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ABSTRACT

Objective: Given evidence from genetic studies, we hypothesized that there may be a shared component to the role of myeloid function in Parkinson and Alzheimer disease (PD and AD) and assessed whether PD susceptibility variants influenced protein expression of well-established AD-associated myeloid genes in human monocytes.

Methods: We repurposed data in which AD-related myeloid proteins CD33, TREM1, TREM2, TREML2, TYROBP, and PTK2B were measured by flow cytometry in monocytes from 176 participants of the PhenoGenetic Project (PGP) and Harvard Aging Brain Study. Linear regression was used to identify associations between 24 PD risk variants and protein expression. The 2 cohorts were meta-analyzed in a discovery analysis, and the 4 most strongly suggestive results were validated in an independent cohort of 50 PGP participants.

Results: We discovered and validated an association between the PD risk allele rs12456492^{G} in the *RIT2* locus and increased CD33 expression ($p_{joint} = 3.50 \times 10^{-5}$) and found strongly suggestive evidence that rs11060180^{A} in the *CCDC62/HIP1R* locus decreased PTK2B expression ($p_{joint} = 1.12 \times 10^{-4}$). Furthermore, in older individuals, increased CD33 expression on peripheral monocytes was associated with a greater burden of parkinsonism ($p = 0.047$), particularly bradykinesia ($p = 6.64 \times 10^{-3}$).

Conclusions: We find that the rs12456492 PD risk variant affects expression of AD-associated protein CD33 in peripheral monocytes, which suggests that genetic factors for these 2 diseases may converge to influence overlapping innate immune-mediated mechanisms that contribute to neurodegeneration. Furthermore, the effect of the rs12456492^{G} PD risk allele on increased CD33 suggests that the inhibition of certain myeloid functions may contribute to PD susceptibility, as is the case for AD. ***Neurol Genet* 2016;2:e90; doi: 10.1212/NXG.0000000000000090**

GLOSSARY

AD = Alzheimer disease; **DLPFC** = dorsolateral prefrontal cortex; **eQTL** = expression quantitative trait locus; **GWAS** = genome-wide association study; **HABS** = Harvard Aging Brain Study; **PD** = Parkinson disease; **PGP** = PhenoGenetic Project; **pQTL** = protein quantitative trait locus; **ROS-MAP** = Religious Orders Study and Memory and Aging Project; **SNP** = single nucleotide polymorphism.

Parkinson disease (PD) and Alzheimer disease (AD) are clinically distinct neurodegenerative diseases; however, their pathologic features (e.g., Lewy bodies and tau tangles) and certain clinical traits (e.g., parkinsonism and dementia) are often found together in older individuals with these syndromic diagnoses.[1–3] In addition, microglia and macrophages have been implicated in both PD and AD pathogenesis, and the myeloid-specific gene *TREM2* has been implicated in both PD and AD susceptibility,[4,5] suggesting a common role for the innate immune system in both diseases.[6–8] To underscore further a role for the innate immune system in both PD and AD,

Supplemental data at Neurology.org/ng

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our group recently found that multiple PD and AD susceptibility variants influenced the RNA expression of nearby genes: they were *cis*-expression quantitative trait loci (eQTLs) primarily in monocytes.[9,10] Thus, it appears that the functional consequences of AD and PD risk alleles may converge in influencing innate immune pathways.[11]

Given the results of our initial eQTL analysis, we hypothesized that PD-associated single nucleotide polymorphisms (SNPs) could have protein QTL effects with innate immune AD susceptibility genes *in trans*. To test this, we took advantage of a previously generated data set of protein expression levels of AD-related myeloid genes, *TREML2*, *TREM1*, *TREM2*, *TYROBP*, *PTK2B*, and *CD33* in monocytes from 226 genotyped participants of the PhenoGenetic Project (PGP) and the Harvard Aging Brain Study (HABS).[12] In the analyses presented here, we determined whether 24 validated PD susceptibility SNPs (table 1) influenced the expression of these 6 AD-related proteins in a discovery analysis and then validated the top results in an independent set of participants.

