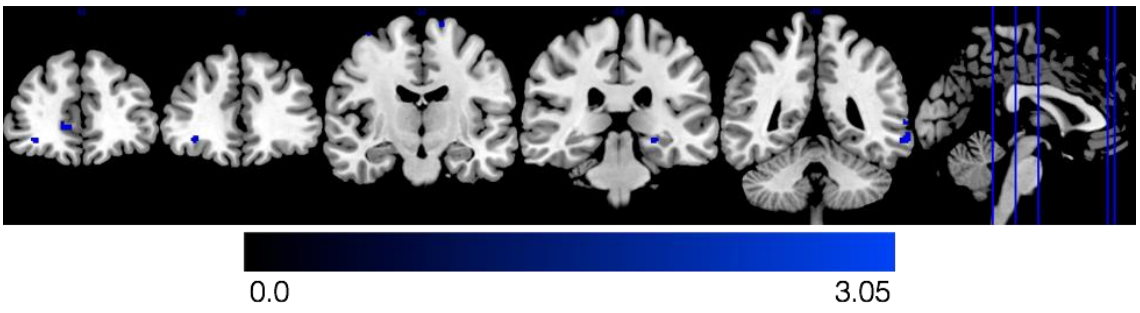


Figure 13. Cortical thickness and neuronal loss related to weighted polygenic risk score in independent cohorts from UPenn.

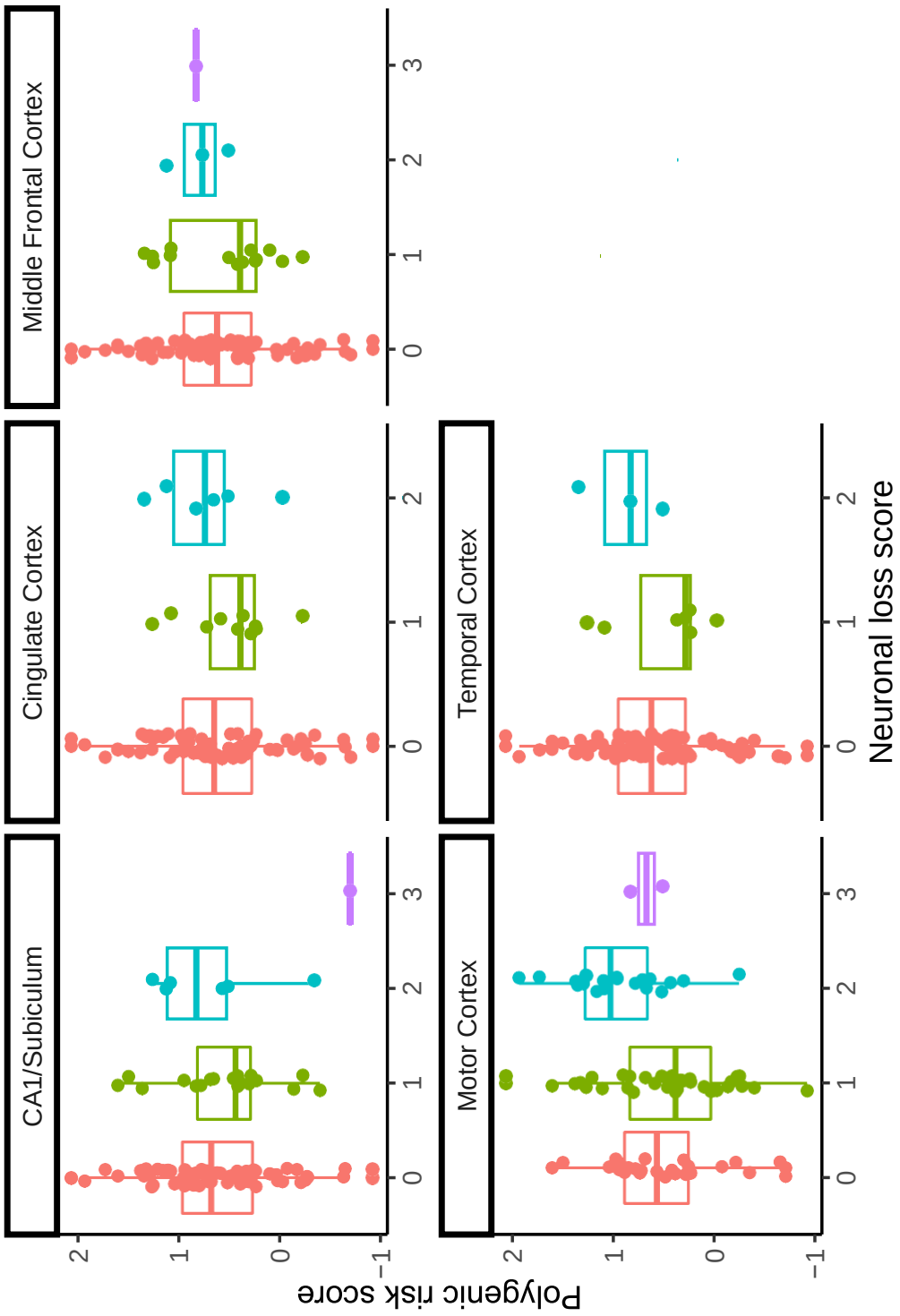
A) ALS patients (N=114) from an independent cohort at UPenn displayed widespread cortical thinning relative to age, sex, and education-matched healthy controls (N=114) in the frontal and temporal lobes. The heatmap indicates the associated T-statistic for each voxel, with light yellow representing the highest value.



