The *TMEM106B* locus and TDP-43 pathology in older persons without FTLD

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ABSTRACT

Objective: To determine the independent association of the *TMEM106B* variants with transactive response DNA binding protein 43 (TDP-43) pathology in older persons without frontotemporal lobar degeneration (FTLD) and to explore functional pathways that link the risk variants to the pathology, including a *GRN* mRNA pathway.

Methods: Data came from 544 autopsied participants without FTLD in 2 community-based studies of aging. Participants underwent uniform neuropathologic evaluations, including TDP-43 cytoplasmic inclusions. We examined the association of *TMEM106B* variants with a semiquantitative measure of TDP-43 pathology in a series of regression analysis. We explored potential pathways by leveraging genetic, brain DNA methylation, miRNA, and transcriptomic data collected from this same group of participants.

Results: TDP-43 pathology was identified in 51.7% of the participants. The index single-nucleotide polymorphism (SNP), rs1990622^{A}, was associated with more advanced TDP-43 pathology. Top hits from fine mapping of the locus were in linkage disequilibrium of the index SNP. The association remained significant after adjustment for other neuropathologies including Alzheimer disease and hippocampal sclerosis (odds ratio = 1.351, 95% confidence interval = 1.068–1.709, $p = 0.012$). *GRN* expression was upregulated in rs1990622$^{AA/AG}$ carriers, and was associated with more advanced TDP-43 pathology. The *TMEM106B* variants were associated with lower level of DNA methylation in an active enhancer in *GRN*.

Conclusions: Common variants in *TMEM106B* serve as a distinct risk factor for TDP-43 pathology in older persons without FTLD. The role of *GRN* expression and epigenetic mechanisms associating *TMEM106B* in the accumulation of TDP-43 in older persons require further study.

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GLOSSARY

AD = Alzheimer disease; **ALS** = amyotrophic lateral sclerosis; **CBD** = corticobasal degeneration; **CERAD** = Consortium to Establish a Registry for Alzheimer's Disease; **DLPFC** = dorsolateral prefrontal cortex; **FTLD** = frontotemporal lobar degeneration; **GWAS** = genome-wide association studies; **MAP** = Memory and Aging Project; **miRNA** = micro RNA; **OR** = odds ratio; **PSP** = progressive supranuclear palsy; **ROS** = Religious Orders Study; **SNP** = single-nucleotide polymorphism; **TDP-43** = transactive response DNA binding protein 43; **TMEM106B** = transmembrane protein 106B.

Transactive response DNA binding protein 43 (TDP-43) is the major disease protein in frontotemporal lobar degeneration (FTLD) with ubiquitin-positive inclusions and amyotrophic lateral sclerosis (ALS).[1] TDP-43 immunoreactivity is also present in other neurodegenerative diseases such as Alzheimer disease (AD), Lewy body disease, and hippocampal sclerosis.[2–5] Recent genome-wide association studies (GWAS) suggest that common variants in the transmembrane protein 106B (*TMEM106B*) locus is a risk factor for susceptibility of FTLD with TDP inclusions (FTLD-TDP).[6,7] However, it is unclear whether the same risk variants are associated with the much more common TDP-43 pathology found in community-based older persons without FTLD. Further, as comorbid pathologies are common in the aging brain[8–10]

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Supplemental data at Neurology.org

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and the *TMEM106B* variants are implicated in patients with pathologic AD diagnosis,[11] it is important to determine whether such an association is independent of other neuropathologies.

Notably, the physiologic function of the *TMEM106B* locus in TDP-43 pathology remains elusive. Initial studies report that *TMEM106B* RNA expression is elevated in FTLD-TDP and could be *cis* regulated by the risk variants[6,12]; however, these findings are inconclusive.[7] Separately, *TMEM106B* variants are posited to influence the risk of FTLD-TDP by potentially regulating expression of progranulin (*GRN*), a mendelian risk gene for FTLD[13,14]; yet there are also reports that *GRN* level is not altered by *TMEM106B* expression.[15] In addition, epigenetic mechanisms such as micro RNA (miRNA) and DNA methylation could also be involved.[16,17]

In this study, we first tested the hypothesis that the *TMEM106* risk variants are associated with TDP-43 pathology in older persons without FTLD. We confirmed the association after controlling for potential confounding due to other age-related pathologies, including AD, Lewy bodies, infarcts, and hippocampal sclerosis. Next, we explored potential functional pathways that may link the *TMEM106B* risk variants to downstream TDP-43 pathology through DNA methylation, miRNA, or mRNA expression.

