APOE $\epsilon 2\epsilon 4$ genotype, incident AD and MCI, cognitive decline, and AD pathology in older adults

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Abstract

Objective

To examine the association of the *APOE* $\epsilon 2\epsilon 4$ genotype with incident Alzheimer disease (AD), mild cognitive impairment (MCI), cognitive decline, and AD pathology in older adults.

Methods

We used data from 2,151 older adults of European ancestry who were free of dementia at baseline and underwent structured annual clinical evaluation in a longitudinal study for incident AD and MCI, and cognitive decline. Postmortem examination in decedents documented pathologic AD and quantified β -amyloid and neurofibrillary tangles. Participants were stratified into 4 groups based on *APOE* genotyping: $\epsilon 2\epsilon 4$, $\epsilon 4$ ($\epsilon 4\epsilon 4$, $\epsilon 4\epsilon 3$), $\epsilon 2$ ($\epsilon 2\epsilon 2$, $\epsilon 2\epsilon 3$), with $\epsilon 3\epsilon 3$ carriers serving as the reference group. We used Cox proportional hazards models to examine the association of *APOE* genotype with incident AD and MCI. Linear mixed-effect models were used to examine the association with cognitive decline. Logistic and linear regression models were used to examine AD pathology. All the models controlled for age, sex, and education.

Results

Of the 2,151 participants included in this study, $\epsilon 2\epsilon 4$ accounted for 2.1%, $\epsilon 3/4$ and $4/4$ 21.8%, $\epsilon 2/3$ and $2/2$ 14.0%, and $\epsilon 3\epsilon 3$ 62.1%. We did not observe a difference in the risk of AD for $\epsilon 2\epsilon 4$ compared to $\epsilon 3\epsilon 3$. In cases without cognitive impairment at baseline, $\epsilon 2\epsilon 4$ carriers had an increased risk of incident MCI (hazard ratio 2.13, 95% confidence interval 1.34–3.39, $p = 0.002$) and a faster rate of cognitive decline (estimate -0.047 , SE 0.018, $p = 0.008$) compared to $\epsilon 3\epsilon 3$ carriers. In decedents ($n = 1,100$), $\epsilon 2\epsilon 4$ showed a 3-fold increased odds of pathologic AD and a higher β -amyloid load than $\epsilon 3\epsilon 3$.

Conclusion

APOE $\epsilon 2\epsilon 4$ genotype in older adults is associated with risk of MCI, cognitive decline, and a greater burden of AD pathology, especially β -amyloid.

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Glossary

A β = β -amyloid; AD = Alzheimer disease; CI = confidence interval; HR = hazard ratio; MAP = Memory and Aging Project; MCI = mild cognitive impairment; NCI = no cognitive impairment; ROS = Religious Orders Study; TR = targeted replacement.

The *APOE* gene is the best known genetic risk factor for late-onset Alzheimer disease (AD). It has 3 common alleles, ϵ 2, ϵ 3, and ϵ 4. Prior work has shown that ϵ 4 carriers (ϵ 4 ϵ 4 or ϵ 3 ϵ 4) are associated with about a 3-fold increased risk of AD dementia,¹ more rapid cognitive decline,¹ and a higher burden of AD pathology.² By contrast, ϵ 2 carriers (ϵ 2 ϵ 2 or ϵ 2 ϵ 3) are associated with a decreased risk of AD dementia,³ slower cognitive decline,⁴ and lower burden of AD pathology.⁵ Prior studies have generally excluded the small number of individuals with the ϵ 2 ϵ 4 genotype, or have included few ϵ 2 ϵ 4 carriers without specific analyses for their association with AD dementia. Therefore, the association of ϵ 2 ϵ 4 genotype with cognitive function and AD pathology in older adults is unknown.

