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Association Between Brain Gene Expression, DNA Methylation, and Alteration of Ex Vivo Magnetic Resonance Imaging Transverse Relaxation in Late-Life Cognitive Decline

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Additional Information: To obtain data from the Religious Orders Study and the Rush Memory and Aging Project for research use, please visit the Rush Alzheimer's Disease Center Research Resource Sharing Hub (http://www.radc.rush.edu).

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Abstract

IMPORTANCE—Alteration of ex vivo magnetic resonance imaging transverse relaxation is associated with late-life cognitive decline even after controlling for common neuropathologic conditions. However, the underlying neurobiology of this association is unknown.

OBJECTIVE—To investigate the association between brain gene expression, DNA methylation, and alteration of magnetic resonance imaging transverse relaxation in late-life cognitive decline.

DESIGN, SETTING, AND PARTICIPANTS—Data came from 2 community-based longitudinal cohort studies of aging and dementia, the Religious Orders Study, which began in 1993, and the Rush Memory and Aging Project, which began in 1997. All participants agreed to undergo annual clinical evaluations and to donate their brains after death. By October 24, 2016, a total of 1358 individuals had died and had brain autopsies that were approved by board-certified neuropathologists. Of those, 552 had undergone ex vivo imaging. The gene expression analysis was limited to 174 individuals with both imaging and brain RNA sequencing data. The DNA methylation analysis was limited to 225 individuals with both imaging and brain methylation data.

MAIN OUTCOMES AND MEASURES—Maps of ex vivo magnetic resonance imaging transverse relaxation were generated using fast spin echo imaging. The target was a composite measure of the transverse relaxation rate (R_2) that was associated with cognitive decline after controlling for common neuropathologic conditions. Next-generation RNA sequencing and DNA methylation data were generated using frozen tissue from the dorsolateral prefrontal cortex. Genome-wide association analysis was used to investigate gene expression and, separately, DNA methylation for signals associated with the R_2 measure.

RESULTS—Of the 552 individuals with ex vivo imaging data, 394 were women and 158 were men, and the mean (SD) age at death was 90.4 (6.0) years. Four co-expressed genes (*PADI2* [Ensembl ENSG00000117115], *ZNF385A* [Ensembl ENSG00000161642], *PSD2* [Ensembl ENSG00000146005], and *A2ML1* [Ensembl ENSG00000166535]) were identified, of which higher expressions were associated with slower R_2. The association of R_2 with cognitive decline was attenuated when the gene expression signals were added to the model, such that the mean (SE) coefficient of association was reduced from 0.028 (0.008) ($P < .001$) to 0.019 (0.009) ($P = .03$). The DNA methylation scan did not detect a genome-wide significant signal, but it revealed an anticorrelation between R_2 and DNA methylation in many of the cytosine-guanine dinucleotides.

CONCLUSIONS AND RELEVANCE—Brain gene expression and DNA methylation dysregulations are implicated in the alteration of brain tissue properties associated with late-life cognitive decline above and beyond the influence of common neuropathologic conditions.

As approximately 14 million Americans 65 years of age or older will have Alzheimer disease (AD) dementia by 2050,[1] prevention and treatment of AD dementia becomes increasingly important to public health. Alzheimer disease, cerebrovascular disease, Lewy body disease, hippocampal sclerosis, and the more recently observed transactive response DNA-binding protein 43 (TDP-43) are common neuropathologic conditions that contribute to late-life cognitive decline, the clinical hallmark of AD dementia.[2–5] Yet, a majority of person-to-person variations in cognitive decline are not attributable to these pathologic conditions, suggesting that additional determinants of cognitive decline remain to be identified.[6] Ex vivo brain magnetic resonance imaging (MRI) complements common neuropathologic measures by providing information on brain structure and tissue integrity that is not captured by traditional histopathologic findings. In particular, transverse relaxation properties measure the integrity of neural tissue and are important correlates of cognition and memory.[7] A slower transverse relaxation rate (R_2) in white matter regions is associated with poorer cognitive performance and faster cognitive decline.[8–11] The association between R_2 and cognitive decline is in addition to the influence of key neuropathologic measures.[11] Investigation of the neurobiology underlying the R_2 alteration in cognitive decline may lead to novel therapeutic targets to combat late-life cognitive impairment and AD dementia.