METHODS

Participants. Participants are from 2 community-based clinical pathologic cohort studies of aging, the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP).[18,19] Participants enroll without known dementia. At the time of these analyses, 1,363 ROS and MAP participants had died and 1,179 had undergone brain autopsies; the autopsy rate exceeds 85%. Of 1,179 autopsied participants, complete TDP-43 pathology had been collected in 620 (ROS = 294; MAP = 326). Notably, there was no statistical difference in age or common neuropathologic burdens between the participants with and without TDP-43 data (table e-1 on the *Neurology*® Web site at Neurology.org). Of 620, 13 (2%) had FTLD or a related pathology (including 7 with FTLD, 1 with ALS, 2 with progressive supranuclear palsy [PSP], and 3 corticobasal degeneration [CBD]), and were excluded from the analyses. Primary analyses were performed on 544 of 607 participants who also had genotype data. The average age at death was 89.0 years with SD of 6.3 years and range 71.7 to 108.3 years; 365 (67.1%) were female; and average education was 16.1 years (SD 3.6, range 5–28).

Standard protocol approvals, registrations, and patient consent. Studies were approved by the Institutional Review Board of Rush University Medical Center. Each participant signed informed consent for annual clinical evaluations and organ donation at the time of death.

Uniform neuropathologic evaluation. Uniform neuropathologic evaluations were conducted by examiners blinded to age and all clinical data. Brains were removed following a standard protocol, weighed, and cut coronally into 1-centimeter slabs. Presence of chronic macroscopic infarcts was assessed using slabs or pictures from both hemispheres, and verified after dissection and histologic review.[20] Blocks of predetermined brain regions were dissected from one hemisphere. Six-micrometer sections were stained for hematoxylin & eosin to assess presence of chronic microinfarcts[21] and hippocampal sclerosis and α-synuclein immunostain to assess presence of neocortical Lewy bodies.[22] Neuropathologic AD diagnosis was based on Braak staging for neurofibrillary tangles and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) estimate of neuritic plaque density, as recommended by the National Institute on Aging Reagan criteria. AD diagnosis by Reagan criteria required either an intermediate likelihood of AD (i.e., at least Braak stage 3 or 4 and CERAD moderate plaques) or a high likelihood (i.e., at least Braak stage 5 or 6 and CERAD frequent plaques). Neuropathologic diagnoses of FTLD, CBD, and PSP followed standard criteria.[23]

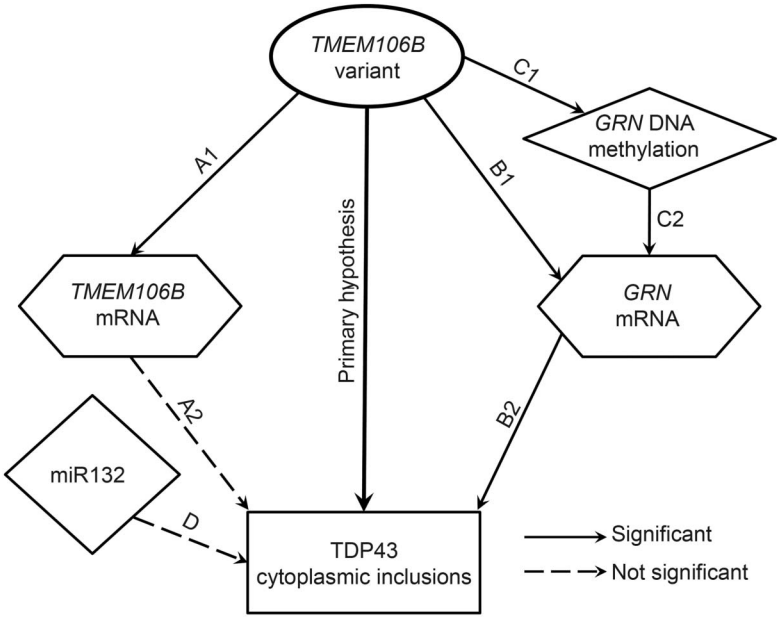
TDP-43 pathology was assessed in 6 predetermined brain regions of interest: amygdala, hippocampus CA1, dentate gyrus, entorhinal cortex, midtemporal cortex, and midfrontal cortex. Six-micrometer sections were stained for monoclonal antibodies to phosphorylated TDP-43 (pS409/410; 1:100) to assess the counts of TDP-43 cytoplasmic inclusions (both neuronal and glial) in each region. A semiquantitative measure capturing the staging of TDP-43 pathology was used across all the analyses. This measure was rated on 4 levels, including no inclusions; inclusions in amygdala only; inclusions in amygdala as well as entorhinal cortex or hippocampus CA1; and inclusions in amygdala, entorhinal cortex, or hippocampus CA1, and neocortex.