Prior work from our group mostly excluded persons with *APOE* ϵ 2 ϵ 4.⁶ In this study, we used data from community-dwelling older adults of European ancestry participating in the Religious Orders Study (ROS) or the Rush Memory and Aging Project (MAP) who were free of dementia at baseline and underwent structured annual clinical evaluation. We examined the associations of *APOE* ϵ 2 ϵ 4 with incident AD dementia, incident mild cognitive impairment (MCI), the rate of cognitive decline, and in decedents, measures of AD pathology.

Methods

Participants

Participants were enrolling in 2 ongoing cohort studies in which they agreed to annual assessments and brain donation at the time of death. ROS began in 1994 and has been recruiting older nuns, priests, and brothers from more than 45 sites across the United States.⁷ MAP began in 1997, and has been enrolling residents from retirement facilities and subsidized housings across the Chicago metropolitan area.⁸

Uniform data collection and autopsy procedures conducted by the same team allow us to analyze ROS and MAP data together. The overall follow-up exceeds 90%, and the combined autopsy rate for both studies exceeds 85%. The association of *APOE* with AD varies in different ethnic groups,³ so we limited our analyses to non-Latino white. At the time of these analyses, 2,151 ROS and MAP participants had completed baseline evaluations and did not have clinical dementia, had valid *APOE* genotyping, and had at least one valid follow-up examination to allow for longitudinal analyses.

Assessment of cognition and clinical diagnoses

Participants underwent a uniform structured annual clinical examination, which included 21 cognitive tests. Standardized scores of 7 tests were combined to create an episodic memory

score, 3 tests used for semantic memory, 3 tests for working memory, 2 tests for perceptual speed, and 2 tests for visuospatial abilities. All 17 tests were summarized into a global cognition score as previously described.⁹ A neuropsychologist and neurologist with expertise in dementia reviewed annual findings and classified cognitive status as no cognitive impairment (NCI), MCI, probable and possible AD, and other dementias.¹⁰

APOE genotyping

DNA was extracted from peripheral blood lymphocytes or frozen postmortem brain tissue. Genotyping was performed at Polymorphic DNA Technologies (Alameda, CA) by investigators blinded to all clinical and pathologic data. The *APOE* genotypes were determined by sequencing rs429358 (codon 112) and rs7412 (codon 158) at exon 4 of the *APOE* gene.¹¹

Assessment of AD neuropathology

The brains of deceased participants were removed and prepared in a standard procedure, as described previously.^{12–14} The brains were coronally cut into 1-cm slabs, and slabs from one hemisphere were fixed in 4% paraformaldehyde for 3 days. β -Amyloid (A β) load and paired helical filament tau immunoreactive neuronal neurofibrillary tangles (tau tangles) were quantified in 8 brain regions (anterior cingulate cortex, superior frontal cortex, midfrontal cortex, inferior temporal cortex, hippocampus, entorhinal cortex, angular gyrus/supramarginal gyrus, and calcarine cortex). Overall A β load was calculated by averaging mean percentage area of A β deposition per region, across multiple brain regions. Likewise, densities of tangles were derived by averaging densities of tangles across corresponding brain regions.¹²

A modified Bielschowsky silver stain was used to visualize neuritic plaques, diffuse plaques, and neurofibrillary tangles in 5 cortical areas (hippocampus, entorhinal, midfrontal, middle temporal, and inferior parietal). A board-certified neuropathologist, blinded to clinical data, determined the pathologic diagnosis of AD based on an intermediate to high likelihood of AD according to National Institute on Aging–Reagan criteria. In addition, a global AD pathology score was determined based on the greatest density of neuritic plaques, diffuse plaques, and neurofibrillary tangles in 1 mm².¹⁵

Statistical analyses

We stratified participants into 4 groups based on their *APOE* genotype: ϵ 2 ϵ 4, ϵ 2 (ϵ 2 ϵ 2 or ϵ 2 ϵ 3), ϵ 4 (ϵ 3 ϵ 4 or ϵ 4 ϵ 4), with ϵ 3 ϵ 3 carriers serving as the reference group.