Table 1 Parkinson disease variants examined in protein quantitative trait locus analysis

SNP	Locus	Alleles (ref/other)
rs10797576	*SIPA1L2*	C/T
rs11060180	*CCDC62/HIP1R*	A/G
rs11158026	*GCH1*	C/T
rs11724635	*BST1*	C/A
rs117896735	*INPP5F*	G/A
rs11868035	*SREBF1-RAI1*	G/A
rs12456492	*RIT2*	A/G
rs12637471	*MCCC1*	G/A
rs14235	*BCKDK-STX1B*	G/A
rs1474055	*STK39*	C/T
rs199347	*GPNMB*	A/G
rs2414739	*VPS13C*	G/A
rs329648	*MIR4697*	T/C
rs34311866	*TMEM175-GAK-DGKQ*	T/C
rs356182	*SNCA*	G/A
rs35749011	*GBA-SYT11*	G/A
rs591323	*FGF20*	G/A
rs6430538	*ACMSD-TMEM163*	C/T
rs6812193	*FAM47E-SCARB2*	C/T
rs76904798	*LRRK2*	C/T
rs8118008	*DDRGK1*	A/G
rs823118	*RAB7L1-NUCKS1*	C/T
rs17649553[a]	*MAPT*	C/T
rs9275326[b]	*HLA-DQB1*	C/T

All single nucleotide polymorphisms (SNPs) were $p \leq 0.05$ in the replication phase of Nalls et al.[19]
[a] Used proxy variant rs113579895.
[b] Used proxy variant rs115462410.

METHODS We used the same methodology as the one used in a previous study.[12] Additional information is included in the e-Methods at Neurology.org/ng.

Standard protocol approvals and patient consent. Experiments, including blood draws, brain autopsies, and data analysis, were done in compliance with protocols approved by either the Partners Human Research Committee or the Rush University Institutional Review Board. Written, informed consent was obtained from all participants.

PhenoGenetic Project. For this study, cryopreserved peripheral blood mononuclear cells derived from healthy, genotyped participants of the PGP, a living biobank, from Brigham and Women's Hospital in Boston, MA, were used. To date, 1,753 self-reported healthy participants, ranging in age from 18 to 50, have been recruited. Of the participants, 71% are Caucasian and 62.7% are female. For the protein quantitative trait locus (pQTL) study performed herein, all samples were derived from PGP participants of European ancestry (n = 165). The EIGENSTRAT program was used with the genome-wide genotype data to determine ancestry.

Harvard Aging Brain Study. Neuroimaging was used to identify cognitively nonimpaired, healthy older individuals with increases in brain amyloid in the longitudinal HABS. Participants range in age from 65 to 90. Currently, 276 participants are enrolled in the study; 81% are Caucasian and 59.4% are female. These individuals undergo clinical and neuroimaging evaluations, as described previously.[13] Of the HABS participants, 161 have been genotyped, and the EIGENSTRAT program was used to determine ancestry. All the individuals in the current study are of European ancestry (n = 61).

Religious Orders Study and Memory and Aging Project. Similar to HABS, the Memory and Aging Project (MAP) and Religious Orders Study (ROS) are longitudinal aging studies. Participants are recruited while cognitively nonimpaired and undergo annual clinical assessments in addition to agreeing to donate their brains at the time of death under the Anatomic Gift Act. Detailed antemortem clinical and neuropathologic assessments are performed for each participant. Parkinsonism was assessed by trained nurses at study entry and was based on 26 items from a modified version of the motor section of the Unified Parkinson's Disease Rating Scale.[14] Four previously established parkinsonian sign scores (bradykinesia, rigidity, tremor, and gait disturbance) were derived from these 26 items, and a summary global parkinsonian sign score was constructed by averaging these 4 scores, as detailed in prior publications.[14,15] The retention rate and autopsy rate of participants exceeds 90% and 80%, respectively. A detailed report of MAP and ROS can be found elsewhere.[16–18] All individuals with CD33 monocyte surface protein expression (n = 151) and those with *PTK2B* RNA expression from the dorsolateral prefrontal cortex (DLPFC) (n = 508) were determined to be of European ancestry by using EIGENSTRAT.

Statistical analysis. In summary, 24 SNPs previously identified in a PD genome-wide association study (GWAS)[19] were selected for the study. Our analysis was limited to 144 SNP:protein pairs (24 SNPs × 6 proteins) in the discovery phase. Four SNP:protein pairs with the lowest, most suggestive p values were selected for follow-up in the validation phase. To identify statistically significant *trans* associations, we used a Bonferroni significance threshold of $p \leq 0.003$ ($p \leq 0.01/4$).