B) ALS patients (N=114) from an independent cohort at UPenn with higher weighted polygenic risk score exhibited greater reduction of cortical thickness in the orbital prefrontal cortex, anterior cingulate cortex, premotor cortex, lateral temporal cortex, and hippocampus. The heatmap indicates the associated T-statistic for each voxel, with light blue representing the highest value.



C) Beeswarm boxplots of ordinal neuronal loss score (0 = none/rare, 3 = severe) in ALS cases at autopsy from an independent cohort at UPenn (N=88) showing that greater ordinal neuronal loss score is associated with higher weighted polygenic risk score.



CHAPTER 5

Research from the past several decades has demonstrated inextricable clinical, biologic, and genetic linkage between ALS and FTD, such that the two diseases are contemporarily conceptualized as occurring as clinical extremes on a continuous spectrum. Affected individuals experience disease onset often in middle age and diagnosis precedes death typically in less than 10 years. There is no cure for disease, and current treatments are only palliative or disease-slowing in nature. Despite advances in modern understanding of the cause and consequence of ALS-FTD spectrum disease, profound phenotypic heterogeneity precludes clinical prognostication and efforts for therapeutic development. Critically, cognition is a major source of phenotypic heterogeneity and impaired cognition confers increased risk for decreased functional ability during disease course and shorter survival. The goal of my thesis work has been to elucidate factors that influence cognition and underlying disease neuroanatomy in ALS-FTD spectrum disease, with the ultimate goal of defining prognostic markers and potential targets for disease-modifying therapies. In a series of three original research studies, I demonstrate strong environmental and genetic contribution to severity of cognitive impairment and frontotemporal disease neuroanatomy in ALS-FTD spectrum disease.

In the first study, I show environmental contribution from lifetime cognitive engagement to verbal executive control and gray matter atrophy in a phenotypically and genetically diverse cohort of patients. Specifically, my results indicate that higher education and occupation – considered singly or cumulatively

in a cognitive reserve index – relate to reduced gray matter atrophy in right-lateralized prefrontal cortical regions functionally linked to executive processes, and relate to less impaired performance on a measure of verbal executive control. I further demonstrate through analysis of data from healthy controls that this association is specific to patients. In answer to my first research question, “How does lifetime cognitive engagement relate to cognition and disease neuroanatomy in ALS-FTD spectrum disease?”, these findings suggest that lifetime cognitive engagement contributes to heterogeneity of verbal executive control and underlying neurodegeneration in functionally-associated prefrontal cortical regions.

In the second study, I demonstrate converging, multimodal evidence that common genetic variation at rs12608932 in *UNC13A*, a previously-identified risk locus for ALS and FTD, further contributes to frontotemporal disease in patients with sporadic ALS-FTD spectrum disease with initial ALS. My findings indicate that patients carrying the rs12608932 minor allele exhibit more severe *in vivo* cortical thinning in prefrontal, motor, and temporal cortices and greater impairment on a measure of working memory under both additive and recessive minor allele modeling. Consistent with *in vivo* findings, analyses in a separate autopsy cohort show that carriers of the rs12608932 minor allele have greater burden of *post mortem* TDP-43 pathology in the middle frontal cortex, middle temporal cortex, and motor cortex. In answer to my second research question, “Do common genetic variants that confer risk for ALS-FTD spectrum disease relate to cognition and disease neuroanatomy?”, my results demonstrate

converging multimodal evidence of contribution via common, single-allelic genetic variation at rs12608932 to frontotemporal disease phenotype.