We first used discrete-time Cox proportional hazards models, adjusted for age, sex, and education, to estimate the association of *APOE* genotypes with the risk of incident AD dementia. Then, we used the same approach to estimate the

APOE association with incident MCI among persons without cognitive impairment at baseline. In both sets of analyses, the outcomes of interest were time in years until the first diagnosis of AD dementia, and separately the first diagnosis of MCI. Participants with no incident event were right censored at the last evaluation, as has been reported previously.¹⁶

In further analyses, we examined the association of *APOE* genotypes with the rate of cognitive decline.¹⁷ We used linear mixed-effects models to study change in global cognition across years of follow-up. All models also controlled for age, sex, education, and their interaction with annual rate of change in cognition. The models provide 2 sets of estimates. The first set includes an estimate for baseline level of global cognition among the $\epsilon 3\epsilon 3$ carriers and estimates for the differences in baseline global cognition between each of the *APOE* strata ($\epsilon 2\epsilon 4$, $\epsilon 4$, $\epsilon 2$) and $\epsilon 3\epsilon 3$ carriers. The second set includes an estimate for average slope of cognitive change among the $\epsilon 3\epsilon 3$ carriers and estimates for the differences in the slope of cognitive changes between each of the *APOE* strata ($\epsilon 2\epsilon 4$, $\epsilon 4$, $\epsilon 2$) and $\epsilon 3\epsilon 3$ carriers. In further analyses, we repeated the models to study score changes in cognitive domains (episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability) among different *APOE* strata compared with the $\epsilon 3\epsilon 3$ carriers. We subsequently repeated these analyses among persons free of cognitive impairment (NCI) at baseline.

To examine associations of *APOE* genotypes with A β and tau depositions, we used linear regressions, and logistic regression was used to study the association between *APOE* groups and

odds of pathologic AD diagnosis. All of these models also adjusted for age, sex, and education.

Our primary analysis focused on cognition in $\epsilon 2\epsilon 4$ carriers compared to the reference group, $\epsilon 3\epsilon 3$ carriers, so statistical significance was fixed at α level of 0.05. In 2 sets of post hoc comparisons that involved multiple groups (i.e., $\epsilon 2\epsilon 4$ vs $\epsilon 4$ and $\epsilon 2\epsilon 4$ vs $\epsilon 2$), statistical significance was fixed at α level of 0.025 because of multiple testing. The analyses were done using SAS version 9.4 (SAS Institute, Cary, NC).

Standard protocol approvals, registrations, and patient consents

The study was approved by the institutional review board of Rush University Medical Center. Written informed consent and an anatomical gift act for brain donation at the time of death were obtained from all study participants.

Data availability

Anonymized data will be shared by request from any qualified investigator for purposes of replicating procedures and results.

Results

The clinical characteristics of individuals included in these analyses are described more fully in table 1 and appendix e-1 (links.lww.com/WNL/A517). Participants in the analyses ($n = 2,151$) were, on average, nearly 80 years old at the study baseline, more than 70% female, and had averages of 16 years of education and baseline Mini-Mental State Examination