Genetic, epigenetic, and mRNA measures. DNA was extracted from blood, lymphocytes, or frozen brain tissue. Genome-wide genotyping, quality control pipeline, as well as imputation procedures have been described previously.[24] The imputed dosage values of single-nucleotide polymorphisms (SNPs) were used for the primary analyses, and were coded additively in terms of the reference allele. The dosage value captures the level of uncertainty of imputed genotypes at the interrogated sites. We first targeted rs1990622^{A} (allele frequency = 0.58), the index SNP tagging *TMEM106B* risk variants based on the recent GWAS. Subsequent fine mapping of the locus interrogated a total of 260 additional SNPs that covered both genic as well as 10 kB flanking area of the *TMEM106B* gene.

Brain DNA methylation, miRNA, and RNA expression data were generated using frozen postmortem dorsolateral prefrontal cortex brain tissue, as previously described.[25] Details are in e-Methods.

Statistical analyses. Individual testing hypotheses (figure 1) were based on the findings in the FTLD literature. We first fit an ordinal logistic regression model with 4-level TDP-43 stage measure as the categorical outcome and rs1990622^{A} the predictor, adjusted for age at death and sex. Here rs1990622^{A} defines the dosage with respect to the SNP's A allele, and was used to estimate the odds ratio (OR) for more advanced TDP-43 pathology with every additional copy of the A allele. Next, we expanded the model by including pathologic indices of AD, chronic macro and micro infarcts, neocortical Lewy bodies, as

Figure 1 Relationship and potential pathways linking *TMEM106B* variant and transactive response DNA binding protein 43 pathology



Each line in the figure represents a hypothesis tested in this study. The primary hypothesis is that transmembrane protein 106B (*TMEM106B*) variants are related to transactive response DNA binding protein 43 (TDP-43) pathology in aging after controlling for other common age-related pathologies. A (1 and 2) indicates the *TMEM106B* expression pathway; B (1 and 2) indicates granulin expression pathway; and C and D indicate potential epigenetic pathways. Solid lines indicate those relationships that were found to be significant. Dashed lines show relationships that did not reach significance (note miR132 pathway was not significant after controlling for other neurodegenerative pathologies).

well as hippocampal sclerosis. The score tests assessed proportional odds assumption for the ordinal logistic regression. To confirm the signal, we interrogated additional SNPs in the *TMEM106B* locus. Specifically, for each of the 260 SNPs queried, we tested individual SNP association with TDP-43 pathology using an ordinal logistic regression.

Using multiple linear regression models, we examined association of rs1990622 with brain RNA expression of *TMEM106B*, and separately *GRN*. In these models, RNA expression level was the outcome and rs1990622 variant the predictor, adjusted for age at death, sex, postmortem intervals, and RNA degradation measure. We also examined association of RNA expression with TDP-43 pathology using similar ordinal logistic regressions mentioned above. Spearman correlation examined the correlation between the *TMEM106B* and *GRN* expression.

Finally, we explored potential epigenetic mechanisms that link the *TMEM106B* risk variant to the downstream TDP-43 pathology. First, we scanned DNA methylation of CpG sites in the *GRN* locus, and we regressed DNA methylation level of individual CpG sites on rs1990622^{A} dosage, adjusted for age at death, sex, batch, and bisulfite conversion efficiency. Next, we examined *cis* methylation association with RNA expression by fitting linear regression models with *GRN* RNA expression as the outcome and methylation of individual CpG sites the predictor. We also tested the hypothesis for association of miR132 with TDP-43 pathology.