Genes dictate cell function, and gene expression profiling has proved to be fundamental in understanding the genetic architecture of complex human diseases. In addition, gene expression can be regulated by epigenetic markers. DNA methylation, the addition of a methyl group to the cytosine base of cytosine-guanine dinucleotides (CGs), is one of the most common epigenetic markers that regulate access to DNA sequence and, consequently, gene expression. Identifying differentially expressed genes and differentially methylated CGs that are implicated in R_2 and cognitive decline holds the potential to better understand the transcriptomic and epigenetic footprints underlying the association between R_2 and cognitive impairment.

In this study, we investigated gene expression and DNA methylation in postmortem human brains, examined their association with R_2 alteration, and explored the degree to which these markers contribute to the association between R_2 and cognitive decline. Post mortem

specimens came from autopsied participants from 2 ongoing clinicopathologic cohort studies of aging and dementia. Genome-wide gene expression and DNA methylation profiles were generated using frozen tissue from the dorsolateral prefrontal cortex (DLPFC). We targeted a composite ex vivo R_2 measure that was associated with cognitive decline after controlling for common neuropathologic indices.

Methods

Study Participants

Participants came from the Religious Orders Study and the Rush Memory and Aging Project.[12,13] Participants were community-based older persons without known dementia who agreed to annual, detailed clinical and cognitive evaluations and to donate their brains after death. Both studies were approved by the Rush University Medical Center institutional review board. Written informed consent was obtained from each participant, and each participant signed an Anatomical Gift Act consent donating his or her brain.

The Religious Orders Study started in 1993, and the Rush Memory and Aging Project started in 1997. At the time of the analyses (October 24, 2016), 3308 participants were enrolled, 1599 had died, and 1383 had undergone autopsy. The autopsy rate was 86.5%. Of the autopsied participants, 1358 cases were reviewed and approved by board-certified neuropathologists. The collection of ex vivo imaging, RNA sequencing, and DNA methylation data started later. As a result, ex vivo imaging was available for 552 autopsy-approved participants. The analysis for genome-wide gene expression was limited to 174 participants with available imaging and RNA sequencing data. The analysis for genome-wide DNA methylation was limited to 225 participants who had both imaging and brain DNA methylation data. The Venn diagram (eFigure 1 in the Supplement) describes the sample sizes across different data sets. The demographic and neuropathologic characteristics were largely comparable across the samples (Table 1).

Ex Vivo MRI and R_2 Associated With Cognitive Decline

Brains were removed and bisected after death following a standard procedure. Hemispheres chosen for imaging were fixed in 4% paraformaldehyde and stored in refrigerators at 4°C. Scans were performed a mean (SD) of 41.1 (19.2) days (range, 23-235 days) after death, and each scan included a 2-dimensional turbo spin echo sequence with multiple echo times. The original R_2 value was estimated voxel by voxel from the turbo spin echo data by fitting a monoexponential decay function, then normalized within each group of hemispheres imaged on a given MRI scanner.[14,15] Details on scanner-specific normalization are provided in the eAppendix in the Supplement.

We considered but elected not to restrict the R_2 metric to the DLPFC, the actual tissue collection site where RNA sequencing and DNA methylation data were obtained. The literature extensively supports the functional and structural connectivity of the DLPFC with other brain regions, and genes expressed or methylated in 1 region are closely linked to, and possibly even influence, imaging signatures throughout the brain. Therefore, we focused on a composite R_2 measure that captures the overall relaxation associated with cognitive

decline above and beyond the influence of commonly measured neuropathologic conditions.[11]

In brief, person-specific rates of cognitive decline (ie, slopes) were estimated using longitudinal data from annual cognitive assessments prior to death. Next, voxelwise linear regressions were performed by regressing person-specific rates of decline against the R_2 value of each of approximately 400 000 tissue-containing voxels, controlling for the demographics and 9 common neuropathologic indices. A false discovery rate of 0.05 was used to correct for multiple testing. These analyses identified 4 clusters of voxels, each consisting of at least 100 contiguous voxels (100 mm^3) that were associated with cognitive decline (Figure 1). A composite score of R_2 was obtained by averaging individual voxel R_2 values first within and then across clusters and standardized using the overall mean and SD. The use of a composite R_2 measure was supported by the positive intercorrelations among individual clusters and the result from exploratory factor analysis (eFigure 2 and 3 in the Supplement). Specifically, pairwise Pearson correlations between clusters ranged from 0.43 to 0.63 ($P < .001$ for all correlations). Furthermore, only 1 principal component was retained in the factor analysis, which contributes to most (65%) of the total variance.