Table 1 Clinical characteristics of participants at study entry

	All ($n = 2,151$)	$\epsilon 2\epsilon 4$ ($n = 46, 2.1\%$)	$\epsilon 4$ ($n = 469, 21.8\%$)	$\epsilon 2$ ($n = 301, 14.0\%$)	$\epsilon 3\epsilon 3$ ($n = 1,335, 62.1\%$)
Age, y	78.3, 7.4	78.6, 6.5	77.3, 7.2	79.0, 7.9	78.5, 7.3
Female	1,544 (71.8)	36 (78.3)	338 (72.1)	221 (73.4)	949 (71.1)
Education, y	16.3, 3.5	16.6, 3.2	16.8, 3.5	16.0, 3.3	16.2, 3.5
Years of follow-up	8.5, 5.1	7.5, 5.0	8.4, 5.1	8.6, 5.1	8.6, 5.1
Mini-Mental State Examination score	28.3, 1.8	27.8, 2.2	28.2, 1.9	28.3, 1.9	28.3, 1.8
Diagnosis of MCI	534 (24.8)	14 (30.4)	151 (32.2)	66 (21.9)	303 (22.7)
Baseline MCI in decedents ($n = 1,100$)	342 (31.1)	6 (25.0)	97 (40.3)	45 (30.8)	194 (28.2)
Global cognition	0.12, 0.16	-0.02, 0.48	0.08, 0.57	0.16, 0.48	0.12, 0.51
Episodic memory	0.12, 0.21	0.01, 0.65	-0.01, 0.76	0.23, 0.57	0.14, 0.64
Semantic memory	0.13, 0.20	0.07, 0.56	0.15, 0.62	0.14, 0.59	0.13, 0.62
Working memory	0.11, 0.11	-0.02, 0.67	0.13, 0.75	0.12, 0.71	0.10, 0.70
Perceptual speed	0.11, 0.18	-0.13, 0.87	0.10, 0.83	0.14, 0.83	0.12, 0.83
Visuospatial ability	0.12, 0.19	-0.06, 0.85	0.17, 0.75	0.07, 0.78	0.12, 0.78

Data are number (%) or mean, SD.
Abbreviation: MCI = mild cognitive impairment.

score of 28. The most frequent *APOE* group was $\epsilon 3\epsilon 3$ ($n = 1,335$, 62.1%), followed by $\epsilon 4$ ($n = 469$, 21.8%), $\epsilon 2$ ($n = 301$, 14.0%), and $\epsilon 2\epsilon 4$ ($n = 46$, 2.1%).

APOE genotype and incident AD dementia

Over an average of 7.8 years of follow-up, 16 of 46 (35%) $\epsilon 2\epsilon 4$, 196 of 468 (42%) $\epsilon 4$, 68 of 301 (23%) $\epsilon 2$, and 369 of 1,335 (28%) $\epsilon 3\epsilon 3$ carriers developed AD dementia. In a Cox proportional hazards model that controlled for age, sex, and education, the point estimate for the risk of AD dementia was higher among $\epsilon 2\epsilon 4$ compared with $\epsilon 3\epsilon 3$ carriers, but it failed to reach statistical significance (hazard ratio [HR] = 1.54, 95% confidence interval [CI] 0.91–2.59, $p = 0.108$).

By contrast, $\epsilon 4$ carriers had an 87% increased risk of incident AD dementia (HR = 1.87, 95% CI 1.55–2.24, $p < 0.001$) and $\epsilon 2$ carriers had a 30% decreased risk of incident AD dementia (HR = 0.71, 95% CI 0.54–0.92, $p = 0.010$) compared to $\epsilon 3\epsilon 3$ carriers (appendix e-2, links.lww.com/WNL/A517), as we and others have previously reported.

We did not observe a significant difference in the risk of AD dementia between $\epsilon 2\epsilon 4$ and $\epsilon 4$ carriers (HR = 0.82, 95% CI 0.48–1.40, $p = 0.477$); however, $\epsilon 2\epsilon 4$ carriers had double the risk of AD dementia compared to $\epsilon 2$ carriers (HR = 2.18, 95% CI 1.23–3.84, $p = 0.007$).

APOE genotype and incident MCI

Over an average of 6.5 years of follow-up, 23 of 32 (72%) $\epsilon 2\epsilon 4$, 197 of 317 (62%) $\epsilon 4$, 112 of 235 (48%) $\epsilon 2$, and 572 of 1,032 (55%) $\epsilon 3\epsilon 3$ carriers without cognitive impairment developed MCI. In a Cox proportional hazards model that controlled for age, sex, and education, the risk of incident MCI among $\epsilon 2\epsilon 4$ carriers was doubled compared with $\epsilon 3\epsilon 3$ carriers (HR = 2.13, 95% CI 1.34–3.39, $p = 0.002$). $\epsilon 4$ carriers had an increased risk of MCI (HR = 1.25, 95% CI 1.05–1.49, $p = 0.01$) and $\epsilon 2$ carriers had a reduced risk of MCI (HR = 0.72, 95% CI 0.58–0.90, $p = 0.003$) compared to $\epsilon 3\epsilon 3$ carriers (figure 1).

