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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 frontotemporal degeneration (FTD) associated with shorter survival. The genetic polymorphism rs12608932 in *UNC13A* confers increased risk of sporadic ALS and sporadic 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-weighted magnetic resonance imaging (N = 109) relative to controls (N = 113), and observed that minor allele (C) carriers exhibited greater reduction of cortical thickness in the dorsal prefrontal, ventromedial prefrontal, anterior temporal, and middle temporal cortices and worse performance on a frontal lobe–mediated cognitive test (reverse digit span). In sporadic ALS with autopsy data (N = 102), minor allele homozygotes exhibited greater burden of phosphorylated tar DNA-binding protein-43 kda (TDP-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.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a multisystem disorder primarily characterized by progressive degeneration of the upper and lower motor neurons 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 multidomain impairments consistent with frank frontotemporal degeneration (FTD) (Montuschi et al., 2015; Ringholz et al., 2005). Although ALS and FTD commonly feature tar DNA-binding protein-43 kda (TDP-43) pathology (Neumann et al., 2006) and can both be caused by pathogenic *C9orf72* repeat expansions (DeJesus-Hernandez et al., 2011; Renton et al., 2011), the mechanisms influencing the risk of progression from ALS to develop cognitive impairment and FTD have been underevaluated. The presence of FTD is consistently associated with shorter survival in ALS patients (Elamin et al., 2011; Govaarts et al., 2016), 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 et al., 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

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reported. However, loci in *UNC13A* including rs12608932, that is in high linkage disequilibrium 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 (minor allele frequency (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 (Chiò et al., 2013; Diekstra et al., 2012; Vidal-Taboada et al., 2015).

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.

2. Materials and methods

We evaluated 190 patients with sporadic forms of ALS distributed across neuroimaging, neuropathological, and/or neuropsychological cohorts, described in detail below and summarized in Supplementary Fig. 1.

2.1. Neuroimaging cohort

We retrospectively evaluated 109 sporadic (see Genetic Screening) 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.J.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 before 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 Mini-Mental State Exam (MMSE) (Table 1). There were no statistically significant differences in age, education, or sex between controls and ALS (all p values > 0.05). We assessed participant race and ethnicity via self-report. Although population diversity is known to influence allele frequency across individuals, rs12608932 has a relatively equal MAF 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 neuroimaging cohorts are summarized in Table 1.

All participants participated in an informed consent procedure approved by an institutional review board convened at the University of Pennsylvania.

2.2. Autopsy cohort

We evaluated neuropathological data from 102 sporadic ALS autopsy cases (Table 2) who were diagnosed by a board-certified neuropathologist (J.Q.T., E.B.L.) 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 (repeated analyses excluding these individuals summarized in Supplementary Table 1 revealed similar findings; see Supplementary Results). 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

Table 1
Demographic, clinical, and genetic information for neuroimaging cohorts

Variable	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	
Multiracial	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

Key: ALS, amyotrophic lateral sclerosis; ADNI, Alzheimer's disease neuroimaging initiative; ALSFRS-R, ALS functional rating scale-revised; MMSE, mini-mental state exam.

Table 2
Demographic, clinical, and genetic information for autopsy cohort

Variable	ALS
N (F)	102 (40)
Age at death, y	65.01 (10.83)
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)
Disease duration at death, y	4.83(4.98)
rs12608932 genotype, N	
AA	38
AC	47
CC	17

Key: ALS, amyotrophic lateral sclerosis.

(pTDP-43) intraneuronal inclusions (dots, wisps, skeins) for each sampled region on a semiquantitative 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.

2.3. Alzheimer's disease neuroimaging initiative cohort

To evaluate the disease specificity of any observed neuroanatomic and genetic associations, we additionally performed cortical thickness analyses in 84 amyloid-negative (florbetapir standardized uptake value ratio (SUVR) < 1.11) (Landau et al., 2012), cognitively normal healthy controls from the publicly available Alzheimer's Disease Neuroimaging Initiative (ADNI) database (see Table 1 and Supplementary Methods and Materials for details).

2.4. 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 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 3-generation pedigree history as previously reported (Wood et al., 2013). For cases that had 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* (Rosen, 1993), *TARDBP* (Kabashi et al., 2008), *FUS* (Kwiatkowski et al., 2009; Vance et al., 2009), and *VCP* (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, USA). We excluded any individuals identified as having a pathogenic mutation.