Gene Expression and DNA Methylation

Details on gene expression and DNA methylation data processing are provided in the eAppendix in the Supplement. In brief, RNA and DNA samples were extracted from frozen DLPFC tissue, and RNA sequencing was performed on the Illumina HiSeq (Illumina Inc). The gene expression level was measured, with higher value corresponding to higher expression. Genome-wide DNA methylation data were generated using the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc). The methylation level at each CG site was measured by a β value ranging from 0 (unmethylated) to 1 (fully methylated).

Neuropathologic Evaluations

At autopsy, systematic evaluations assessed burdens of common age-related neuropathologic conditions, including AD, macroscopic infarcts, microinfarcts, Lewy bodies, hippocampal sclerosis, cerebral amyloid angiopathy, TDP-43, atherosclerosis, and arteriolosclerosis. Details on the quantification of these pathologic conditions are provided in the eAppendix in the Supplement.

Longitudinal Cognitive Assessments

Uniform cognitive assessments were administered each year at baseline and at follow-up visits. The cognitive testing battery included 17 cognitive tests. Scores on each test were standardized using the baseline mean and SD of the entire cohort. A composite score of global cognition was obtained by averaging individual standardized scores across the tests.[16] Higher score indicates better cognition.

Statistical Analysis

Bivariate associations between the R_2, neuropathologic, and cognitive measures were examined using pairwise correlations, 2-sided *t* tests, or analysis of variance, as appropriate. Multivariable linear regression models were used to examine gene expression. In these

models, the composite R_2 measure was the continuous outcome, and individual gene expression was the predictor. The corresponding regression coefficient estimates the difference in standardized R_2 value with every 1-SD increase in gene expression level. Genome-wide statistical significance for gene expression analysis was determined at an α level of 2×10^{-6} (ie, Bonferroni correction for approximately 23 000 genes targeted).

Similar linear regression models were used to interrogate the association of DNA methylation with R_2. In these models, the R_2 measure was the continuous outcome, and the methylation level of individual CG was the predictor. The regression coefficient estimates the difference in standardized R_2 value with every 1-SD increase in methylation. Genome-wide statistical significance for DNA methylation was determined at an α level of 1×10^{-7} (ie, Bonferroni correction for approximately 500 000 CGs targeted). For both analyses, the models were adjusted for age, sex, postmortem interval to imaging, and other technical confounders, as well as neuropathologic indices for AD, macroscopic infarcts, microinfarcts, Lewy bodies, hippocampal sclerosis, TDP-43, cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis. All analyses were conducted using SAS/STAT software, version 9.3 (SAS Institute Inc), and R software, version 3.3.1 (R Foundation for Statistical Computing). Unless otherwise noted, $P < .05$ was considered significant.

Results

Common Neuropathologic Conditions, Cognitive Decline, and R_2

At autopsy, 376 of the study participants (68.1%) had a pathologic AD diagnosis, 190 (34.4%) had macroscopic infarcts, and 171 (31.0%) had microinfarcts (Table 1). Lewy bodies were present in 127 of the participants (23.0%), hippocampal sclerosis in 65 (11.8%), and TDP-43 in 312 (56.5%). Other neuropathologic conditions are described in Table 1. The mean (SD) Mini-Mental State Examination score of these participants decreased from 27.6 (2.8) at baseline to 20.3 (9.3) proximate to death, consistent with an overall decline in cognition; the annual mean (SE) rate of change in global cognition, as measured by annual cognitive assessments spanning a mean of 9 years, was −0.100 (0.004) standard units ($P < .001$).