Last, in the third study, I demonstrate that common genetic variation further contributes to cognition and underlying disease neuroanatomy in a polygenic manner. Using machine learning conducted in a large, multicenter patient cohort, I find polygenic contribution predominantly from SNPs conferring joint risk for ALS and FTD to cognitive performance in ALS patients both at baseline and longitudinally. I derive a polygenic risk score for cognitive dysfunction based on these results, and show that ALS patients at higher polygenic risk have greater cortical atrophy in the prefrontal, premotor, and temporal cortices and the hippocampus. I further show in an independent autopsy cohort that ALS patients at higher polygenic risk demonstrate more severe neuronal loss in the motor cortex. In answer to the question, “Is there evidence of polygenic contribution via common genetic variants to cognition and disease neuroanatomy?”, this study provides quantitative trait evidence for polygenic contribution from common genetic variants to cognition and frontotemporal disease neuroanatomy in ALS-FTD spectrum disease.

Collectively, this body of work aids scientific understanding of factors shaping heterogeneous cognitive phenotypes in ALS-FTD spectrum disease and offers biomarkers for actionable use in patient prognostication and clinical trials. In this final chapter, I now consider avenues for future research stemming from my thesis work and conclude by remarking on the implications this work holds for the field.

Limitations & Future Directions

My thesis work motivates a number of avenues for future research on factors contributing to heterogeneous phenotypes in ALS-FTD spectrum disease and suggests methods applicable for similar study in other phenotypically heterogeneous neurodegenerative diseases.

The work in this dissertation presents evidence in favor of strong contribution from both lifetime cognitive engagement and common genetic variation to cognition and disease neuroanatomy in ALS-FTD spectrum disease, leading to a subsequent line of inquiry: Do these factors interact to influence phenotypic heterogeneity? A few recent studies, while limited to patients with FTD, suggest that such interaction may occur. Presymptomatic individuals either positive for a pathogenic mutation in *C9ORF72*, *MAPT*, or *GRN* or with a strong familial disease history display higher premorbid frontal and total GM volume relating to greater educational attainment (Gazzina et al. 2019; Premi et al. 2017); interestingly, risk genotype at a SNP near *TMEM106B* was found to enhance this relationship in mutation carriers relative to non-carriers (Premi et al. 2017). Another study in a symptomatic FTD cohort ranging in clinical phenotypes and mutation status showed that risk genotype at a polymorphism near the *SCLA4* gene magnified the association between an index of lifetime cognitive engagement and reduced frontal cerebral blood flow (Premi et al. 2015). With these findings in mind, it is reasonable to surmise that similar interactions may occur between lifetime cognitive engagement and common genetic variation at single loci or considered in a polygenic manner. This train of thought could be

further extended to examine potential interactions between genetic factors and other environmental factors not specifically associated with lifetime cognitive engagement, such as smoking (Sutedja et al. 2007), physical activity (Huisman et al. 2013), and traumatic brain injury (Kalkonde et al. 2012; Rosso, Landweer, et al. 2003), which have been associated with risk for ALS and FTD. In a similar vein, epigenetic modification of genes associated with ALS and FTD (e.g. *C9ORF72* (McMillan et al. 2015; Russ et al. 2015)) could be also explored relative to environmental factors. Thus, one avenue for future work is to examine how environmental and genetic factors may interact or concurrently influence cognition and disease anatomy across ALS-FTD spectrum disease.

A second logical extension of my thesis work regards the broader study of genetic factors. I limited my investigation of common genetic variants in my thesis work to loci previously identified as conferring risk for ALS and FTD to explore quantitative-trait relationships with cognition and disease anatomy in a hypothesis-driven manner. However, in recent years, whole-genome sequencing has become increasingly cost-effective and technological advances have enabled greater computational efficiency for the analysis of extremely large data sets (e.g. 3.2 billion reads from ~38x genome coverage). Another interesting avenue for further study is thus the study of the whole genome relative to cognition and neuroanatomy in ALS-FTD spectrum disease. For example, sparse, machine learning approaches like sCCA could investigate genotype-phenotype relationships between a dataset comprising whole genome sequencing and a dataset comprising quantitative phenotypic characterization

(e.g. neuropsychological evaluation, voxel-wise neuroimaging data). Some intriguing and innovative research studies over the past decade from the Alzheimer's disease literature offer insight into the potential use of genome-wide data relative to complex quantitative phenotypes. These include the development of a voxel-wise GWAS techniques designed to identify genetic loci associated with regional heterogeneity in structural neuroimaging these methods has been applied to identify both SNP-based and gene-based loci associated with brain volume in healthy elderly participants and mixed cohorts of controls and patients from ADNI (Stein et al. 2010; Hibar et al. 2011). More recent studies from the Alzheimer's disease literature also describe techniques for analyzing genome-wide data relative to neuropathological categorization and relative to clinical phenotypic variation (Beecham et al. 2014; Moreno-Grau et al. 2019). It is therefore not unreasonable to suggest these techniques could be adopted to the study of genetic factors influencing heterogeneity in cognition and disease anatomy, ascertained through multiple modalities including neuropsychological evaluation, neuroimaging, and post mortem neuropathological examination, in ALS-FTD spectrum disease.