2.5. SNP genotyping

All neuroimaging and neuropathology cases were genotyped using peripheral blood or brain DNA extracted as mentioned previously for rs12608932 using a custom-designed Pan Neurodegenerative Disease-oriented Risk Allele panel designed to genotype 92 common and risk allele variants identified in association and other studies as modifying disease risk or phenotype for several neurodegenerative diseases, including ALS and FTD, Parkinson's disease, and Alzheimer's disease (Toledo et al., 2014). Although 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., 2014, 2018). Briefly, the 92 SNP type assays were designed by D3 Assay Design tool (Fluidigm). Allele-specific polymerase chain reaction 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 were collected and analyzed using BioMark Genotyping Analysis software.

2.6. 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 et al., 1999) and cognitive function using the Forward and Reverse Digit Span, the Visual-Verbal Test (VVT), letter fluency, and the 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). 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 3 of 4 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 1 minute (excluding proper nouns and numbers); patients did not complete letter fluency if bulbar upper or lower motor symptoms were present upon examination. The MMSE is a 30-point questionnaire that evaluates global dementia severity (Crum et al., 1993); 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 education of our patients when available (Tombaugh et al., 1999; Weintraub et al., 2009) (see Table 1). We report patient performance on the VVT 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 females]; 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.

2.7. Neuroimaging acquisition and processing

High-resolution T1-weighted magnetization prepared rapid acquisition with gradient echo (MPRAGE) structural magnetic resonance imaging (MRI) scans were acquired for ALS and control neuroimaging cohorts using a 3T Siemens Tim Trio scanner with an 8-channel head coil, with T = 1620 ms, T = 3.09 ms, flip angle = 15°, 192 × 256 matrix, and 1 mm^3 voxels. T1-weighted MRI images were then preprocessed using Advanced Normalization Tools (ANTs) cortical thickness software (Tustison et al., 2014). Each individual data set was deformed into a standard local template space in a canonical stereotactic coordinate system. 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 6 tissue classes (cortex, white matter, CSF, subcortical gray structures, brainstem, and cerebellum) using template-based priors and to generate probability maps of each tissue. Voxelwise cortical thickness was measured in millimeters (mm) from the pial surface and then transformed into Montreal Neurological Institute space, smoothed using a 2 sigma

full-width half-maximum Gaussian kernel, and downsampled to 2mm isotropic voxels.

2.8. Neuroimaging analysis

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 neuroimaging cohort. Permutation-based statistical testing is robust to concerns regarding multiple comparisons because, 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 cohort relative to the control cohort, constraining analysis using an explicit mask restricted to high probability cortex (>0.4). We report clusters that survive $p < 0.005$ threshold-free cluster enhancement (Smith and Nichols, 2009) corrected for familywise error 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 $p < 0.01$ (uncorrected) threshold and cluster extent threshold of >20 adjacent voxels relative to 10,000 random permutations; we use 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 previously.

2.9. Statistical analyses

All additional statistical analyses were performed using R (www.r-project.org). 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 the 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.

3. Results

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

A group comparison of ALS patients relative to 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 (see Fig. 1, Table 3).

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 (see Fig. 1, Table 3); we observed similar findings under a minor allele recessive model (see Supplementary Results including Supplementary Table 2, Supplementary Fig. 2). Investigation of the inverse association between rs12608932 genotype (i.e., number of major nonrisk allele (A)) and cortical thickness yielded no relationship for either