The bivariate association between the R_2 measure and neuropathologic indices was consistent with a previous report.[17] As shown in eFigure 4 in the Supplement, slower R_2 was correlated with higher burden of AD pathology. Individuals with macroscopic infarcts or hippocampal sclerosis had slower R_2. Slower R_2 was also associated with more severe TDP-43, atherosclerosis, and arteriolosclerosis. On the other hand, we did not observe a strong association between R_2 and microinfarcts, Lewy body, or cerebral amyloid angiopathy. In a regression analysis with all the neuropathologic measures included in the same model, AD, macroscopic infarcts, hippocampal sclerosis, TDP-43, and arteriolosclerosis remained associated with slower R_2 (eTable 1 in the Supplement). After adjustment for demographics and all 9 neuropathologic indices, slower R_2 was strongly associated with faster decline in cognition (estimate [SE], 0.023 [0.004]; $P < .001$) (Table 2).

Brain Gene Expression and R_2

We investigated the RNA sequencing data by regressing the R_2 measure associated with cognitive decline on individual gene expression. Four gene expression signals (ie, *PADI2* [Ensembl ENSG00000117115], *ZNF385A* [Ensembl ENSG00000161642], *PSD2* [Ensembl ENSG00000146005], and *A2ML1* [Ensembl ENSG00000166535]) reached genome-wide significance. In all cases, higher expression of these genes was associated with slower R_2 (Table 3). Partial residuals plots support a robust negative association between R_2 and individual gene expression (eFigure 5 in the Supplement). The lead gene is *PADI2*, which encodes a peptidyl arginine deiminase enzyme that promotes posttranslational protein citrullination. The overexpression of *PADI2* has been implicated in neurodegeneration and demyelination.[18,19] The other 3 genes are lesser known. *ZNF385A* encodes a regulatory zinc finger protein that functions as a transcription factor, binds RNA, or interacts with other proteins.[20] *PSD2* is involved in phospholipid binding based on gene ontology. *A2ML1* encodes an *N*-glycosylated monomeric protein that acts as a protease inhibitor. These 4 genes were highly coexpressed (eFigure 6 in the Supplement), such that the pairwise Pearson correlation coefficients ranged from 0.56 to 0.74 ($P < .001$ for all correlations). Additional top hits are shown in eTable 2 in the Supplement.

Brain DNA Methylation and R_2

We investigated genome-wide DNA methylation data for differentially methylated CGs in relation to the R_2 measure associated with cognitive decline. A summary of the results is illustrated in eFigure 7 in the Supplement. None of the CGs survived the Bonferroni correction for multiple testing. A total of 6CGs (eTable 3 in the Supplement) reached a suggestive significance level of $\alpha = 1 \times 10^{-5}$. Methylation in all 6 CGs was anticorrelated with the R_2 measure such that higher methylation was associated with slower R_2. Furthermore, the anticorrelation pattern was evident in many of the CGs investigated and was robust against different choices of statistical cutoffs. The results from proportions (χ^2) tests suggest that the number of CGs anticorrelated with R_2 was highly enriched regardless of the cutoffs. To illustrate, Figure 2A shows that, of the 1221 CGs that reached the significance level of $\alpha = 0.001$, 979 (80.2%) were anticorrelated ($P < .001$). A similar pattern was observed in gene expression (Figure 2B).

R_2 Association With Cognitive Decline Attenuated by Gene Expression Signals

We assessed the extent to which R_2 association with cognitive decline was attenuated by the top gene expression signals. To do so, we fit a series of linear mixed models with annual global cognitive scores as the continuous longitudinal outcome. The reference model included only demographics as the predictors; the second model (eTable 4 in the Supplement; model A) included demographics and the neuropathologic indices. The reduction of the variance estimates for random slope between the 2 models captures the variability of person-specific rates of cognitive decline explained by common neuropathologic conditions. In this sample, neuropathologic conditions explained 43% of the variance, and the result is consistent with a previous report for the overall cohorts.[6] The third model included demographics, neuropathologic indices, and the composite R_2 measure (eTable 4 in the Supplement; model B), in which R_2 explained an additional 6% of the

variance. Next, we augmented the model by including gene expression signals identified from the RNA sequencing (eTable 4 in the Supplement; model C). The mean expression averaged across the 4 genes was used, considering the strong co-expression. With the gene expression signal added to the model, we observed an attenuation of R_2 association with cognitive decline such that the mean (SE) coefficient was reduced from 0.028 (0.008) ($P < .001$) to 0.019 (0.009) ($P = .03$). Furthermore, after accounting for the gene expression, the percent variance explained by R_2 decreased to 1%.