A third extension of the work presented here concerns the expansion of data sources and the methods used to analyze them. Based on my defined research questions, I chose to study data from symptomatic patients with ALS-FTD spectrum disease and to use specific nonparametric and machine learning methods. Other data sources, including from biofluid biomarkers (Benatar, Turner, and Wuu 2019; van der Ende et al. 2019) and functional neuroimaging

(Ferraro et al. 2018; Olm et al. 2018; Mutsaerts et al. 2019), reflect disease processes in presymptomatic individuals with familial disease history or genetic mutations. For example, serum and CSF levels of phosphorylated neurofilament heavy (pNfH) and neurofilament light (NfL) increase longitudinally in advance of clinically manifest ALS (Benatar et al. 2019). A potential future line of research could thus investigate environmental and genetic factors relative to cognition and frontotemporal disease anatomy in presymptomatic individuals. Results from this proposed research in presymptomatic ALS-FTD could then be subsequently investigated in symptomatic patient cohorts, with analyses stratified for mutation-negative and sporadic forms of disease. In addition to data sources reflecting early disease stage, my thesis work could be extended to utilize other unsupervised machine learning methods to identify genetic factors associated with disease. These include methods integrating prior knowledge (e.g. GWAS effect sizes for loci associated with disease risk) into model estimates (Blatti et al. 2020), and methods designed to identify quantitative trait loci associated with data collected from patients with different disease groups (H. Wang et al. 2012). In the context of ALS-FTD spectrum disease, such machine learning methods could be used to integrate effect sizes from ALS and FTD GWAS into model estimates of genetic risk for impaired cognition, and to identify loci associated with multiple phenotypes (e.g. identify loci associated with impaired cognition *and* frontotemporal atrophy).

A final avenue for future research stems from the ubiquitous need for independent replication of scientific findings, including those presented in the

current body of work. As noted in the introduction, I have endeavored to adopt robust statistical approaches including the incorporation of multiple modalities in an attempt to validate my findings across datasets, given the absence at the time of study of additional, independent cohorts sufficient data necessary for true replication. I have incorporated analysis of complementary, independent datasets (e.g. from ADNI, the CReATe Consortium, and the UPenn Integrated Neurodegenerative Disease Biobank) to evaluate null hypothesis in control cohorts and seek converging evidence from additional biomarkers for my results, when possible. Tremendous efforts for multicenter, cross-institutional, and international observational research studies have been made through the recent formation of entities including the CReATe consortium and the ARTFL–LEFFTDS Longitudinal Frontotemporal Lobar Degeneration (ALLFTD) research consortium (H. J. Rosen, Boeve, and Boxer 2020). True replication of the environmental and genetic influence on cognition and disease anatomy that I demonstrate through my work will likely only be accomplished through continued support for and research participation in these, and other, collaborative endeavors.

Conclusions and Implications for the Field

The research studies I have pursued for my thesis addressed the critical clinical need for the identification of factors influencing heterogeneity in cognition and disease anatomy in ALS-FTD spectrum disease. The availability of rich, multimodal data from large patient cohorts was critical to my study design and to the strength of evidence I was able to present in support of robust contribution

from environmental and genetic factors to cognition and underlying disease anatomy in ALS-FTD spectrum disease.

My thesis work holds implications for future advancements in clinical care and therapeutic development and stimulates further research. The environmental and genetic factors elucidated through my research hold potential to improve prognostication in clinical care and therapeutic development with appropriate validation. Routine patient genotyping, generated through a minimally-invasive procedure in an increasingly cost-effective manner, may be used to investigate pharmaco-genomic interactions in ongoing clinical trials. Patient genotyping and measures of lifetime cognitive engagement (e.g. education and occupation) could further be used to stratify patients in clinical trials to alleviate potential confounds associated with the profound phenotypic heterogeneity observed in ALS-FTD spectrum disease. In addition to stratification, environmental and genetic factors might also serve as potential therapeutic targets. For example, cognitive engagement and associated frontal cortical function might be targeted early in disease course through digital (e.g. smartphone applications) or neuromodulatory (e.g. transcranial direct current stimulation) strategies. With these clinical and therapeutic implications in mind, my work stimulates future research on genetic and environmental factors influencing heterogeneity in patient phenotype. My work has demonstrated the critical utility of large, multimodal datasets in elucidating disease-modifying factors, and accordingly implicates that further support and maintenance of collaborative data-collection on patient populations is necessary to conduct scientifically-rigorous and clinically-meaningful studies.

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