Further analyses showed that $\epsilon 2\epsilon 4$ carriers had a higher risk of incident MCI than $\epsilon 2$ carriers (HR = 2.94, 95% CI 1.79–4.84, $p < 0.001$), but $\epsilon 2\epsilon 4$ carriers were not significantly different from $\epsilon 4$ carriers (HR = 1.70, 95% CI 1.05–2.75, $p = 0.031$).

APOE genotype and cognitive decline

We next used a linear mixed model to examine the effect of $\epsilon 2\epsilon 4$ on the annual rate of cognitive decline as compared to that of $\epsilon 3\epsilon 3$. On average, global cognitive scores annually declined by 0.080 standard units in $\epsilon 3\epsilon 3$ carriers. Although baseline level of global cognition was 0.149 standard units lower among $\epsilon 2\epsilon 4$ carriers, there was no difference between the rate of cognitive decline in $\epsilon 2\epsilon 4$ and $\epsilon 3\epsilon 3$ consistent with

Figure 1 Cumulative hazard curves showing risk of mild cognitive impairment during this study among participants with *APOE* $\epsilon 2\epsilon 4$ genotype (A) compared with other *APOE* genotype groups (B–D)

the results of incident AD dementia (appendix e-3, [links.lww.com/WNL/A517](https://www.alzdiscovery.com/WNL/A517)).

We next examined the association of $\epsilon 2\epsilon 4$ on the rate of cognitive decline among participants with NCI at baseline, excluding those with MCI. At baseline, the level of global cognition was similar in all *APOE* groups (table 2, figure 2). On average, global cognitive scores declined by 0.067 standard units per year in $\epsilon 3\epsilon 3$ (table 2). By contrast, the $\epsilon 2\epsilon 4$ carriers showed a 70% more rapid decline in global cognition compared to $\epsilon 3\epsilon 3$ carriers (table 2, figure 2). $\epsilon 2\epsilon 4$ carriers also had a more rapid rate of decline in episodic memory, semantic memory, and visuospatial ability, but not for working memory and perceptual speed compared to $\epsilon 3\epsilon 3$ carriers (table 2).

Among participants with NCI at baseline, there was no significant difference in the rate of cognitive decline between $\epsilon 2\epsilon 4$ and $\epsilon 4$ carriers (estimate = -0.007 , SE = 0.018 ,

$p = 0.685$), but $\epsilon 2\epsilon 4$ carriers had faster decline than $\epsilon 2$ carriers (estimate = -0.057 , SE = 0.019 , $p = 0.002$).

***APOE* genotype and AD pathology**

At the time of analyses, 1,230 participants had died, of which 1,105 had undergone brain autopsy (autopsy rate 89.8%), and of these, 1,100 had been completed. The mean postmortem interval was 8.8 hours (SD = 7.4), and the last clinical evaluation, done on average 11.0 months before death (SD = 1.3), showed that 38% of participants had clinical AD dementia, 27% had MCI, and 35% had NCI.