Since the presence of the apolipoprotein E (*APOE* [Ensembl ENSG00000130203]) ε4 is strongly associated with cognitive decline and neuropathologic conditions, we conducted secondary analyses to examine whether our results were affected by the presence of *APOE* ε4. Consistent with findings in prior reports,[21,22] the phenotypic associations of ε4 were diminished after adjusting for AD and other neuropathologic conditions. Moreover, the primary results for R_2 association with cognitive decline were unchanged after the models were controlled for *APOE* ε4 (eTable 5 and 6 in the Supplement).

Discussion

Much of the person-to-person variation in late-life cognitive decline is not due to AD or other common neuropathologic conditions.[6] Identification of additional factors associated with cognitive decline may lead to novel therapeutic targets to prevent loss of cognition in old age. Alteration of R_2 measured using ex vivo MRI captures change in brain tissue integrity, which provides a unique window into the neurobiology of cognitive decline.[11] We previously showed that the association of R_2 with cognitive decline persisted after controlling for multiple neuropathologic indices, suggesting that slowing R_2 represents a separate determinant of cognitive decline.[11] In this study, we explored genome-wide transcriptomic and epigenetic correlates that underlie this association. New findings and implications are discussed.

First, multiple genome-wide significant gene signals are identified. The top gene, *PADI2*, is of particular relevance. *PADI2* encodes a posttranslational modification enzyme that catalyzes citrullination, a process that converts arginine residues into citrullines in proteins. Citrullination in myelin basic protein in particular destabilizes myelin structure. A transgenic mouse model shows that overexpressed *PADI2* leads to demyelination in the central nervous system.[23] Thus, the gene plays a key role in demyelinating diseases, such as multiple sclerosis,[24] for which T_2 (reciprocal of R_2) lesions are a radiologic hallmark.[25] Evidence shows that, in brains of individuals with multiple sclerosis, *PADI2* expression was elevated and the *PADI2* promoter was hypomethylated.[19,26] We targeted CG sites in the *PADI2* locus but did not find a significant association with R_2 using an omnibus test.[27] Upregulations of *PADI2* expression and citrullinated proteins have also been implicated in neurodegenerative diseases, including prion diseases and AD.[18,28,29] Using Western blot analyses, a prior study detected citrullinated proteins in hippocampal tissues from patients with AD but not healthy controls, and the amount of PAD2 was significantly higher in ADs.[18] The other 3 genes, namely *ZNF385A, PSD2*, and *A2ML1*, are strongly coexpressed with *PADI2;* however, their biological connections with R_2 alteration are unclear.

Second, the investigation of individual CGs did not yield a genome-wide significant signal for R_2 associated with cognitive decline. One contributing factor is that, compared with gene expression, epigenomic markers, such as DNA methylation, are further away from the phenotype along the biological causal chain. Consequently, the association is likely weaker and requires a larger sample size to detect. Rather than targeting site-specific signals, an alternative approach focusing on methylation module networks helps to reduce the burden for multiple testing and to reveal a clearer picture of the role of DNA methylation. These works are currently ongoing. We observed that higher DNA methylation tends to be associated with slower R_2; the direction of this association was consistent across a massive number of CGs. This pattern suggests that the association between DNA methylation and R_2 may not be loci specific. A popular hypothesis for the independent association of R_2 with late-life cognitive decline is that a prolonged brain transverse relaxation time represents brain pathologies not captured by our current neuropathologic indices. Considering that R_2 alteration is a manifestation of tissue damage due to pathologic conditions, global DNA hypermethylation may be a biological consequence of such an insult. Similar findings have been previously reported with respect to chronological age,[30,31] in which brain DNA methylation is elevated with aging and the association tends to be global.