Flow cytometry protein expression was collected in discrete experiments. Each experiment consisted of multiple batches, which contained 7–12 participants per batch. The discovery data set consisted of 176 unique PGP individuals (n = 115) as well as the HABS cohort (n = 61) separated into 4 experiments. The validation cohort included 50 unique PGP participants and was analyzed in a single experiment. PTK2B was not measured in 49 PGP individuals, creating a PTK2B discovery sample size of n = 127 and a total of n = 177. Prior to the meta-analysis of all experiments, each experiment was analyzed separately. In each experiment, expression levels were gaussianized using equation 1, thus decreasing the weight and potential bias of any outlying observations.

$$Y^{\tau}_{ijk} = \varphi^{-1}\left(\left[r_{ijk} - 3/8\right] \Big/ \left[N_{jk} - 6/8 + 1\right]\right) \quad (1)$$

r_{ijk} = rank, N_{jk} = sample size, and Y_{ijk} = expression level; where i indexes participants, j indexes experiment, and k indexes protein.

Before analyzing each SNP:protein combination, we implemented Combat version 2.0 (with the sva R package[20]) to control for batch. Each SNP:protein combination was then analyzed via linear regression with expression modeled as the outcome and SNP modeled as the predictor variable. To reduce potential confounding, we also included cell viability, age, and sex in the linear model. This was done for each experiment. The resulting t statistic for each pQTL combination was then meta-analyzed using the Stouffer method (equation 2), providing discovery p values. The Stouffer method is commonly used in GWAS meta-analysis.[21] To validate the SNP:protein combinations chosen from discovery, we applied the same transformations and linear regression analysis that was used in discovery. After the validation analysis was complete, we meta-analyzed across all 5 experiments to produce ultimate joint p values. To check for possible inflation of type I error, we also determined empirical p values via permutation analysis, repeating the analysis 10,000 times after randomly sampling genotypes.

Before analyzing ROS-MAP mRNA levels as measured by RNA-seq, multiple QC steps were taken. First, using Combat to adjust for batch, FPKM values were quantile-normalized. Second, the effects of technical and demographic factors (RNA integrity number, log2(total aligned reads), postmortem interval, age, sex, cohort, genotype PCs, and genotyping platform) were removed by creating residuals with linear regression, using mRNA expression as the outcome variable. These residuals were then gaussianized by using equation 1. R was used in all statistical analysis, and GraphPad Prism 6 was used for all plots.

$$Z = \frac{\sum_i Z_i w_i}{\sqrt{\sum_i Z_i w_i^2}}$$
$$w_i = \sqrt{n_i}, \quad (2)$$
$$n_i = \text{sample size of experiment i.}$$

Table 2 Summary of top protein quantitative trait locus effects

SNP	Locus	Protein	p_{disc} [a]	p_{val} [b]	p_{joint} [c]
rs11060180	*CCDC62/HIP1R*	CD33	7.38×10^{-4}	0.572	6.68×10^{-3}
rs12456492	*RIT2*	CD33	2.14×10^{-3}	2.39×10^{-3}	3.50×10^{-5}
rs11060180	*CCDC62/HIP1R*	PTK2B	0.011	0.028	1.12×10^{-4}
rs12637471	*MCCC1*	TYROBP	0.011	0.253	5.43×10^{-3}

Abbreviations: HABS = Harvard Aging Brain Study; PGP = PhenoGenetic Project; SNP = single nucleotide polymorphism.
Complete results can be found in tables e-1–e-3.
[a] Meta-analysis p value for the discovery phase (discovery PGP and HABS participants combined).
[b] The 4 associations were tested independently in a validation phase (Bonferroni cutoff is $p \leq 2.5 \times 10^{-3}$ [$p \leq 0.01/4$]).
[c] Joint p value combining discovery and validation phases.

RESULTS Protein expression of genes thought to be important for AD (*TREML2*, *TREM1*, *TREM2*, *TYROBP*, and *CD33*) was previously measured using flow cytometry in primary human monocytes from 115 younger, healthy participants of PGP and 61 older, cognitively nonimpaired participants from HABS in a quantitative trait study with AD susceptibility variants; *PTK2B* protein expression was also measured in a subset of 66 PGP participants and in all HABS participants.[12] We repurposed this data set to determine whether the 24 PD susceptibility variants previously identified in GWAS (table 1) were associated with measured protein expression. We meta-analyzed the 2 cohorts (PGP and HABS) in a discovery phase analysis to identify suggestive results and then validated these results in an independent cohort consisting of 50 PGP participants. A joint analysis combining individuals from the discovery and validation phases was also performed to summarize all available data. Together, the 165 PGP participants had a mean age of 33.7 (±10.59) years and were 40.6% male, while HABS participants had a mean age of 76.3 (±6.08) years and were 50.8% male.