RESULTS

TDP-43 pathology in older persons without FTLD. In 544 older persons without FTLD, 263 (48.3%) had no TDP-43 cytoplasmic inclusions; 113 (20.8%) had inclusions in amygdala only; 82 (15.1%) had inclusions in both amygdala and hippocampus CA1 or entorhinal cortex; and in the remaining 86 participants (15.8%), TDP-43 inclusions extended from the mesial temporal lobe to the neocortex. The distribution of TDP-43 pathology was not different between male and female participants. Older age at death was associated with more advanced TDP-43 pathology ($p < 0.0001$). Chronic infarcts, macroscopic or microscopic, were not associated with TDP-43 pathology, nor were neocortical Lewy bodies. In contrast, AD and hippocampal sclerosis were associated with TDP-43 pathology (table 1).

Table 1 Presence of neurodegenerative diseases by TDP-43 pathology[a]

	TDP stage 0 (n = 263)	TDP stage 1 (n = 113)	TDP stage 2 (n = 82)	TDP stage 3 (n = 86)	*p*
Age at death, y	87.5 (6.5)	88.8 (5.8)	91.6 (5.3)	91.3 (5.6)	<0.0001[b]
rs1990622^{A} dosage	1.10 (0.70)	1.13 (0.71)	1.19 (0.66)	1.31 (0.69)	0.016[c]
AD diagnosis	136 (51.71%)	72 (63.72%)	61 (74.39%)	71 (82.56%)	<0.0001[d]
Macroscopic infarcts	85 (32.32%)	43 (38.05%)	34 (41.46%)	38 (44.19%)	0.162[d]
Microinfarcts	73 (27.76%)	24 (21.24%)	21 (25.61%)	31 (36.05%)	0.136[d]
Neocortical Lewy bodies	25 (9.51%)	14 (12.39%)	15 (18.29%)	14 (16.28%)	0.122[d]
Hippocampal sclerosis	10 (3.82%)	6 (5.31%)	19 (23.17%)	35 (40.70%)	<0.0001[d]
***TMEM106B* expression**	3.91 (1.19)	3.78 (0.90)	3.72 (1.02)	3.59 (0.93)	0.284[b]

Abbreviations: AD = Alzheimer disease; TDP-43 = transactive response DNA binding protein 43; *TMEM106B* = transmembrane protein 106B.
Values are mean (SD) or n (%).
[a] Stage 0: no inclusions; stage 1: inclusions in amygdala only; stage 2: inclusions in amygdala as well as entorhinal cortex or hippocampus CA1; stage 3: inclusions in amygdala, entorhinal cortex or hippocampus CA1, and neocortex.
[b] *p* Values were assessed using analysis of variance.
[c] *p* Values were assessed using Kruskal-Wallis test.
[d] *p* Values were assessed using χ^2 test, all with 3 degrees of freedom.

***TMEM106B* variants and TDP-43 pathology.** The rs1990622 dosage, coded for the risk allele (A allele), differed by stages of TDP-43 pathology in older persons without FTLD ($\chi^2 = 10.36$, $df = 3$, $p = 0.016$). In a logistic regression model adjusted for age at death and sex, higher dosage of rs1990622^{A} was associated with more advanced TDP-43 pathology ($p = 0.019$). Next, we expanded the model by adjusting for common pathologic indices, and the association of rs1990622^{A} with TDP-43 pathology was robustly retained (table 2). Specifically, each additional A allele increased the odds of having more advanced TDP-43 stages by approximately one-third (OR 1.351, 95% confidence interval 1.068–1.709, $p = 0.012$). Further adjustment for *APOE* genotype in the model did not change the result. In addition, we did not find evidence of a separate SNP association with pathologic AD diagnosis (table 3).

We performed fine mapping by interrogating 260 additional SNPs in *TMEM106B* (table e-2). The top hit rs6460895^{C} was associated with advanced TDP-43 stages ($p = 0.0105$), consistent with the result for rs1990622^{A}. The SNP is an intronic variant in linkage disequilibrium of rs1990622, and the Spearman correlation of the 2 SNP dosages was 0.89. Further, a total of 59 SNPs had smaller p values than rs1990622, all of which were highly correlated with rs1990622 such that the pairwise correlations range from 0.86 to 0.99.

