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RESEARCH ARTICLE

Age-dependent effects of *APOE* ϵ 4 in preclinical Alzheimer's disease

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Abstract

Objective: The ϵ 4 allele of apolipoprotein E (*APOE*) is the strongest known common genetic risk factor for Alzheimer's disease (AD) and alters age of onset in retrospective studies. Here, we longitudinally test the effects of *APOE* ϵ 4 genotype and age during progression from normal cognition to AD. **Methods:** Using data from 5381 cognitively normal older individuals and Cox proportional hazards models, we longitudinally tested the effects of *APOE* genotype on progression from normal cognition to mild cognitive impairment (MCI) or AD in four age strata (<60, 60–70, 70–80, 80+) and with a sliding window approach between ages 60 and 85. **Results:** We found that *APOE* ϵ 4 carrier status and dosage significantly influenced progression to MCI or AD in all four age groups and that *APOE* ϵ 4-associated progression risk peaked between ages 70 and 75. We confirmed *APOE* ϵ 4-associated progression risk in a subset of the cohort with pathologically proven diagnoses. **Interpretation:** Our findings indicate that in clinically normal individuals, *APOE* ϵ 4 status significantly predicts progression to MCI or AD across older adulthood and that this risk varies with age. This information will be useful as therapeutic interventions become available and clinical decisions can be individually tailored based on age and genetic data.

Introduction

Evidence from genetic at-risk cohorts and clinically normal older individuals suggests that the pathobiological process underlying Alzheimer's disease (AD) begins years – if not decades – prior to a clinical diagnosis of dementia.¹ As preventative and therapeutic interventions are developed, it will be increasingly important to identify clinically normal individuals who are at greatest risk for AD (preclinical AD). Of the known common genetic risk factors for AD, carrying the ϵ 4 allele of apolipoprotein E (*APOE*) is the strongest predictor of AD risk^{2,3} and has been shown to moderate amyloid-related memory decline in preclinical AD.^{4,5} Retrospective studies indicate that *APOE* ϵ 4 modifies risk in an age-dependent manner in AD^{6–9} and other diseases, including cardiovascular disease.^{10,11} However, it is unknown whether *APOE* ϵ 4 has age-dependent effects among cognitively normal older individuals in the earliest stages of AD.

Strong evidence from case-control studies links *APOE* genotype to AD risk, and longitudinal studies have shown it to influence progression from normal cognition to mild cognitive impairment (MCI) or AD. Of the representative survival analyses completed to date,^{12–18} none have attempted to test for age-dependent effects of *APOE* ϵ 4. Furthermore, to the best of our knowledge, there have been no survival analyses that follow *APOE* ϵ 4 carriers and noncarriers from normal cognition through autopsy to pathologically confirm their genotype-dependent risk of progression to AD.

In this study, we hypothesized that *APOE* genotype – especially *APOE* ϵ 4 carrier status – would predict progression to MCI and AD in a longitudinal cohort of cognitively normal individuals and that this conversion risk would be different based on age. We employed a survival analysis framework to test our hypothesis using a large, multi-site longitudinal aging and dementia dataset provided by the National Alzheimer's Coordinating Center (NACC). We split the cohort into four age groups and tested for age-dependent effects using a windowed analysis. We tested the validity our findings in a subset of NACC participants with available pathology data.

Materials and Methods

Participants and clinical characterization

We evaluated data obtained through the NACC, which contains cross-sectional and longitudinal clinical and *APOE* data from past and present Alzheimer's Disease Centers (ADC) funded by the National Institute of

Aging (NIA).¹⁹ There were 28371 individuals in the dataset obtained from the NACC as of February 2015. Individuals were seen at ADCs between January 2005 and November 2014. A total of 19690 of these individuals had longitudinal data available for analysis. Of the participants with longitudinal data available, 15503 had *APOE* genotypes.

We conducted a review of the available clinical data to determine which individuals were cognitively normal at entry into the study. We excluded individuals with preexisting conditions to maximize our ability to determine whether an individual progressed to MCI or AD based on *APOE* genotype. We excluded all individuals with an initial diagnosis of MCI (determined by a clinical dementia rating scale²⁰ [CDR] value of 0.5) or preexisting neurodegenerative condition (determined by a CDR value of 1 or greater). The remaining individuals were all cognitively normal (CDR = 0). We excluded individuals with a diagnosis of Parkinson's disease, an active psychiatric condition, or stroke/history of stroke. The resultant cohort consisted of 5381 individuals.

We divided the cohort of 5381 individuals into four strata according to their baseline age. The age groups were as follows: less than or equal to 60, greater than 60 and less than or equal to 70, greater than 70 and less than or equal to 80, and greater than 80 years of age.

We defined clinical progression to MCI as a change of CDR from 0 to 0.5 and a primary suspected etiology of probable or possible AD. We defined clinical progression to AD as a change of CDR from 0 to 1 or greater and primary suspected etiology of probable or possible AD. We did not allow for “reversions” once an individual had met criteria for MCI or AD; in other words, if an individual progressed from CDR 0 to 0.5 during one visit but then reverted to CDR 0 at a subsequent visit, the individual was still considered a “converter.”

For pathological assessment of neuritic plaques (NPs) and neurofibrillary tangles (NFTs), we followed protocols set forth by CERAD²¹ and Braak and Braak,²² respectively. We adapted pathological criteria for AD from criteria previously set forth in Beecham, et al. (2014).²³ Briefly, a pathologically confirmed case required a diagnosis of MCI or AD as well as an NP score of moderate/frequent and an NFT Braak stage of III–VI. To be included as a control, a subject must not have had a clinical diagnosis of MCI or AD during their participation in the study as well as an NP score of none/sparse and an NFT Braak stage of 0, I, or II. If no NPs were identified, then an NFT Braak stage of III or IV was permitted. After applying these criteria to participants who met clinical data requirements, there were 44 cases and 88 controls for analysis.

Statistical analysis

We modeled clinical progression risk using a Cox proportional hazards model.²⁴ We accounted for ties using the Breslow method. We performed Cox regression analyses to test the effects of *APOE* genotype on progression to MCI or AD in each of the four age groups. We tested the effects of *APOE* genotype under two frameworks: (1) carriers versus noncarriers of the *APOE* ϵ 4 allele and (2) number of copies of the *APOE* ϵ 4 allele (0,1, and 2). To further explore the relationship between *APOE* ϵ 4 and age, we assessed an interaction between *APOE* ϵ 4 and age across the four strata by both *APOE* ϵ 4 carrier status and allele dosage, and tested whether the age strata were different from one another by both *APOE* ϵ 4 carrier status and allele dosage using the “metafor” package in R. We ensured that one clinical group did not drive our findings by performing the aforementioned comparisons in individuals who progressed to MCI or AD, separately. In the AD group, cognitively normal individuals who did not progress directly to AD were allowed to pass through MCI criteria prior to reaching AD criteria. To ensure the accuracy of our AD cohort analysis, we also analyzed the individuals who passed through MCI criteria prior to reaching AD criteria. In the above analyses, we included baseline age, sex, and education as covariates on time to progression to MCI or AD. For the pathologically confirmed individuals, we continued to use time to clinical conversion for the analysis rather than time to death. Furthermore, we did not analyze the pathologically confirmed cohort by age strata due to the small sample size. In the pathologically confirmed analysis, we adjusted for right truncation due to sampling of subjects who died by implementing “time reversal” and methods for delayed entry for model testing.²⁵ We included age, sex, and education as covariates on time to progression to MCI or AD.

After these analyses, we explored age-dependent effects of *APOE* ϵ 4 carrier status across older adulthood. We analyzed the effects of age using a sliding window approach starting from age 60 up to age 85. We limited our analyses to ages between 60 and 85 to focus on the ages in which AD is most prevalent²⁶ and the cohorts with the most available data. We created groups composed of all ages \pm 7 years from each age point of interest

(i.e., for the age 60 analysis, individuals aged 53–67 were analyzed). We performed Cox regression analyses in each age group and then plotted the hazard ratios by age. For illustrative purposes, a best fit line was fitted to the calculated hazard ratios at each age. Sensitivity of our plots to window size was tested using \pm 3 and \pm 5 windows.

We performed analyses in R (version 3.2.2) using the “survival”²⁷ and “metafor”²⁸ packages.

Results

Participants

We divided the cohort into four age groups representing decades of older adulthood in order to account for differences in *APOE* ϵ 4 risk by age.^{2,3,10,11} Demographic data for the entire cohort and each of the four subgroups are summarized in Table 1. Overall, the groups were fairly matched on demographics. The smallest group was the \leq 60-year-olds and the largest group was the 70–80-year-olds. *APOE* genotype data are summarized for the entire cohort and each of the four subgroups in Table 2. Consistent with prior observations,³ *APOE* ϵ 4 allele frequency declined with increasing age, reflecting the age-dependent effects of this allele in the baseline cohort. Of the 5381 cognitively normal individuals included in the study, 984 converted to MCI or AD during the observation period. Progression counts and rates by age group are summarized in Table 3.

Model generation and testing

We tested the proportional hazard assumption in all analyses and found that it was valid for all of the survival models’ covariates in all age groups. In the pathologically confirmed cohort, we found the proportional hazards assumption was similarly valid.

APOE ϵ 4 carrier status and allele dosage influence progression risk to MCI and AD

We found that *APOE* ϵ 4 carrier status significantly influenced risk of progression to MCI or AD in all four age groups (hazard ratio [HR] range: 1.50–1.99) (Fig. 1,

Table 1. Cohort demographics are summarized by age strata and for the entire cohort.

	Age group				
	Age \leq 60	60 < Age \leq 70	70 < Age \leq 80	80 < Age	All ages
<i>N</i>	687	1797	1879	1018	5381
Age \pm SD	53.8 \pm 6.9	66.1 \pm 2.7	75.1 \pm 2.9	85.62 \pm 3.87	71.4 \pm 10.3
Edu \pm SD	16.2 \pm 2.6	15.9 \pm 2.8	15.8 \pm 3.0	15.3 \pm 2.9	15.8 \pm 2.9
Sex (%F)	71.9%	70.1%	65.0%	64.1%	67.4%

Ages provided are from the baseline visit. Edu, education; F, female; SD, standard deviation.

Table 2. Cohort genetic characteristics are summarized by apolipoprotein E genotype and allele count for all age groups and the entire cohort.

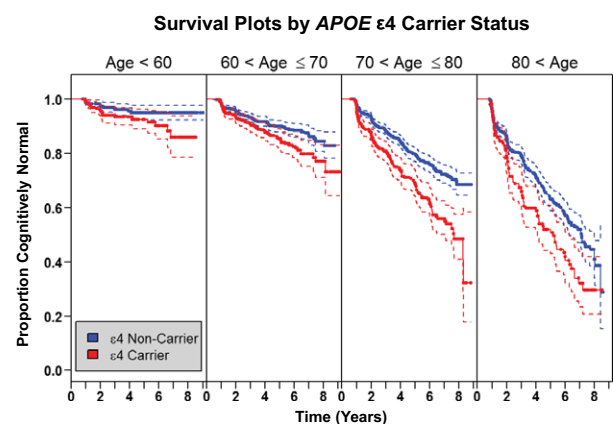
	Age group									
	Age \leq 60		60 < Age \leq 70		70 < Age \leq 80		80 < Age		All ages	
<i>N</i>	687		1797		1879		1018		5381	
	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%
Genotype										
$\epsilon 2\epsilon 2$	1	0.15%	10	0.56%	12	0.64%	7	0.69%	30	0.56%
$\epsilon 2\epsilon 3$	59	8.6%	204	11%	222	12%	149	15%	634	12%
$\epsilon 2\epsilon 4$	21	3.1%	57	3.2%	39	2.1%	23	2.3%	140	2.6%
$\epsilon 3\epsilon 3$	329	48%	997	55%	1129	60%	645	63%	3100	58%
$\epsilon 3\epsilon 4$	233	34%	472	26%	442	24%	190	19%	1337	25%
$\epsilon 4\epsilon 4$	44	6.4%	57	3.2%	35	1.9%	4	0.39%	140	2.6%
Allele count										
$\epsilon 2$	82	6.0%	281	7.8%	285	7.6%	221	10.9%	834	7.7%
$\epsilon 3$	950	69%	2670	74.3%	2922	77.8%	1629	80.0%	8171	75.9%
$\epsilon 4$	342	25%	643	17.9%	551	14.7%	186	9.1%	1757	16.3%

Table 3. Conversion into MCI or AD is summarized by age grouping and for the entire cohort.

	Age group									
	Age \leq 60		60 < Age \leq 70		70 < Age \leq 80		80 < Age		All ages	
# included	687		1797		1879		1018		5381	
Conversion	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%
MCI & AD	36	5.2%	179	10%	408	22%	361	35%	984	18%
MCI only	36	5.2%	176	10%	400	21%	345	34%	957	18%
AD only	3	0.44%	10	0.56%	41	2.2%	79	7.8%	133	2.5%

MCI, mild cognitive impairment; AD, Alzheimer's disease.

Table 4). *APOE* $\epsilon 4$ allele dose also significantly influenced risk of progression to MCI or AD in all four age groups (HR range: 1.52–1.81) (Fig. 2, Table 4). Of the 5381 cognitively normal individuals in the cohort, 957 converted to MCI. When limited to progression to MCI only, our

**Figure 1.** Survival plots by *APOE* $\epsilon 4$ carrier status. Survival plots are shown by *APOE* $\epsilon 4$ carrier status for each age group. 95% confidence intervals are provided as dotted lines.

results remained consistent – *APOE* $\epsilon 4$ carrier status and dose, both significantly influenced risk of progression to MCI ($\epsilon 4$ carrier status HR range: 1.47–1.99; $\epsilon 4$ dose HR range: 1.52–1.78) (Table 5). Our findings were not driven solely by progression to MCI; 133 individuals in our study converted to AD. *APOE* $\epsilon 4$ carrier status and *APOE* $\epsilon 4$ allele dose, both significantly predicted risk of progression to AD in all age groups greater than 60-years-old ($\epsilon 4$ carrier status HR range: 1.91–7.52; $\epsilon 4$ allele dose HR range: 1.75–4.67) but not in the less than or equal to 60-years-old group (Table 5). Our findings in the subset of individuals who progressed through MCI prior to reaching AD closely mirrored the analyses above. *APOE* $\epsilon 4$ carrier status and *APOE* $\epsilon 4$ allele dose, both significantly predicted risk of progression to AD in all age groups greater than 60-years-old ($\epsilon 4$ carrier status HR range: 2.21–10.91; $\epsilon 4$ allele dose HR range: 1.99–3.58) but not in the less than or equal to 60-years-old group. There was not a significant interaction between age and *APOE* $\epsilon 4$ dosage ($P = 0.50$) and carrier status ($P = 0.55$) across the four age strata. Similarly, there was not a significant difference between hazard ratios for the four age strata by *APOE* $\epsilon 4$ dosage ($P = 0.61$) and carrier status ($P = 0.53$).

Table 4. Summarized results for the APOE ε4 carrier status (yes/no) and dosage (0, 1, or 2 copies of the ε4 allele) analysis.

	Age group							
	Age ≤ 60		60 < Age ≤ 70		70 < Age ≤ 80		80 < Age	
	HR (Conf.)	P-value	HR (Conf.)	P-value	HR (Conf.)	P-value	HR (Conf.)	P-value
APOE ε4 carrier status analysis								
Age	1.02 (0.97–1.08)	0.41	1.06 (1.00–1.12)	0.05	1.05 (1.01–1.08)	0.01	1.10 (1.07–1.13)	3.45×10^{-14}
Sex	0.56 (0.29–1.10)	0.09	0.59 (0.43–0.79)	5.59×10^{-4}	0.68 (0.56–0.83)	1.50×10^{-4}	0.97 (0.78–1.21)	0.79
Education	0.89 (0.80–0.99)	0.03	0.94 (0.89–0.99)	0.03	0.93 (0.90–0.96)	1.56×10^{-5}	0.98 (0.95–1.02)	0.40
APOE ε4 carrier status	1.99 (1.01–3.90)	0.05	1.50 (1.11–2.01)	0.01	1.85 (1.51–2.26)	1.78×10^{-9}	1.54 (1.21–1.95)	4.01×10^{-4}
APOE ε4 dosage analysis								
Age	1.02 (0.96–1.08)	0.39	1.05 (1.00–1.12)	0.05	1.05 (1.01–1.08)	0.01	1.10 (1.07–1.13)	3.43×10^{-14}
Sex	0.56 (0.29–1.08)	0.09	0.58 (0.43–0.79)	2.07×10^{-4}	0.68 (0.56–0.83)	1.68×10^{-4}	0.97 (0.78–1.22)	0.82
Education	0.90 (0.81–0.99)	0.04	0.94 (0.90–0.99)	0.03	0.93 (0.90–0.96)	1.69×10^{-5}	0.98 (0.95–1.02)	0.40
APOE ε4 dosage	1.72 (1.05–2.81)	0.03	1.57 (1.24–1.99)	2.08×10^{-4}	1.81 (1.52–2.15)	9.51×10^{-12}	1.52 (1.21–1.91)	3.77×10^{-4}

HR, hazard ratio; Conf., 95% confidence interval range.

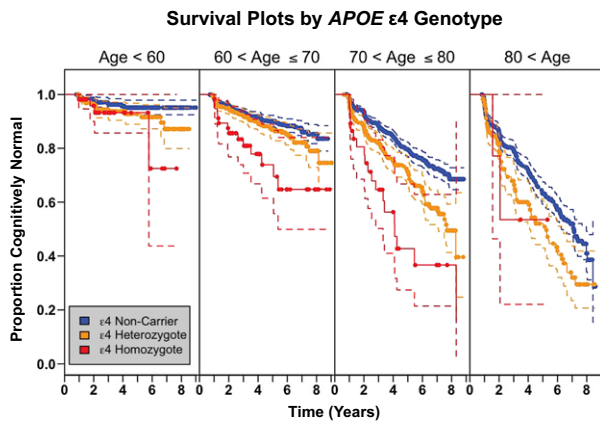


Figure 2. Survival plots by APOE ε4 dosage. Survival plots are shown by APOE ε4 dosage for each age group. 95% confidence intervals are provided as dotted lines.

APOE ε4 influences progression risk to MCI and AD as a function of age

In the above analyses, we observed that the relationship between APOE ε4 carrier status and allele dose appeared most robust in the age 70–80 group. We plotted the HR values from the sliding window analysis for APOE ε4 carrier status and found that they changed nonlinearly as a function of age (Fig. 3). HR values started at approximately 1.4 at age 60 and increased until reaching a peak HR of about 1.8 centered between ages 70 and 75. After those ages, progression risk decreased with increasing age until it approached values similar to those seen at age 60. Changing the window size to ± 5 or ± 3 years did not

change our results. HR error estimates at each age are shown in Figure S1.

APOE ε4 carrier status influences progression risk in pathologically confirmed AD and controls

Finally, we tested whether the estimated progression risk conferred by APOE ε4 was consistent in a subset of the original cohort with confirmed AD or normal brain pathology. There were 44 individuals with pathological AD and 88 individuals that were pathologically normal based on established criteria. We only tested the influence of APOE ε4 carrier status on risk of progression because there were no APOE ε4 homozygotes in the pathological dataset. APOE ε4 carrier status significantly influenced risk of progression to AD ($P = 0.0024$).

Discussion

Our findings illustrate that APOE ε4 carrier status and dosage predict progression to MCI and AD as a function of age, with peak risk between ages 70 and 75. To the best of our knowledge, this is the first longitudinal survival study to illustrate the age-dependent effects of APOE ε4 in cognitively normal individuals who go on to develop AD. These results provide insights into preclinical AD and suggest that the ε4 allele of APOE influences the earliest stages of the AD process.

Our study benefits from a systematic procedure for assigning clinical diagnoses, a large cohort size, detailed clinical and pathological characterization, and a clinically focused statistical approach. By combining CDR with

Table 5. Summarized results for the APOE ε4 carrier status and genotype analysis for MCI and AD.

	Age group											
	Age ≤ 60			60 < Age ≤ 70			70 < Age ≤ 80			80 < Age		
	HR (Conf.)	P-value		HR (Conf.)	P-value		HR (Conf.)	P-value		HR (Conf.)	P-value	
MCI												
APOE ε4 carrier status analysis												
Age	1.02 (0.97–1.08)	0.41		1.05 (1.00–1.11)	0.07		1.04 (1.01–1.07)	0.02		1.10 (1.07–1.13)	7.73 × 10 ⁻¹⁴	
Sex	0.56 (0.29–1.09)	0.09		0.59 (0.43–0.79)	6.39 × 10 ⁻⁴		0.67 (0.54–0.82)	8.32 × 10 ⁻⁵		0.96 (0.76–1.20)	0.7	
Education	0.89 (0.80–0.99)	0.03		0.94 (0.90–1.00)	0.05		0.94 (0.90–0.97)	1.11 × 10 ⁻⁴		0.98 (0.94–1.02)	0.36	
APOE ε4 carrier status	1.99 (1.01–3.90)	0.05		1.47 (1.10–2.00)	0.01		1.83 (1.49–2.24)	5.23 × 10 ⁻⁹		1.56 (1.22–1.98)	3.57 × 10 ⁻⁴	
APOE ε4 genotype analysis												
Age	1.02 (0.97–1.08)	0.39		1.05 (1.00–1.12)	0.06		1.04 (1.01–1.08)	0.02		1.10 (1.07–1.13)	7.73 × 10 ⁻¹⁴	
Sex	0.56 (0.29–1.08)	0.09		0.59 (0.43–0.80)	6.08 × 10 ⁻⁴		0.67 (0.55–0.82)	9.44 × 10 ⁻⁵		0.96 (0.77–1.21)	0.74	
Education	0.90 (0.81–0.99)	0.04		0.95 (0.90–1.00)	0.04		0.94 (0.90–0.97)	1.16 × 10 ⁻⁴		0.98 (0.94–1.02)	0.37	
APOE ε4 genotype	1.72 (1.05–2.81)	0.03		1.52 (1.20–1.94)	6.70 × 10 ⁻⁴		1.78 (1.49–2.11)	8.43 × 10 ⁻¹¹		1.53 (1.21–1.93)	3.37 × 10 ⁻⁴	
AD												
APOE ε4 carrier status analysis												
Age	0.96 (0.80–1.15)	0.67		1.13 (0.88–1.45)	0.35		1.12 (1.01–1.25)	0.03		1.13 (1.07–1.19)	7.28 × 10 ⁻⁶	
Sex	0.33 (0.03–3.84)	0.38		1.31 (0.27–6.24)	0.74		1.10 (0.56–2.18)	0.78		1.15 (0.70–1.89)	0.59	
Education	0.60 (0.39–0.93)	0.02		0.91 (0.73–1.13)	0.40		0.84 (0.76–0.92)	3.62 × 10 ⁻⁴		0.98 (0.90–1.07)	0.64	
APOE ε4 carrier status	6.89 (0.31–151.23)	0.22		7.52 (1.59–35.48)	0.01		4.42 (2.36–8.26)	3.06 × 10 ⁻⁶		1.91 (1.17–3.13)	9.91 × 10 ⁻³	
APOE ε4 genotype analysis												
Age	0.98 (0.82–1.17)	0.80		1.13 (0.88–1.46)	0.32		1.13 (1.01–1.25)	0.03		1.13 (1.07–1.19)	8.41 × 10 ⁻⁶	
Sex	0.29 (0.03–3.32)	0.32		1.36 (0.29–6.43)	0.70		1.12 (0.56–2.21)	0.75		1.16 (0.70–1.90)	0.57	
Education	0.62 (0.41–0.94)	0.02		0.89 (0.72–1.12)	0.32		0.84 (0.76–0.92)	2.45 × 10 ⁻⁴		0.98 (0.90–1.07)	0.66	
APOE ε4 genotype	3.83 (0.39–37.89)	0.25		4.67 (1.87–11.64)	9.52 × 10 ⁻⁴		3.77 (2.34–6.07)	4.83 × 10 ⁻⁸		1.75 (1.10–2.79)	0.02	

MCI, mild cognitive impairment; HR, hazard ratio; Conf., 95% confidence interval range; APOE, apolipoprotein E.

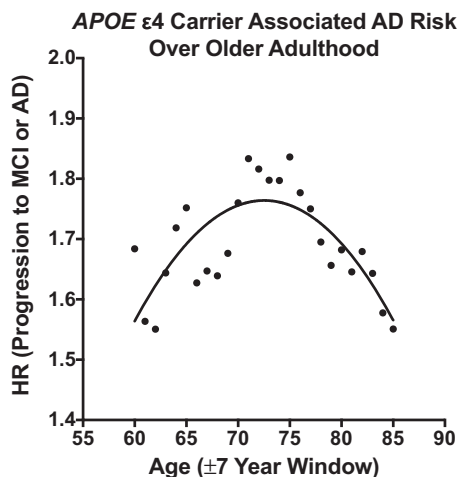


Figure 3. *APOE* ϵ 4 carrier-associated AD risk over older adulthood. Hazard ratios for the windowed analysis are plotted by age. HR, hazard ratio; AD, Alzheimer's disease. A line of best fit was added to the diagram for illustrative purposes.

etiologic diagnosis, we had a more quantitative approach for determining MCI or AD status rather than relying solely upon clinical diagnoses, which could vary across the multiple study sites included in the dataset. The CDR has high interrater reliability^{29,30} and is thereby an optimal measure of progression in a multicenter study like ours. Furthermore, when compared to retrospective studies that rely upon subjective impressions of disease onset during patient interviews, our study was able to more accurately capture clinical onset as each participant progressed through a uniform, quantifiable measure of cognitive impairment. Our large cohort and conservative inclusion criteria likely improved our ability to detect the effects of *APOE* ϵ 4 on conversion into MCI and AD. Time to progress to MCI or AD was measured from baseline visit rather than age; this approach allowed us to estimate HRs that are more useful in a clinical setting and to directly assess whether risk varies based on age. The HR we estimated for each age using the sliding window approach is amenable to extrapolation for use in clinical trials, where risk estimates can be specifically calculated for each individual over the study period based on enrollment age.

Our findings are consistent with our hypothesis that *APOE* ϵ 4 modulates AD progression risk in an age-dependent manner. Our estimates of *APOE* ϵ 4 carrier status risk as a function of age extend and expand upon the results from a large meta-analysis of cross-sectional studies that support these findings.³ In our longitudinal study and in previous cross-sectional studies,³ *APOE* ϵ 4-associated progression risk was U-shaped, increasing with age to a peak and subsequently decreasing (for example, see Fig. 3). In our study, the risk of progression by *APOE* ϵ 4

status was much more pronounced between ages 70 and 80. This provides a likely explanation for why we observed increasing progression risk for the earlier ages in our windowed analysis – individuals carrying one or two copies of the ϵ 4 allele were more likely than noncarriers to progress to MCI or AD at an earlier age. We hypothesize that the subsequent decrease in progression risk at later ages (greater than 80) may in part be due to fewer numbers of individuals alive in this stratum and decreased ϵ 4 frequency in this group (i.e., people with *APOE* ϵ 4 develop AD at an earlier age). This is particularly evident in the oldest age strata in Figures 1 and 2. Consistent with recent evidence,²⁶ one possible explanation for this finding is that *APOE* ϵ 4 carriers who successfully pass through peak risk years possess “protective” genetic, lifestyle, or other factors that may delay progression to cognitive impairment. Our data also suggest that progression to cognitive impairment occurs irrespective of genotype status (Figs. 1, 2); carrying the ϵ 4 allele just predisposes AD development at an earlier age. In this framework, “protection” implies delayed onset, rather than prevention of disease.

Our finding that *APOE* ϵ 4-associated risk changes as a U-shaped function of age has important clinical ramifications. Although there are currently no disease-modifying treatments for AD, identifying individuals at greatest genetic risk for AD may help with clinical diagnosis and prognosis and help initiate a dialog on future planning. As effective treatments for AD become available, clinicians could incorporate a patient's *APOE* genotype and age into treatment regimens – preemptively identifying individuals most at risk for AD prior to reaching the ages where they are most vulnerable to progression to cognitive impairment.

In addition to their clinical relevance, our findings have notable scientific implications. Given that *APOE* ϵ 4-associated AD risk does not change linearly across age, future studies and trials that utilize *APOE* ϵ 4 status may choose to include only individuals within the highest age bracket of *APOE* ϵ 4-associated risk to improve their power to detect disease-modifying intervention effects. Recent evidence in murine models of AD suggests that the age-dependent effects of *APOE* ϵ 4 are linked to an NMDA receptor pathway³¹ and reducing ϵ 3 and ϵ 4 *APOE* protein attenuates age-dependent A β accumulation.³² Given this and developing *APOE* ϵ 4-targeted treatments,³³ clinicians may soon be able to proactively identify cognitively normal *APOE* ϵ 4 carriers and others progressing toward AD for enrollment in clinical trials.

There are some limitations to this study. First, we focused our analyses on *APOE* ϵ 4 carrier status and dosage as a predictor of cognitive decline. Additional cellular and neuroanatomical biomarkers may provide better

estimations of AD risk, particularly if used in combination. Second, data provided by the NACC is not community-based – individuals in the study are generally healthy, and self-selected to participate in research studies. For example, our cohort had a larger proportion of females in it than would be expected in the general population. Given this, our estimates of AD risk may not extrapolate to the general population, so future community-based studies will be required to improve these estimates. Additionally, we do not have information on what factors incentivize an individual to participate in AD research studies. For instance, a cognitively normal 60-year-old individual may be participating in research for different reasons than a cognitively normal 80-year-old individual. Whether one age group participates in research studies due to differing AD risk profiles remains open to further research. We did not incorporate information on other genetic markers known to be associated with AD; future studies that incorporate additional common and rare variants, in combination with *APOE*, may prove even more informative. Our sliding window analysis is limited by sample size and nonsignificant test statistics below the age of 60 and above 80. We speculate that, with sufficient data, future studies may be able to better determine the shape of the plotted risk curve and ascertain the earliest age at which *APOE* ϵ 4 begins influencing AD risk. Future studies may also provide better estimates of progression risk to AD rather than to AD or MCI as done in our study. We observed 133 conversions to AD out of 5381 cognitively normal individuals and of these, only 13 individuals were aged less than 70. Our estimates of AD risk in the youngest two groupings are relatively uncertain when compared to our other findings and should be treated with caution. Finally, we did not incorporate specific biomarkers of AD pathology like molecular imaging or cerebrospinal fluid amyloid/tau levels into our analyses. However, we did successfully replicate our primary findings in a pathologically confirmed subcohort of cases and controls suggesting that diagnostic specificity within the entire dataset may have been sufficient to provide accurate estimates of *APOE* ϵ 4-associated risk.

In summary, we emphasize the importance of *APOE* ϵ 4 as a predictor of AD risk and for the first time, identify its age-specific effects on progression to cognitive impairment. Our study shows that individuals between ages 70 and 75 have the highest ϵ 4-associated risk of progression to MCI or AD. This finding is in line with previously published cross-sectional data. Our results are an important step toward a more personalized and disease-specific treatment strategy. Age-based *APOE* ϵ 4 risk estimates could provide stronger opportunities to identify disease-modifying treatments and could be especially valuable once effective therapies are available. Finally, our findings

set the stage for more detailed characterization of already identified AD risk variants in a comprehensive, age-dependent framework.

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Conflicts of Interest

Nothing to report.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. *APOE* ϵ 4 carrier-associated AD risk over older adulthood.