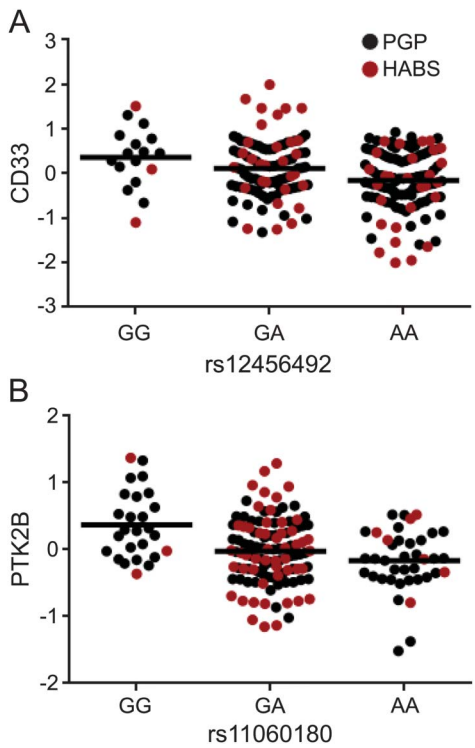
In the discovery phase, 4 PD SNP:AD protein pairs with the lowest, most suggestive p values (rs11060180:CD33 [$p_{disc\_meta} = 7.38 \times 10^{-4}$], rs12456492:CD33 [$p_{disc\_meta} = 2.14 \times 10^{-3}$], rs11060180:PTK2B [$p_{disc\_meta} = 0.011$], and rs12637471:TYROBP [$p_{disc\_meta} = 0.011$] [table 2; table e-1]) were selected for validation in an independent experiment (n = 50). Among the 4 SNP:protein pairs, only the rs12456492:CD33 association was validated after Bonferroni correction ($p_{cut\text{-}off} \leq 2.5 \times 10^{-3}$ [$p \leq 0.01/4$]) ($p_{val} = 2.39 \times 10^{-3}$, $p_{joint} = 3.50 \times 10^{-5}$; table 2; table e-2) with the direction of effect being consistent in the discovery and validation analyses. Although the influence of rs11060180 on PTK2B expression was only suggestive in the validation phase ($p_{val} = 0.028$; table 2), it was the only other association aside from rs12456492:CD33 in the joint analysis to pass the global Bonferroni threshold ($p_{joint} = 1.12 \times 10^{-4}$; table 2; table e-3) and had a consistent direction of effect in both data sets. Furthermore, we applied

a permutation-based analysis to all 144 hypotheses tested, in which the false discovery rate (FDR) was derived from 10,000 permutations of the data (genotype was permuted). In this analysis, both the rs11060180:PTK2B and rs12456492:CD33 associations were the only associations to yield FDR ≤0.05 (table e-4). Thus, our 2 main results were unlikely to be chance observations.

Consistent with the previously reported rs3865444^{C} CD33 AD risk allele,[13] the PD risk allele rs12456492^{G} in the *RIT2* locus was associated with increased CD33 surface expression on monocytes (figure, A) and explained 7.6% of the variance in CD33. Conversely, the suggestive rs11060180^{A} risk allele near *CCDC62/HIP1R* was associated with decreased PTK2B expression (figure, B) and explained 8.5% of the variance in PTK2B; this result contrasts with the *PTK2B* and *NME8* AD susceptibility variants rs28834970^{C} and rs2718058^{A}, respectively, which we have previously reported to be associated with higher levels of PTK2B expression in this data set.[12] To determine whether these *trans* associations were present at the mRNA level, we analyzed mRNA expression in monocyte ImmVar data[13] and in DLPFC tissue from the Religious Orders Study and Memory and Aging Project (ROS-MAP). Brain tissue was analyzed because infiltrating CNS monocytes and brain-resident microglia, cell types that are known to express the measured proteins, are thought to play a critical role in the accumulation of AD pathology. Neither rs12456492 nor rs11060180 had an association with *CD33* or *PTK2B* mRNA, respectively, in either data set.