***TMEM106B* variant, RNA expression, and TDP-43 pathology.** Since prior literature suggests that *TMEM106B* variant influences FTLD susceptibility by regulating *GRN* level,[11] we examined whether rs1990622^{A} was associated with brain RNA expression of *TMEM106B* and *GRN* in dorsolateral prefrontal cortex (DLPFC) and subsequently whether expression of these genes was associated with TDP-43 pathology. RNA-Seq data were currently processed in a subset of the sample used in the main analysis (n = 345). As shown in figure 2A, both *TMEM106B* and *GRN* were expressed in postmortem brain and were negatively correlated (Spearman correlation = −0.270, $p < 0.0001$).

Higher dosage of rs1990622^{A} was associated with higher level of *TMEM106B* RNA expression ($\beta = 0.173$, $p = 0.0235$), and the association was independent of other neuropathologies. However, association of *TMEM106B* expression with odds of TDP-43 pathology did not reach statistical significance. Separately, we did not find direct association of rs1990622^{A} with *GRN* expression. We explored the genotypic association of the SNP by collapsing rs1990622^{A} dosage into a binary variable for rs1990622^{GG} homozygosity (table e-3). Interestingly, the *GRN* RNA expression level was lower in rs1990622^{GG} homozygous participants (figure 2C). In a linear regression model adjusted for age at death, sex, postmortem intervals, RNA degradation, and other common neuropathologies, the *GRN* expression was about 1.5 units higher in participants with rs1990622$^{AA/AG}$ than rs1990622^{GG} ($\beta = 1.536$, $p = 0.0392$). This result was marginally significant considering that the association of rs1990622 with the *GRN* expression was tested at both allelic and genotypic levels ($\alpha = 0.05/2 = 0.025$). Interestingly, higher level of *GRN* expression was associated with higher odds for more advanced TDP-43 stages (OR 1.059, $p = 0.0024$), and the association was unchanged after adjustment for other neuropathologies. In this reduced sample, the association of rs1990622^{A} with TDP-43 pathology (OR 1.230) was comparable to the result from the larger sample, but the p value was only suggestive ($p = 0.1679$).

***TMEM106B* risk variant and *GRN* DNA methylation.** To investigate whether the association of the *TMEM106B* risk variant with *GRN* expression may work through an alteration of methylation levels, we interrogated the 132 CpG sites found in the *GRN* locus that were sampled by our BeadChip. We first performed multiple linear regressions of DNA methylation on rs1990622^{A}. After correction for multiple testing ($\alpha = 0.05/132 = 0.0004$), we found 5 CpG sites in the *GRN* locus where the rs1990622^{A} dosage was associated with a small but significant decrease in methylation (table e-4). Four of these sites were located in the gene body, 3 of which were in an active enhancer (cg20727623, cg00628375, and cg16861818) based on our chromatin map of DLPFC generated from 2

Table 2 Association of *TMEM106B* variant with TDP-43 pathology

Model terms	OR (95% CI)	p
Age at death, y	1.066 (1.036, 1.096)	<0.0001
Male sex	0.876 (0.613, 1.253)	0.469
Pathologic AD diagnosis	1.888 (1.324, 2.693)	0.001
Macroscopic infarcts	1.231 (0.865, 1.751)	0.248
Microinfarcts	0.856 (0.586, 1.252)	0.424
Neocortical Lewy bodies	1.549 (0.954, 2.516)	0.077
Hippocampal sclerosis	6.590 (3.979, 10.917)	<0.0001
rs1990622^{A} dosage	1.351 (1.068, 1.709)	0.012

Abbreviations: AD = Alzheimer disease; CI = confidence interval; OR = odds ratio; TDP-43 = transactive response DNA binding protein 43; *TMEM106B* = transmembrane protein 106B. The model was conducted using ordinal logistic regression models with TDP-43 grade as the outcome and age at death, sex, AD pathologic diagnosis, macro and micro infarcts, neocortical Lewy bodies, hippocampal sclerosis, and rs1990622^{A} dosage as the predictors. The logits model the odds of more advanced TDP pathology against less advanced TDP pathology (that is, 1, 2, or 3 vs 0; 2 or 3 vs 0 or 1; and 3 vs 0, 1, or 2). Note that in addition to the relation of the index single-nucleotide polymorphism and TDP-43, age, AD, and hippocampal sclerosis also are separately related to TDP-43 stage (trend for neocortical Lewy bodies). Score test was performed to ensure the proportional odds assumption was satisfied ($\chi^2 = 20.989$, $df = 16$, $p = 0.179$).

Table 3 Association of *TMEM106B* variant with pathologic AD diagnosis

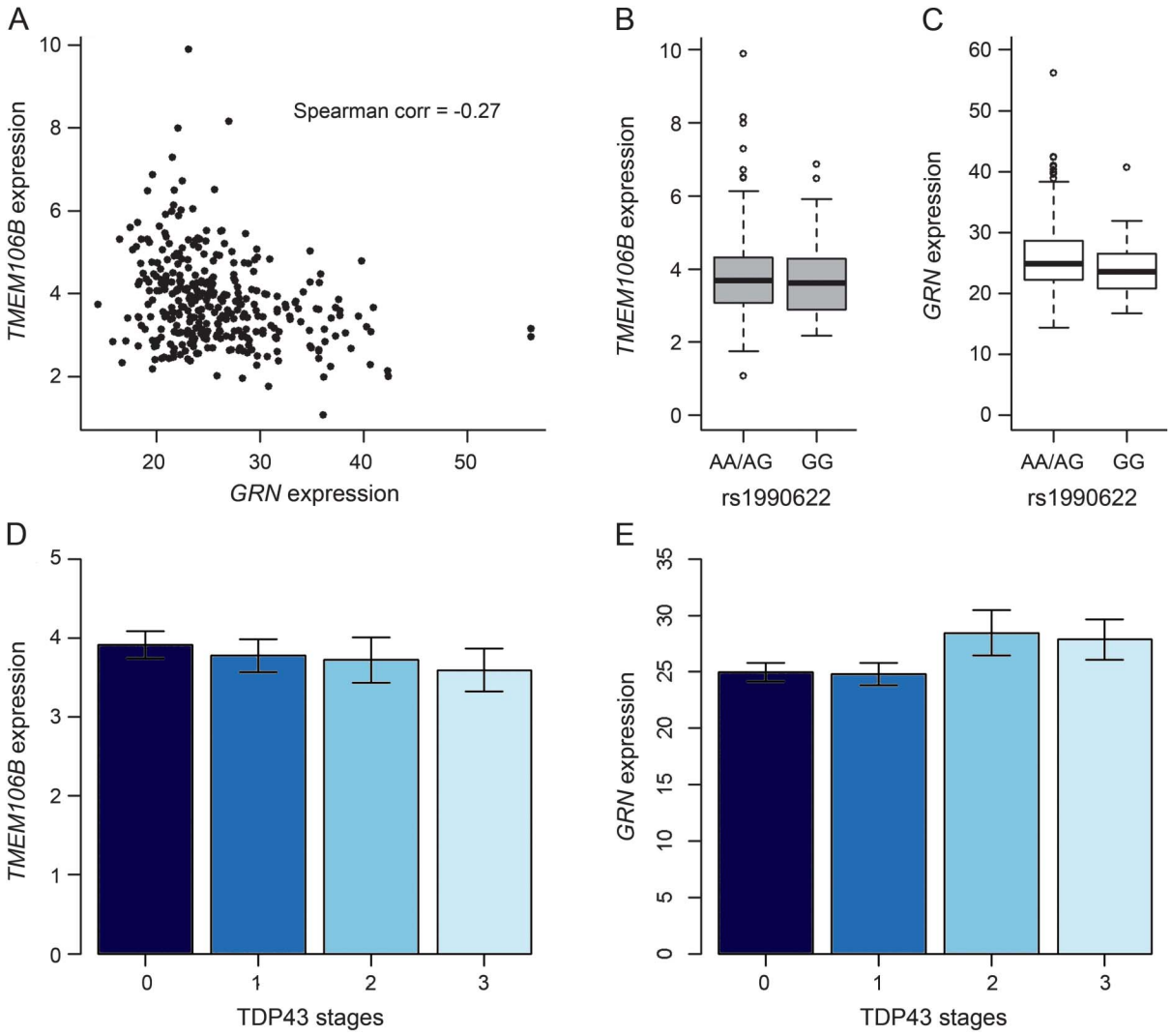
Model terms	OR (95% CI)	*p*
Age at death, y	1.045 (1.013, 1.077)	0.005
Male sex	0.810 (0.550, 1.192)	0.285
TDP-43 pathology	1.548 (1.293, 1.855)	<0.001
rs1990622[A] dosage	0.891 (0.685, 1.158)	0.386