Pathologic diagnosis of AD

More than 60% of decedents (686/1,100, 62.4%) met pathologic diagnostic criteria of AD ($\epsilon 2\epsilon 4$ carriers: 20/24, 83.3%; $\epsilon 4$ carriers: 193/241, 80.1%; $\epsilon 2$ carriers: 67/146, 45.9%; and $\epsilon 3\epsilon 3$ carriers: 406/689, 58.9%). Using logistic regression models that controlled for age, sex, and education, $\epsilon 2\epsilon 4$ and

Table 2 Association of *APOE* genotype and the annual rate of change in global cognition and 5 cognitive abilities among participants with no cognitive impairment (NCI) at baseline

A Cognitive outcome	B $\epsilon 2\epsilon 4$			C $\epsilon 4$			D $\epsilon 2$			E $\epsilon 3\epsilon 3$		
	Estimate	SE	P Value	Estimate	SE	P Value	Estimate	SE	P Value	Estimate	SE	P Value
Global cognition												
Baseline level	-0.108	0.069	0.117	0.034	0.024	0.158	0.020	0.027	0.474	0.364	0.014	<0.001
Annual change	-0.047	0.018	0.008	-0.040	0.006	<0.001	0.010	0.007	0.148	-0.067	0.003	<0.001
Episodic memory												
Baseline level	-0.080	0.080	0.327	0.009	0.028	0.752	0.034	0.032	0.280	0.473	0.016	<0.001
Annual change	-0.056	0.023	0.014	-0.048	0.008	<0.001	0.014	0.009	0.095	-0.068	0.004	<0.001
Semantic memory												
Baseline level	-0.083	0.087	0.338	0.069	0.031	0.024	-0.001	0.035	0.983	0.312	0.017	<0.001
Annual change	-0.035	0.017	0.045	-0.038	0.006	<0.001	0.005	0.006	0.419	-0.059	0.003	<0.001
Working memory												
Baseline level	-0.117	0.111	0.294	0.053	0.040	0.178	0.015	0.044	0.730	0.259	0.022	<0.001
Annual change	-0.014	0.015	0.336	-0.029	0.005	<0.001	0.003	0.005	0.599	-0.052	0.003	<0.001
Processing speed												
Baseline level	-0.301	0.129	0.020	0.022	0.045	0.620	-0.008	0.051	0.868	0.298	0.025	<0.001
Annual change	-0.037	0.020	0.061	-0.026	0.006	<0.001	0.003	0.007	0.657	-0.098	0.004	<0.001
Visuospatial												
Baseline level	-0.144	0.110	0.191	0.025	0.039	0.531	0.037	0.044	0.400	0.192	0.022	<0.001
Annual change	-0.031	0.015	0.042	-0.017	0.005	<0.001	0.002	0.005	0.697	-0.041	0.003	<0.001

Columns B–D show the association of a different *APOE* genotype with the results of 6 separate linear mixed-effect models for global cognition or one of 5 cognitive abilities listed in column A. For each cognitive outcome, one cell shows the estimate (standard error [SE]) for the association of a different *APOE* genotype with the baseline level of global cognition (baseline level) and with the annual rate of change in global cognition (annual change) or one of the 5 cognitive abilities. The β estimate in each model compares the annual rates of cognitive decline between 2 average participants with $\epsilon 2\epsilon 4$, $\epsilon 2$, or $\epsilon 4$ carriers vs $\epsilon 3\epsilon 3$ carriers (columns B, C, and D). Column E shows the results for the reference group $\epsilon 3\epsilon 3$ carriers alone. Each model also included 6 additional terms (not shown) controlling for age, sex, education, and their interaction with annual rate of cognitive change.

Figure 2 Relation of *APOE* genotype with the annual rate of change in global cognition and 5 cognitive abilities during this study among participants with no cognitive impairment at baseline

ε4 carriers were 3-fold more likely show pathologic AD compared to ε3ε3 carriers (table 3). ε2 carriers showed a 50% reduced odds of having pathologic AD compared to ε3ε3 carriers (table 3).

Compared to ε2 carriers, ε2ε4 carriers had 6.5 times greater odds of a pathologic diagnosis of AD controlling for age, sex, and education. However, no significant difference was found in the odds of a pathologic diagnosis of AD between ε2ε4 and ε4 carriers.