Intrigued by the evidence suggesting that AD-associated proteins CD33 and PTK2B may play a role in PD, we examined the extent of association of PD-related pathologic (neuronal loss in the substantia nigra, burden of Lewy bodies) and clinical (parkinsonism as well as its component measures of bradykinesia, rigidity, and gait impairment) traits with ROS-MAP monocyte CD33 surface expression (repurposing a previous data set[13]) and ROS-MAP DLPFC *PTK2B* mRNA expression (the only *PTK2B* expression data available from this cohort). We did not observe an association of *PTK2B* mRNA expression in DLPFC with these traits. However, we did note a nominal association of increasing CD33 monocyte protein surface expression with a greater global measure of parkinsonism ($p = 0.047$) that appeared to be primarily driven by an effect on bradykinesia ($p = 6.64 \times 10^{-3}$).

Figure **Parkinson disease risk variants influence expression of Alzheimer-related proteins CD33 and PTK2B in human monocytes**



(A) CD33 and (B) PTK2B protein expression in monocytes was quantified through flow cytometry and plotted against each participant's rs12456492 and rs11060180 genotype, respectively; the *y*-axis represents normalized median fluorescence intensity (MFI) and the horizontal line denotes mean MFI. Each dot represents one individual from either the PhenoGenetic Project (PGP) or Harvard Aging Brain Study (HABS) cohort.

DISCUSSION We conducted a *trans*-pQTL study in human ex vivo monocytes examining the relationship between PD risk variants and expression of AD-related proteins TREM1, TREM2, TREML2, TYROBP, PTK2B, and CD33 to detect whether AD and PD pathophysiology share common innate immune mechanisms. We discovered that the PD risk allele rs12456492^{G} in the *RIT2* locus was associated with increased CD33 surface expression and found highly suggestive evidence that the rs11060180^{A} PD risk allele in the *CCDC62/HIP1R* locus was associated with lower PTK2B expression. Of interest, these *trans* associations were not observed at the mRNA level, highlighting the importance of protein-level QTL studies, which can capture effects of posttranslational regulation.[22]

PTK2B, a member of the focal adhesion kinase family, is a protein tyrosine kinase that is rapidly activated by a number of mediators including lipopolysaccharide, cytokines, and cell adhesion[23,24] and is thought to play an important role in the phagocytosis and migration of monocytes and macrophages through its involvement in cytoskeletal signaling pathways.[25] In a GWAS, rs28834970 in the *PTK2B* locus was identified as having a significant association with AD susceptibility,[26] and we have been able to confirm that rs28834970 has both a *cis*-eQTL and *cis*-pQTL effect on *PTK2B* expression in monocytes.[9,16] Although the effect of the *CCDC62/HIP1R* rs11060180 PD risk allele on decreasing PTK2B is opposite of that in AD

(in which risk variants are associated with increased PTK2B), the effect of rs11060180 was consistent across our discovery and validation studies. Thus, while certain immune molecular pathways may be implicated in both PD and AD, they may not have the same role in the 2 diseases. This is not unlike a number of susceptibility alleles that are associated with different inflammatory diseases but have effects in opposite directions (being risk-associated for one disease and protective for another).[12,27] In addition, we did not detect an association between *PTK2B* mRNA cortical expression and PD-related traits in the ROS-MAP cohort; however, an analysis of a PD-specific cohort would provide a complementary analysis that would be more relevant for the context of PD.

The AD-associated protein CD33 is a siglec expressed on the surface of myeloid cells and contains putative immunoreceptor tyrosine–based inhibitory motifs that are known to suppress cellular activity such as proinflammatory cytokine secretion and amyloid beta uptake.[13,28] Not only has *CD33* been implicated in AD through GWAS[26,29–31] but the effect of the *CD33* rs3865444^{C} risk allele on increased CD33 protein expression[13,32] as well as CD33's effect on amyloid accumulation in humans and mouse models of AD[13,33] has been reported. In our study, we found that the rs12456492^{G} Parkinson risk allele in the *RIT2* locus was associated with increased CD33 expression, consistent with the *CD33* AD risk allele, which suggests that myeloid suppression is associated with PD susceptibility, as is the case for AD. Of note, we found that CD33 protein surface expression on monocytes of older individuals was modestly associated with bradykinesia, implicating CD33 in PD pathogenesis in addition to AD. These intriguing results will be important in guiding future studies examining the role of CD33 in both diseases.