Finally, the R_2 association with cognitive decline is attenuated by the lead gene expression signals. This finding lends support to the idea that dysregulation of genes, including *PADI2*, is associated with late-life cognitive impairment above and beyond the influence of common neuropathologic conditions. If confirmed, our findings could provide potential gene targets for neuropathologic conditions that cause R_2 alteration and, in turn, cognitive impairment and AD dementia. Earlier evidence from the multiple sclerosis mouse model demonstrated that protein hypercitrullination is reversible and disease progression is attenuated by inhibiting the peptidyl-arginine deiminase enzymes.[32]

Transverse relaxation properties can be measured in vivo and ultimately may serve as a biomarker in research on aging. A pilot study compared in vivo and ex vivo T_2 maps using data from 28 individuals.[17] The results showed strong linear correlations between the 2 sets of values across multiple brain regions, suggesting that ex vivo findings in this study may be generalizable to in vivo cases. Furthermore, while ex vivo R_2 is the most readily available metric in our current data pool that can be matched with autopsied cases from which brain RNA sequencing, DNA methylation, and neuropathologic data are also available, other imaging markers might account for additional variation in cognitive decline above and beyond that explained by neuropathologic findings. In vivo MRI measures will eventually be available for a greater number of autopsied cases, but at the moment, these numbers are fewer than 100. Using preliminary data, we observed positive correlations between R_2 and in vivo hippocampal volume and entorhinal cortex thickness. Other postmortem MRI modalities and metrics, such as R_2*, diffusion imaging, and volumetrics, have not yet been developed as extensively as R_2, although work is also ongoing to expand into these areas. Thus, as sufficient data accrue, we will be able to examine the association between various imaging markers, neuropathologic conditions, genomics, and cognitive decline.

Limitations

This study has some limitations. We applied the voxelwise analyses to empirically identify the regions of interest for the R_2 measure. Because the genomic data are generated using DLPFC tissue, the current approach lacks the intuitive appeal of spatially matching with the actual tissue collection site. Future studies on DLPFC-specific R_2 metrics are warranted to better understand the association of ex vivo brain integrity with brain genomics. Our DNA methylation data, generated using the Illumina 450 assay, preferentially target regions with a high frequency of CG sites, and the assay probes only a small fraction of the epigenome. In addition, the assay does not differentiate between cytosine methylation and hydroxymethylation. Furthermore, both the Religious Orders Study and the Rush Memory and Aging Project are voluntary cohorts, and the participants are generally older and have higher education levels. Our findings await replications from other clinicopathologic studies.

Conclusions

Taken together, these results suggest that both DNA methylation and gene expression dysregulations are involved in R_2 alteration in late-life cognitive impairment. To our knowledge, this is the first study to investigate the correlates of genome-wide gene expression and DNA methylation with transverse relaxation in postmortem brains. Ex vivo imaging techniques provide novel metrics for brain structure and tissue integrity that are complementary to traditional histopathologic indices. By leveraging both transcriptomic and epigenomic data measured from the same participants in whom we have detailed clinical, neuropathologic, and imaging data, this study offers further insight into the genetic footprints that underlie the association of brain tissue properties with cognitive impairment and dementia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

Question

What are the associations between brain gene expression, DNA methylation, and alteration of ex vivo magnetic resonance imaging transverse relaxation in late-life cognitive decline above and beyond common neuropathologic conditions?

Findings

From analysis of data from longitudinal clinicopathologic cohort studies of aging, 4 genes were identified (ie, *PADI2*, *ZNF385A*, *PSD2*, and *A2ML1*), of which higher expression was associated with a slower transverse relaxation rate. Furthermore, an anticorrelation pattern between the transverse relaxation rate and DNA methylation in many of the cytosine-guanine dinucleotides was observed.

Meaning

Gene expression and DNA methylation are implicated in the alteration of brain tissue properties associated with cognitive decline.