Postmortem AD indices

Appendix e-4 (links.lww.com/WNL/A517) shows boxplots of postmortem indices of AD pathology for *APOE* genotypes. Using regression models that controlled for age, sex, and education, ε2ε4 and ε4 had higher levels and ε2 had lower levels of global AD pathology compared to ε3ε3 (table 3). ε2ε4 and ε4 had a higher burden of Aβ and ε2 had lower burden of Aβ compared to ε3ε3. We did not observe

significant differences in paired helical filament tau tangle density between ε2ε4 and ε3ε3 (table 3). In contrast, ε4 had higher tangle density and ε2 had lower tangle density vs ε3ε3 (table 3).

Compared to ε2, ε2ε4 carriers had significantly more Aβ deposition and higher burden of global AD pathology and showed a trend for increased tangle density. By contrast, ε2ε4 carriers had significantly fewer tangles and showed a trend for less global AD pathology compared to ε4 carriers, with no significant difference in Aβ deposition.

Discussion

Using data from more than 2,000 older non-Latino white adults without dementia, we found that individuals with the *APOE* ε2ε4 genotype had an increased risk of incident MCI and a faster rate of cognitive decline among NCI persons.

Table 3 Association of *APOE* genotype with postmortem indices of AD and the pathologic diagnosis of AD

Pathology measure	ε2ε4		ε4		ε2	
	Est. (SE)	p Value	Est. (SE)	p Value	Est. (SE)	p Value
β-Amyloid, β (SE)	0.512 (0.229)	0.026	0.746 (0.082)	<0.001	-0.456 (0.099)	<0.001
Tangles density, β (SE)	0.183 (0.240)	0.446	0.778 (0.088)	<0.001	-0.306 (0.107)	0.004
Global AD pathology, β (SE)	0.140 (0.073)	0.055	0.272 (0.026)	<0.001	-0.178 (0.032)	<0.001
Pathologic AD, OR (95% CI)	3.4 (1.1–10.1)	0.031	3.2 (2.2–4.6)	<0.001	0.51 (0.35–0.75)	<0.001

Abbreviations: AD = Alzheimer disease; CI = confidence interval; OR = odds ratio. Estimates (Est.) are derived from linear regression models β (standard error [SE]) or from logistic regressions OR (95% CI). All the models controlled for age, sex, and education (not shown), and the results in each cell represent the associations for each of the *APOE* genotypes in each column compared to the reference group ε3ε3.

Moreover, decedents with $\epsilon 2\epsilon 4$ showed increased odds of a pathologic diagnosis of AD and more severe burden of A β load but not tau tangle density. By contrast, $\epsilon 4$ carriers not only had an increased risk of MCI and more severe burden of A β , but they were also more prone to develop AD dementia and had more severe deposition of tau tangle density. In addition, $\epsilon 2$ carriers had decreased risk of MCI and AD dementia, slower cognitive decline, and decreased burden of AD brain pathologies compared to $\epsilon 3\epsilon 3$ carriers. These results suggest that despite the presence of a single copy of the $\epsilon 2$ allele, which is protective in $\epsilon 2$ carriers, adults with the $\epsilon 2\epsilon 4$ genotype manifest an increased risk of late-life cognitive impairment and accumulate a higher burden of AD pathology in the brain in a way that is much more similar to $\epsilon 4$ than $\epsilon 2$.

Since $\epsilon 2$ carriers and $\epsilon 4$ carriers are associated with opposite effects and the $\epsilon 2\epsilon 4$ genotype is rare, many previous studies, including those by our group, excluded this variant from prior analyses.^{6,18} Other studies have included few $\epsilon 2\epsilon 4$ carriers without specific analyses for their association with AD dementia.^{5,19–21} A single cross-sectional, case-control study that examined the association of $\epsilon 2\epsilon 4$ with AD dementia reported no association but included only 18 $\epsilon 2\epsilon 4$ carriers,²² and a meta-analysis found that $\epsilon 2\epsilon 4$ carriers had an increased odds of AD.³ Thus, there is a paucity of data about the clinical associations of $\epsilon 2\epsilon 4$ genotype in older adults with late-life cognitive impairment, and we were unable to identify any prior study examining the association of $\epsilon 2\epsilon 4$ with incident MCI, change in cognition, or measures of AD pathology.

Our results were interesting not only for what we found, but also for what we did not find. Despite associations with incident MCI, change in cognition, AD pathology, and A β , we did not find an association of $\epsilon 2\epsilon 4$ with risk of incident AD or with rate of cognitive decline when persons with MCI were included in the analyses. Also, we did not find an association of allele status with the density of tau tangles. These data suggest that $\epsilon 2\epsilon 4$ may preferentially affect the early course of AD disease. We previously reported that the effect of A β was mediated by tau tangles such that when they are both included in the same model, A β is no longer significant.^{12,14} Only when restricting analyses to participants without cognitive impairment did we find associations of A β with cognition with tau tangles in the model.²³ Thus, finding an association of $\epsilon 2\epsilon 4$ with incident MCI and A β , but not incident AD dementia and tangle density, suggests that the effect of allele status is very early.

Preclinical data suggest that apoE may also be involved in deposition and/or clearance of A β from the brain. For example, apoE, and apoE $\epsilon 4$ in particular, have been shown to enhance spontaneous fibrillogenesis of A β in vitro,²⁴ and apoE has been found to form complexes with soluble A β in AD brains.²⁵ By using targeted replacement (TR) mice expressing human apoE isoforms, Shinohara et al.¹⁸ showed that memory preservation in apoE $\epsilon 2$ -TR mice was not

related with markers of synaptic integrity, neuroinflammation, or oxidative stress. In contrast, apoE $\epsilon 4$ -TR mice have been shown to express more systemic and CNS inflammation²⁶ and more deficits in synaptic integrity^{27,28} than apoE $\epsilon 3$ -TR mice. These data suggest that the disparate clinical associations of $\epsilon 2$ and $\epsilon 4$ genotypes may be linked differences in their underlying neurobiology. We found no preclinical study addressing cellular and molecular mechanisms underlying association of $\epsilon 2\epsilon 4$ with cognitive impairment.

The study has several strengths. Uniform structured procedures were followed, examiners were blinded to previously collected data, and all personnel collecting postmortem, genetic, and clinical data were blinded to all other data, further reducing the potential for bias.

Nonetheless, this study has several limitations. Most important, we had only 46 $\epsilon 2\epsilon 4$ carriers in our analytic sample and fewer with the subsample of incident MCI that was small relative to the other more common genotypes, and might account for the high proportion of cases with MCI at baseline and during the course of the study. Although far larger than prior publications, the study will need replicating with even larger samples. Level of education and lifestyles of participants in the study are not representative of the general population. We restricted our analyses to non-Latino white people since previous studies have shown differential associations between APOE genotypes and AD risk in different populations. These studies will need to be extended to other populations.³ We only quantified the fibrillary form of A β and paired helical filament tau-positive tangles by antibodies against single epitopes. It is possible that other epitopes or soluble forms of amyloid or tau are also associated with cognitive decline and risk of AD.

Author contributions

Dr. Oveisgharan: drafting manuscript, analyses/interpretation of data. Dr. Buchman: drafting manuscript, analyses/interpretation of data, study supervision. Dr. Yu: critical revision of the manuscript, analyses/interpretation of data, statistical analyses. Dr. Farfel: critical revision of the manuscript. Dr. Hachinski: critical revision of the manuscript. Dr. Gaiteri: critical revision of the manuscript. Dr. De Jager: critical revision of the manuscript, data acquisition. Dr. Schneider: critical revision of the manuscript, data acquisition. Dr. Bennett: study concept/design, critical revision of the manuscript, analyses/interpretation of data, study supervision, obtaining funding, data acquisition.

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Disclosure

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