Growing evidence indicates that the innate immune system plays an important role in PD and AD pathophysiology, but it remains unclear whether overlapping signaling pathways are involved. Our pQTL study revealing that the AD-associated protein CD33 is modulated by a PD susceptibility locus and that increased CD33 monocyte surface expression is correlated with both PD and AD clinical traits suggests that, although PD and AD causal variants are distinct, some of them may converge on the same signaling pathways. Furthermore, the association of increased CD33 with PD risk provides the insight that myeloid suppression is a risk factor for PD. It is also not clear whether the effects we report in peripheral monocytes contribute to PD directly by influencing the behavior of infiltrating cells that differentiate into macrophages or whether these associations reflect shared effects with resident microglia. While we report some overlap between the effects of AD and PD variants in monocytes, it is important to note that the bulk of the monocyte molecular pathways implicated in each disease appears to be unique (as evidenced by opposing relationships in PTK2B expression between PD and AD risk alleles), which suggests that therapeutic approaches to target immune pathways in PD and AD may have to be considered carefully to avoid provoking another neurodegenerative process as an adverse event. Although more work will be needed to verify these associations in macrophages and microglia at the sites of pathology, our findings begin to shed light onto common immune mechanisms contributing to both PD and AD, which may aid in the development of therapeutic strategies used to target each disease.

AUTHOR CONTRIBUTIONS

Gail Chan: designed the study; performed experiments; analyzed and interpreted data; drafted and revised manuscript. Charles C. White, Joseph M. Replogle, and Lori B. Chibnik: performed statistical analyses; interpreted data; revised manuscript. Phoebe A. Winn, Maria Cimpean, and Katie J. Ryan: performed experiments; revised manuscript. Laura R. Glick and Nicole E. Cuerdon: coordinated clinical visits and managed participant information; revised manuscript. Keith A. Johnson and Reisa A. Sperling: provided HABS blood samples and genetic/clinical data; revised manuscript. Julie A. Schneider and David A. Bennett: provided ROS-MAP clinical, genetic, and postmortem data; revised manuscript. Philip L. De Jager: designed study; coordinated access to all cohorts; interpreted data; revised manuscript. Elizabeth M. Bradshaw: designed study; interpreted data; revised manuscript.

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DISCLOSURE

Dr. Chan, Dr. White, Ms. Winn, Ms. Cimpean, Mr. Replogle, Ms. Glick, Ms. Cuerdon, and Dr. Ryan report no disclosures. Dr. Johnson has consulted for/served on scientific advisory boards for Lundbeck, Piramal Healthcare, Biogen, Siemens, Abbvie, Roche, Avid/Lilly, AZTherapies, Isis Pharmaceuticals, and Novartis; has received travel funding/speaker honoraria from GEHC, Biogen, and Lundbeck; has been involved with clinical procedures/imaging studies regarding the following: (1) Division of Nuclear Medicine and Molecular Imaging, Department of Imaging, Massachusetts General Hospital, Boston, MA, <5%, 2002–2011; and (2) Memory Disorders Unit, Department of Neurology, Brigham and Women's Hospital, Boston, MA, <5%, 1993–2011; has received research support from Avid/Lilly, Biogen, NIH/NIA, the Alzheimer Association, the Marr Foundation, Fidelity Biosciences, and the Harvard NeuroDiscovery Center; and receives royalties from the publication of the book *The Whole Brain Atlas*. Dr. Schneider is a consultant for Navidea Biopharmaceuticals and AVID Radiopharmaceuticals, serves on the editorial board of the *Journal of Neuropathology and Experimental Neurology*; is a monitoring editor of *Journal of Histochemistry and Cytochemistry*; has been a consultant for AVID Radiopharmaceuticals and Navidea Biopharmaceuticals; has been an Alzheimer's expert panel member for Eli Lilly and Company; and has received research support from AVID Radiopharmaceuticals and NIH/NIA. Dr. Bennett has served on the scientific advisory board of Vigorous Minds and Takeda Pharm; has served on the editorial boards of *Neurology*, *Current Alzheimer Research*, and *Neuroepidemiology*; and has received research support from NIH. Dr. Chibnik has received research support from NIH/NIA.

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