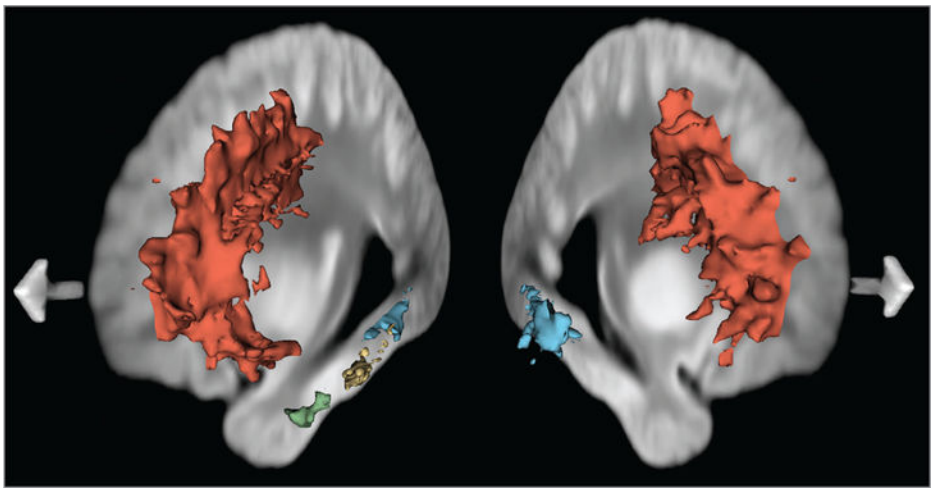

Figure 1. Transverse Relaxation Rate (R_2) Regions Associated With Cognitive Decline

Two separate views are shown of the 4 white matter regions (colorized) in which the R_2 was associated with late-life cognitive decline (global cognition). To provide spatial context, the 3-dimensional regions are superimposed on a 2-dimensional sagittal slice of an exvivo brain hemisphere template, shown in gray scale. The same 2-dimensional slice is used in both views, with the arrow oriented in the anterior direction in both cases. The left image is captured from a more medial camera position, while the right image is captured from a more lateral camera position.

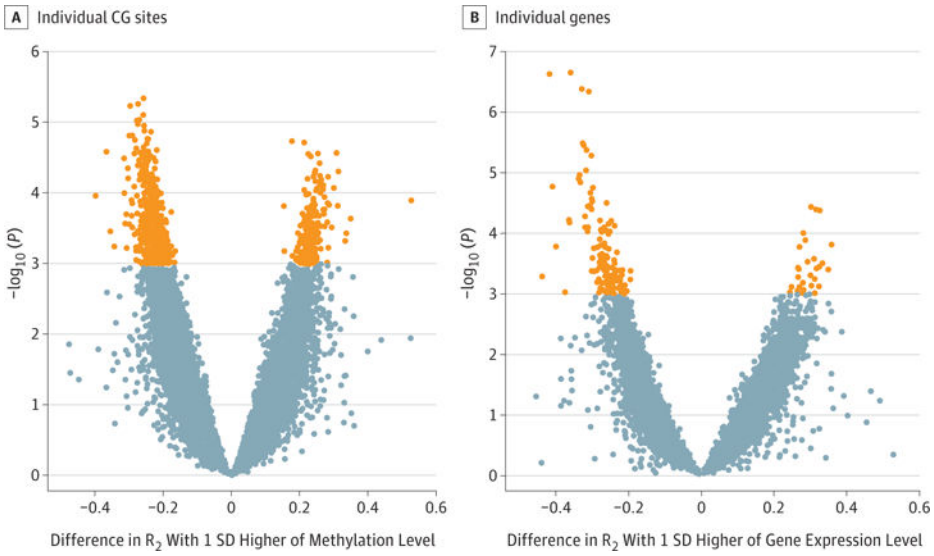

Figure 2. GenomicAssociations With the Transverse Relaxation Rate(R_2) Measure

A, Significance level vs the regression estimates for individual cytosine-guanine dinucleotide (CG) sites. B, Significance level vs the regression estimates for individual genes. Orange dots represent CGs (A) and genes (B) that reached the significance level of $P < .001$. In both cases, most of the top signals are anticorrelated with R_2 such that the higher the methylation (or gene expression), the slower the relaxation rate.