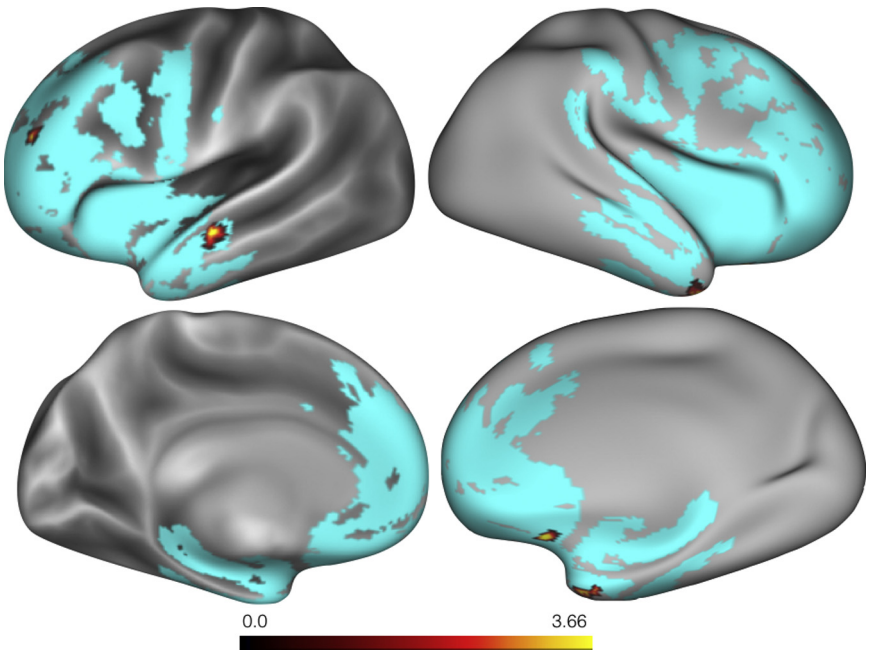

Fig. 1. Reduced cortical thickness associated with rs12608932. In an analysis restricted to regions of reduced cortical thickness identified in sporadic ALS (N = 109) relative to controls (N = 113) (light blue regions), patients with sporadic ALS who are carriers of the minor allele (C) exhibited greater reduction of cortical thickness in dorsal prefrontal, ventromedial prefrontal, anterior temporal, and middle temporal cortices (regions indicated in red-yellow heatmap). The heatmap indicates the associated T-statistic for each voxel, with yellow representing the highest value. Abbreviation: ALS, amyotrophic lateral sclerosis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Regions of reduced cortical thickness for ALS relative to controls and associated with rs12608932 in ALS

Neuroanatomic region (BA)	L/R	MNI coordinates			*T* Statistic	*p* value	Voxels
		x	y	z			
Reduced cortical thickness in ALS relative to controls[a]*:*							25,627
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	
Ventral medial orbital frontal cortex (11)	R	12	14	−20	5.91	0.0001	
Ventral medial 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:							
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

Key: ALS, amyotrophic lateral sclerosis; MNI, Montreal Neurological Institute.
[a] Cortical regions identified from peak voxel coordinates in an effort to describe subpeaks within a larger, contiguous cluster.

additive or recessive models (not shown), suggesting that the rs12608932 minor allele is specifically associated with reduced cortical thickness in these regions.

To examine the disease specificity of the relationship between rs12608932 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).

3.2. 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. Under a minor allele additive model, minor allele heterozygotes (AC) ($\beta = 3.41$, $p = 0.04$) but not minor allele homozygotes (CC) ($\beta = 1.97$, $p = 0.35$) demonstrated higher ALSFRS-R total score compared to major allele homozygotes (AA); disease duration ($\beta = -0.037$, $p = 0.1$), age ($\beta = 0.059$, $p = 0.38$), sex ($\beta = 1.53$, $p = 0.33$), and cognitive diagnosis (ALSci: $\beta = -2.34$, $p = 0.37$; ALS-FTD: $\beta = -0.88$, $p = 0.62$) 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 ($\beta = 0.34$, $p = 0.87$) 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) ($\beta = -0.81$, $p = 0.086$) performed worse than major allele homozygotes (AA) on reverse digit span after accounting for cognitive diagnosis (ALSci: $\beta = -1.73$, $p = 0.0014$; ALS-FTD: $\beta = -2.09$, $p < 0.00001$); heterozygous genotype (AC) ($\beta = 0.32$, $p = 0.38$), disease duration ($\beta = -0.0022$, $p = 0.64$), ALSFRS-R total score ($\beta = -0.012$, $p = 0.60$), and age ($\beta = -0.0021$, $p = 0.89$) did not relate to reverse digit span performance in this model.

Under a minor allele recessive model, minor allele homozygotes at rs12608932 (CC) ($\beta = -0.96$, $p = 0.029$) performed significantly worse than major allele homozygotes (AA) and heterozygotes (AC) on reverse digit span after accounting for cognitive diagnosis (ALSci: $\beta = -1.69$, $p = 0.0017$; ALS-FTD: $\beta = -2.08$, $p < 0.00001$); disease duration ($\beta = -0.0018$, $p = 0.69$), ALSFRS-R total score ($\beta = -0.0013$, $p = 0.95$), age ($\beta = -0.0013$, $p = 0.76$), and sex ($\beta = 0.55$, $p = 0.11$) did not significantly relate to reverse digit span performance in this model.

Performance on other neuropsychological tests was not found to associate with rs12608932, including forward digit span (CC: $\beta = -0.03$, $p = 0.93$; CA: $\beta = 0.40$, $p = 0.20$), letter fluency (CC: $\beta = 0.26$, $p = 0.85$; CA: $\beta = 1.61$, $p = 0.15$), MMSE (CC: $\beta = 0.036$, $p = 0.28$; CA: $\beta = 0.006$, $p = 0.82$), and the VVT (CC: $\beta = -0.22$, $p = 0.67$; CA: $\beta = -0.40$, $p = 0.32$). We observed similar results under minor allele recessive models: forward digit span (CC: $\beta = -0.22$, $p = 0.57$), letter fluency (CC: $\beta = -0.39$, $p = 0.76$), MMSE (CC: $\beta = 0.034$, $p = 0.26$), and the VVT (CC: $\beta = 0.12$, $p = 0.80$).

3.3. pTDP-43 pathologic burden associated with rs12608932 in middle frontal and temporal cortices

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 (see Fig. 2), which were associated with rs12608932 in the above neuroimaging analyses.

Minor allele homozygotes (CC) were 8.26 times (95% CI: 2.01, 38.64; $p = 0.0043$) more likely and heterozygotes (AC) were 5.53 times (95% CI: 1.78, 21.21; $p = 0.0057$) 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; $p = 0.022$) 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; $p = 0.056$) 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 > 0.1).

4. Discussion

In this study, we evaluated whether rs12608932 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)

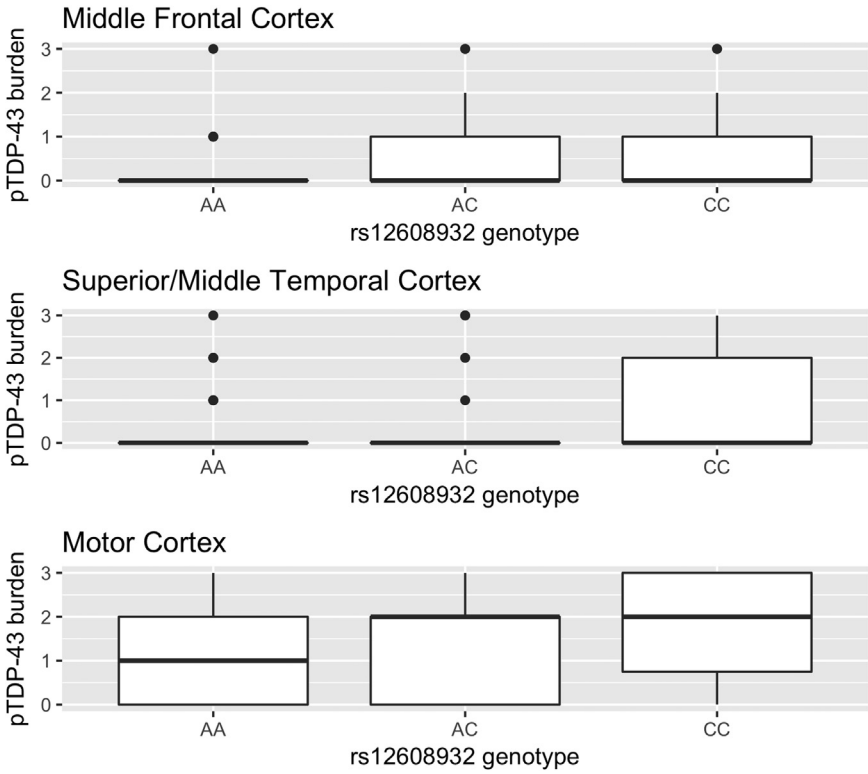

Fig. 2. Greater burden of pTDP-43 pathology associated with rs12608932. Box and whisker plots of pTDP-43 pathologic burden in our sporadic ALS autopsy cohort (N = 102) show that minor allele homozygotes (CC) and heterozygotes (AC) feature greater burden of pathology in the middle frontal cortex. Minor allele homozygotes but not minor allele heterozygotes also feature greater burden of pathology in the superior/middle temporal cortex and motor cortex. Abbreviations: ALS, amyotrophic lateral sclerosis; pTDP-43, phosphorylated TDP-43.

show reduced cortical thickness in regions including the dorsal and ventromedial prefrontal cortices, anterior and middle temporal cortices, 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 2-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 (Chiò et al., 2013; Diekstra et al., 2012; 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 linkage disequilibrium 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 which compare only categorical clinical designations (e.g., ALS vs. healthy controls) and allows detailed phenotypic characterization associated with rs12608932.

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 (Keil et al., 2012; Kwan et al., 2013; Menke et al., 2014; Müller et al., 2016; Senda et al., 2011; Verstraete et al., 2012, 2014), and that degree of frontotemporal cortical thinning relates to cognitive-behavioral phenotype (Agosta et al., 2016; Schuster et al., 2014). In addition, staging of neuropathological burden suggests stereotyped propagation of TDP-43 from motor regions (brainstem, spinal cord) to frontal neocortex and temporal neocortex over the course of

disease (Braak et al., 2013; Brettschneider et al., 2013). Our finding of greater TDP-43 pathologic burden associated with the rs12608932 in frontal neocortex 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 (Elamin et al., 2011; Govaarts et al., 2016).

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), whereas 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 et al., 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 (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 (Gallagher et al., 2014), and confer risk for impaired executive function in ALS (Vass et al., 2010).

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. Although 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 (Li et al., 2016). In regard 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 (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. Although 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.

Disclosure statement

All authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.09.031.

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