Abbreviations: AD = Alzheimer disease; CI = confidence interval; OR = odds ratio; TDP-43 = transactive response DNA binding protein 43; *TMEM106B* = transmembrane protein 106B. The model was conducted using logistic regression with binary pathologic AD diagnosis as the outcome, and age at death, sex, TDP-43 pathology, and rs1990622[A] dosage as the predictors. The logits model the odds of AD present against absent.

older cognitively nonimpaired subjects with minimal neuropathologies.[26] The fourth one (cg13120756) was located next to the *GRN* transcription start site that is an active conformation in this map.

Next, we examined the *cis* association of CpGs in the *GRN* locus with the gene expression (table e-5). The methylation at the 5 CpGs influenced by rs1990622 was not found to be associated with *GRN* expression, so we could not formally evaluate the possibility that the effect of rs1990622[A] on *GRN* expression is mediated through methylation, probably because of our modest sample size. Looking at association of other CpGs with *GRN* RNA expression, the top site was cg24457026 ($p = 0.0003$), where methylation was associated with lower level of *GRN* expression. This CpG is located in the 5′UTR of the *GRN* gene that is in a strongly transcribed conformation in our map. This result suggests the existence of multiple distinct genomic features that likely influence the level of *GRN* expression. Notably, the association of *TMEM106B* variant with *GRN* RNA expression was slightly attenuated after

Figure 2 Single-nucleotide polymorphism rs1990622, *TMEM106B*, and *GRN* expression



(A) Scatterplot of *GRN* and transmembrane protein 106B (*TMEM106B*) expression level. (B) Box plot of *TMEM106B* expression level by rs1990622$^{AA/AG}$ vs rs1990622^{GG}. (C) Box plot of *GRN* expression level by rs1990622$^{AA/AG}$ vs rs1990622^{GG}. (D) Bar chart of *TMEM106B* expression level by transactive response DNA binding protein 43 (TDP-43) stages. (E) Bar chart of *GRN* expression level by TDP-43 stages.

additional adjustment for methylation at cg24457026 ($\beta = 1.420$, $p = 0.0549$).

miR132 expression and TDP-43 pathology. *TMEM106B* is a primary target of miR132, so we examined the association of miR132 expression with TDP-43 pathology. In a logistic regression model adjusted for age at death and sex, higher expression of miRNA was associated with less burden of TDP-43 pathology (OR 0.702, $p = 0.0173$). Since the miRNA has been reported to be downregulated in AD and FTLD,[27,28] we refit the model by further adjusting for other neuropathologies. Indeed, the association of miR132 with TDP-43 pathology was no longer significant after additional neuropathologic indices were added ($p = 0.7940$).

DISCUSSION It has been increasingly recognized that pathologic TDP-43 is a common pathology in aging, and contributes to late-life cognitive decline[29] and dementia.[30] In this study, more than half of our sample showed varying stages of accumulation of TDP-43 cytoplasmic inclusions. The major allele of the *TMEM106*B index SNP, rs1990622^A, which is associated with susceptibility to FTLD-TDP, also increases the risk for accumulation of TDP-43 pathology in older persons without FTLD-TDP. Fine mapping of the locus suggests that top variants, including rs1990622, are mapped onto a single haplotype without a clear candidate causal variant.

TDP-43 pathology is comorbid with other neuropathologies. It has been reported that the *TMEM106B* variant is associated with hippocampal sclerosis in people with pathologic diagnosed AD, and a separate polymorphism in the *ABCC9* gene is also shown to be associated with hippocampal sclerosis.[31] In our sample, over 85% of the cases with hippocampal sclerosis have TDP-43 inclusions. Interestingly, we find little evidence for a direct association of rs1990622^A with hippocampal sclerosis after adjusting for TDP-43 pathology. As contrast, we show that the association of the *TMEM106B* risk variant with TDP-43 is independent of AD and hippocampal sclerosis. There are several factors that might contribute to this finding. First, it is unknown whether these 2 pathologies share the same underlying neurobiology, and our results suggest that the *TMEM106B* variants target specifically at TDP-43, independent of hippocampal sclerosis. Second, hippocampal sclerosis is much less common than TDP-43 pathology, and the sample size in this study might not be sufficient to detect an association with hippocampal sclerosis. Further studies are warranted to disentangle the relationship of *TMEM10B* variant with TDP-43 and hippocampal sclerosis.

The biological mechanisms underlying the variant association in FTLD remain an active area of investigation. Prior studies on FTLD have proposed a potential pathway such that *TMEM106B* variants upregulate the *TMEM106B* expression,[6] which subsequently downregulates the *GRN* expression,[11] and GRN protein in general is neuronal protective.[32] In our brain sample, we did not find association of *TMEM106B* expression with TDP-43 pathology; on the other hand, our result shows upregulation of *GRN* expression in participants who are heterozygous or homozygous for rs1990622^A. Subsequently, higher level of *GRN* expression is related to more advanced TDP-43 pathology. Increased expression of *GRN* has been reported in patients with ALS.[33] A possible explanation is that *GRN* is overexpressed in response to the neuronal inflammation or degeneration. Such overexpression is hypothesized to be the result of microglial activation.[34] Microglia data collection is in progress (n = 122), and these preliminary data suggest a trend for a positive association of activated microglia and *GRN* expression (data not shown).

Epigenetic factors are also implicated in the pathway from the *TMEM106B* variants to FTLD-TDP, which include promoter DNA methylation in *GRN* and miR132/212 cluster. Data from this study suggest that *TMEM106B* risk variant downregulates DNA methylation in an active enhance region of the *GRN* locus, and methylation at a strong transcription region in 5′UTR of the *GRN* gene is associated with a lower level of *GRN* expression. In addition, our result on miR132 suggests that its association with TDP-43 pathology might be confounded by the role of miR132 in other neuropathologies, potentially AD. Clarification of directionality and elucidating biological mechanisms, including RNA expression and brain epigenetic changes, that potentially link *TMEM106B* to TDP-43 pathology will require larger numbers and further study.

The study has strengths as well as limitations. Although we excluded persons with a neuropathologic diagnosis of FTLD, the nosology of FTLD continues to evolve and the relationship between TDP-43 of aging and FTLD-TDP may not always be clear. Moreover, early disease and mixed pathologies may obscure an FTLD diagnosis. Regardless of nosology, the current data show that TDP-43, a common and deleterious pathology in aging, is independently related to *TMEM106B* variants, expanding the significance of this variant and related biological pathways. To our knowledge, this is the first study that investigates and confirms an independent association of *TMEM106B* variants with TDP-43 pathology in older persons without FTLD. Multilevel genetic, brain epigenetic, and RNA expression data are analyzed in combination with neuropathologic

measures collected from hundreds of autopsied participants. These data allow testing the primary hypothesis on genetic variant association with TDP-43, and also provide an opportunity to explore multiple pathways between *TMEM106B* and TDP-43 pathology in aging. Additional studies are needed to replicate these findings and further elucidate the genetic basis of TDP-43 pathology in aging.

AUTHOR CONTRIBUTIONS

Drafting/revising the manuscript for content: Drs. Yu, De Jager, Yang, Trojanowski, Bennett, and Schneider. Study concept or design: Drs. Yu, Bennett, and Schneider. Analysis or interpretation of the data: Drs. Yu, De Jager, Yang, Trojanowski, Bennett, and Schneider. Acquisition of data: Drs. De Jager, Bennett, and Schneider. Statistical analysis: Dr. Yu. Study supervision or coordination: Dr. Schneider. Obtaining funding: Drs. Bennett and Schneider.

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DISCLOSURE

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