Table 1

Characteristics of the Study Participants

Characteristic	Participants, No. (%)[a]		
	N_0 = 552	N_1 = 174	N_2 = 225
Age, mean (SD), y	90.4 (6.0)	90.1 (5.9)	89.7 (5.8)
Female sex	394 (71.4)	122 (70.1)	156 (69.3)
Educational level, mean (SD), y	15.8 (3.6)	15.8 (3.6)	15.7 (3.6)
Non-Hispanic white	532 (96.6)	171 (98.3)	221 (98.2)
MMSE scores at baseline, mean (SD)	27.6 (2.8)	27.2 (3.1)	27.2 (3.1)
MMSE scores proximate to death, mean (SD)	20.3 (9.3)	20.5 (9.2)	19.9 (9.4)
NIA-Reagan AD	376 (68.1)	108 (62.1)	148 (65.7)
Macroscopic infarcts	190 (34.4)	59 (33.9)	79 (35.1)
Microinfarcts	171 (31.0)	45 (25.9)	59 (26.2)
Lewy bodies	127 (23.0)	33 (19.0)	45 (20.0)
Hippocampal sclerosis	65 (11.8)	22 (12.6)	27 (12.0)
TDP-43	312 (56.5)	105 (60.3)	132 (58.6)
Moderate to severe CAA	194 (35.1)	63 (36.2)	79 (35.1)
Moderate to severe atherosclerosis	153 (27.7)	76 (43.7)	98 (43.6)
Moderate to severe arteriolosclerosis	146 (26.5)	62 (35.6)	75 (33.3)

Abbreviations: AD, Alzheimer disease; CAA, cerebral amyloid angiopathy; MMSE, Mini-Mental State Examination; N_0, number of participants with ex vivo imaging data; N_1, number of participants with both ex vivo imaging and RNA gene expression data; N_2, number of participants with both ex vivo imaging and DNA methylation data; NIA-Reagan AD, pathologic AD diagnosis based on modified National Institute on Aging Reagan criteria; TDP-43, transactive response DNA-binding protein 43.

[a]Data are presented as number (percentage) of participants unless otherwise indicated.

Table 2

Transverse Relaxation Rate (R_2) and Cognitive Decline Controlling for Common Neuropathologic Indices[a]

Characteristic	Estimate (SE)	*P* Value
Age	0.002 (0.0006)	<.001
Male sex	0.006 (0.008)	.46
Education	0.002 (0.001)	.009
Alzheimer disease	−0.056 (0.006)	<.001
Macroscopic infarcts	−0.023 (0.008)	.003
Microinfarcts	0.007 (0.008)	.35
Lewy bodies	−0.026 (0.008)	.002
Hippocampal sclerosis	−0.044 (0.012)	<.001
TDP-43	−0.006 (0.003)	.08
Cerebral amyloid angiopathy	−0.003 (0.003)	.44
Atherosclerosis	−0.017 (0.004)	<.001
Arteriolosclerosis	−0.006 (0.004)	.14
R_2	0.023 (0.004)	<.001

Abbreviation: TDP-43, transactive response DNA-binding protein 43.

[a]For a total of 522 patients. The row estimates were obtained from a linear mixed model with annual global cognitive scores as the continuous longitudinal outcome.

Table 3

Top Genes Associated With the Transverse Relaxation Rate (R_2) Measure[a]

Gene	Ensembl Gene ID	Estimate (SE)	*P* Value
PADI2	ENSG00000117115	−0.358 (0.066)	2.234×10^{-7}
ZNF385A	ENSG00000161642	−0.417 (0.077)	2.313×10^{-7}
PSD2	ENSG00000146005	−0.329 (0.062)	4.105×10^{-7}
A2ML1	ENSG00000166535	−0.309 (0.059)	4.641×10^{-7}

[a]For a total of 174 patients. The row estimates were obtained from separate linear regression models with the R_2 measure as the continuous outcome and individual gene expression as the predictor. All the models were adjusted for age, sex, postmortem interval to imaging, and RNA integrity number scores, as well as neuropathologic indices for Alzheimer disease, macroscopic infarcts, microinfarcts, Lewy bodies, hippocampal sclerosis, transactive response DNA-binding protein 43, cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis.