ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

RESEARCH ARTICLE

Age-dependent effects of *APOE* ε4 in preclinical Alzheimer's disease

Luke W. Bonham[1], Ethan G. Geier[1], Chun C. Fan[2], Josiah K. Leong[3], Lilah Besser[4], Walter A. Kukull[4], John Kornak[5], Ole A. Andreassen[6], Gerard D. Schellenberg[7], Howard J. Rosen[1], William P. Dillon[8], Christopher P. Hess[8], Bruce L. Miller[1], Anders M. Dale[2], Rahul S. Desikan[8,a] & Jennifer S. Yokoyama[1,a]

[1]Memory and Aging Center, Department of Neurology, University of California, San Francisco, San Francisco, California
[2]Departments of Cognitive Sciences and Neurosciences, University of California, San Diego, La Jolla, California
[3]Department of Psychology, Stanford University, Palo Alto, California
[4]National Alzheimer's Coordinating Center, Department of Epidemiology, University of Washington, Seattle, Washington
[5]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California
[6]NORMENT, Institute of Clinical Medicine, University of Oslo and Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway
[7]Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania
[8]Neuroradiology Section, Department of Radiology and Biomedical Imaging, University of California, San Francisco, San Francisco, California

Correspondence
Jennifer S. Yokoyama, Memory and Aging Center, University of California, San Francisco, 675 Nelson Rising Lane, Suite 190, San Francisco, CA 94158, USA. Tel: (415) 476-5565; Fax: (415) 502-7588; E-mail: jennifer.yokoyama@ucsf.edu
Rahul S. Desikan, Neuroradiology Section, Department of Radiology and Biomedical Imaging University of California, San Francisco, 505 Parnassus Avenue, San Francisco, CA 94143, USA. Tel: (415)-353-1079; Fax: 415-353-8593; E-mail: rahul.desikan@ucsf.edu

Funding information
Primary support for data analyses was provided by the Larry L. Hillblom Foundation 2012-A-015-FEL (JSY), AFTD Susan Marcus Memorial Fund Clinical Research Grant (JSY), NIA K01 AG049152 (JSY), and the Tau Consortium. The University of California, San Francisco Academic Senate provided support for publication of this work through the Open Access Publishing Fund. Samples from the National Cell Repository for Alzheimer's Disease (NCRAD), which receives government support under a cooperative agreement grant (U24 AG21886) awarded by the National Institute on Aging (NIA), were used in this study.

Received: 6 May 2016; Revised: 3 June 2016; Accepted: 14 June 2016

doi: 10.1002/acn3.333

[a]equal contribution; corresponding authors

Abstract

Objective: The ε4 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* ε4 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* ε4 carrier status and dosage significantly influenced progression to MCI or AD in all four age groups and that *APOE* ε4-associated progression risk peaked between ages 70 and 75. We confirmed *APOE* ε4-associated progression risk in a subset of the cohort with pathologically proven diagnoses. **Interpretation**: Our findings indicate that in clinically normal individuals, *APOE* ε4 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 ε4 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* ε4 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* ε4 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* ε4. Furthermore, to the best of our knowledge, there have been no survival analyses that follow *APOE* ε4 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* ε4 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 scale[20] [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 CERAD[21] 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* ε4 allele and (2) number of copies of the *APOE* ε4 allele (0,1, and 2). To further explore the relationship between *APOE* ε4 and age, we assessed an interaction between *APOE* ε4 and age across the four strata by both *APOE* ε4 carrier status and allele dosage, and tested whether the age strata were different from one another by both *APOE* ε4 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* ε4 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 prevalent[26] and the cohorts with the most available data. We created groups composed of all ages ±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 ±3 and ±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* ε4 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 ≤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* ε4 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 ε4 carrier status and allele dosage influence progression risk to MCI and AD

We found that *APOE* ε4 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 ≤ 60	60 < Age ≤ 70	70 < Age ≤ 80	80 < Age	All ages
N	687	1797	1879	1018	5381
Age ± SD	53.8 ± 6.9	66.1 ± 2.7	75.1 ± 2.9	85.62 ± 3.87	71.4 ± 10.3
Edu ± SD	16.2 ± 2.6	15.9 ± 2.8	15.8 ± 3.0	15.3 ± 2.9	15.8 ± 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 ≤ 60		60 < Age ≤ 70		70 < Age ≤ 80		80 < Age		All ages	
N	687		1797		1879		1018		5381	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%
Genotype										
ε2ε2	1	0.15%	10	0.56%	12	0.64%	7	0.69%	30	0.56%
ε2ε3	59	8.6%	204	11%	222	12%	149	15%	634	12%
ε2ε4	21	3.1%	57	3.2%	39	2.1%	23	2.3%	140	2.6%
ε3ε3	329	48%	997	55%	1129	60%	645	63%	3100	58%
ε3ε4	233	34%	472	26%	442	24%	190	19%	1337	25%
ε4ε4	44	6.4%	57	3.2%	35	1.9%	4	0.39%	140	2.6%
Allele count										
ε2	82	6.0%	281	7.8%	285	7.6%	221	10.9%	834	7.7%
ε3	950	69%	2670	74.3%	2922	77.8%	1629	80.0%	8171	75.9%
ε4	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 ≤ 60		60 < Age ≤ 70		70 < Age ≤ 80		80 < Age		All ages	
# included	687		1797		1879		1018		5381	
Conversion	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
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* ε4 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 results remained consistent – *APOE* ε4 carrier status and dose, both significantly influenced risk of progression to MCI (ε4 carrier status HR range: 1.47–1.99; ε4 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* ε4 carrier status and *APOE* ε4 allele dose, both significantly predicted risk of progression to AD in all age groups greater than 60-years-old (ε4 carrier status HR range: 1.91–7.52; ε4 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* ε4 carrier status and *APOE* ε4 allele dose, both significantly predicted risk of progression to AD in all age groups greater than 60-years-old (ε4 carrier status HR range: 2.21–10.91; ε4 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* ε4 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* ε4 dosage ($P = 0.61$) and carrier status ($P = 0.53$).

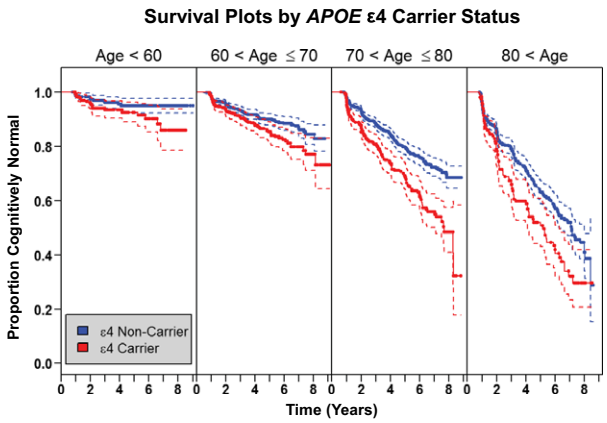

Figure 1. Survival plots by *APOE* ε4 carrier status. Survival plots are shown by *APOE* ε4 carrier status for each age group. 95% confidence intervals are provided as dotted lines.

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.

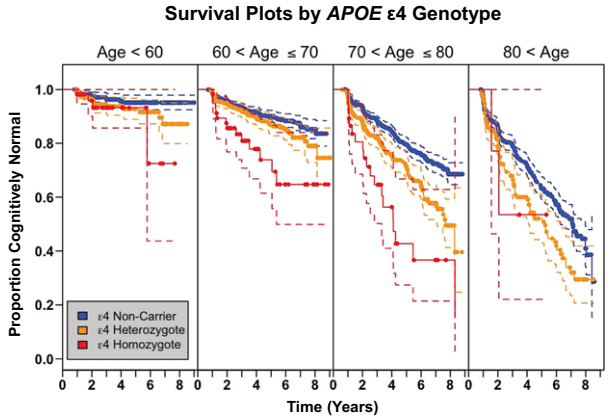

Figure 2. Survival plots by *APOE* ε4 dosage. Survival plots are shown by *APOE* ε4 dosage for each age group. 95% confidence intervals are provided as dotted lines.

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.

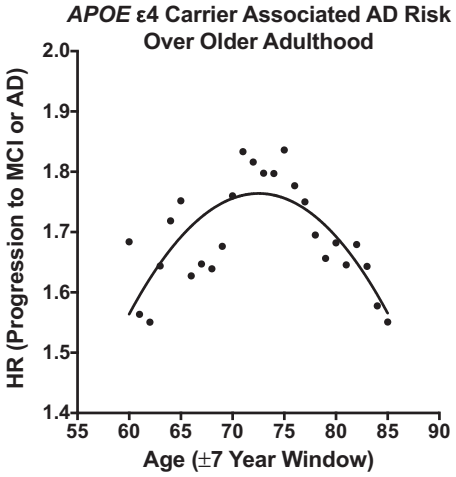

Figure 3. *APOE* ε4 carrier-associated AD risk over older adulthood. Hazard ratios for the windowed analysis are plotted by age. HR, hazard ratio; AD, Alzheimer's disease. A line of best fit was added to the diagram for illustrative purposes.

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* ε4 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* ε4 modulates AD progression risk in an age-dependent manner. Our estimates of *APOE* ε4 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* ε4-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* ε4 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 ε4 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 ε4 frequency in this group (i.e., people with *APOE* ε4 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* ε4 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 ε4 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* ε4-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* ε4-associated AD risk does not change linearly across age, future studies and trials that utilize *APOE* ε4 status may choose to include only individuals within the highest age bracket of *APOE* ε4-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* ε4 are linked to an NMDA receptor pathway[31] and reducing ε3 and ε4 APOE protein attenuates age-dependent Aβ accumulation.[32] Given this and developing APOE ε4-targeted treatments,[33] clinicians may soon be able to proactively identify cognitively normal *APOE* ε4 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* ε4 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* ε4 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* ε4-associated risk.

In summary, we emphasize the importance of *APOE* ε4 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 ε4-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* ε4 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.

Acknowledgments

We thank all research participants for their long-standing dedication to their respective studies. Primary support for data analyses was provided by the Larry L. Hillblom Foundation 2012-A-015-FEL (JSY), AFTD Susan Marcus Memorial Fund Clinical Research Grant (JSY), NIA K01 AG049152 (JSY), National Alzheimer's Coordinating Center (NACC) Junior Investigator (JI) Award (RSD) and the Tau Consortium. The University of California, San Francisco Academic Senate provided support for publication of this work through the Open Access Publishing Fund. The NACC database is funded by NIA/NIH Grant U01 AG016976. NACC data are contributed by the NIA funded ADCs: P30 AG019610 (PI Eric Reiman, MD), P30 AG013846 (PI Neil Kowall, MD), P50 AG008702 (PI Scott Small, MD), P50 AG025688 (PI Allan Levey, MD, PhD), P50 AG047266 (PI Todd Golde, MD, PhD), P30 AG010133 (PI Andrew Saykin, PsyD), P50 AG005146 (PI Marilyn Albert, PhD), P50 AG005134 (PI Bradley Hyman, MD, PhD), P50 AG016574 (PI Ronald Petersen, MD, PhD), P50 AG005138 (PI Mary Sano, PhD), P30 AG008051 (PI Steven Ferris, PhD), P30 AG013854 (PI M. Marsel Mesulam, MD), P30 AG008017 (PI Jeffrey Kaye, MD), P30 AG010161 (PI David Bennett, MD), P50 AG047366 (PI Victor Henderson, MD, MS), P30 AG010129 (PI Charles DeCarli, MD), P50 AG016573 (PI Frank LaFerla, PhD), P50 AG016570 (PI Marie-Francoise Chesselet, MD, PhD), P50 AG005131 (PI Douglas Galasko, MD), P50 AG023501 (PI Bruce Miller, MD), P30 AG035982 (PI Russell Swerdlow, MD), P30 AG028383 (PI Linda Van Eldik, PhD), P30 AG010124 (PI John Trojanowski, MD, PhD), P50 AG005133 (PI Oscar Lopez, MD), P50 AG005142 (PI Helena Chui, MD), P30 AG012300 (PI Roger Rosenberg, MD), P50 AG005136 (PI Thomas Montine, MD, PhD), P50 AG033514 (PI Sanjay Asthana, MD, FRCP), P50 AG005681 (PI John Morris, MD), and P50 AG047270 (PI Stephen Strittmatter, MD, PhD). The Alzheimer's Disease Genetics Consortium supported the collection and genotyping of samples used in this study through National Institute on Aging (NIA) grants U01AG032984 and RC2AG036528. Data for this study were prepared, archived, and distributed by the National Institute on Aging Alzheimer's Disease Data Storage Site (NIAGADS) at the University of Pennsylvania (U24-AG041689-01), funded by the National Institute on Aging. Samples from the National Cell Repository for Alzheimer's Disease (NCRAD), which receives government support under a cooperative agreement grant (U24

AG21886) awarded by the National Institute on Aging (NIA), were used in this study.

Author Contributions

Conception and design of the study: L. W. B, E. G. G, C. C. F, J. K. L, W. A. K., J. K., O. A. A., A. M. D., W. P. D., C. P. H., R. S. D., J. S. Y. Acquisition and analysis of data: L. W. B, L. B., W. A. K., G. D. S., H. J. R., B. L. M., R. S. D., J. S. Y. Drafting a significant portion of the manuscript or figures: L. W. B, R. S. D., J. S. Y.

Conflicts of Interest

Nothing to report.

References

1. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging and the Alzheimer's Association workgroup. Alzheimer's Dement 2011;7:280–292.
2. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–923.
3. Farrer LA, Cupples LA, Haines JL, et al. Effects of Age, Sex, and Ethnicity on the Association Between Apolipoprotein E Genotype and Alzheimer Disease: a Meta-analysis. JAMA J Am Med Assoc 1997;278:1349–1356.
4. Lim YY, Villemagne VL, Pietrzak RH, et al. APOE ε4 moderates amyloid-related memory decline in preclinical Alzheimer's disease. Neurobiol Aging 2015;36:1239–1244.
5. Mormino EC, Betensky RA, Hedden T, et al. Amyloid and APOE e4 interact to influence short-term decline in preclinical Alzheimer disease. Neurology 2014;82:1760–1767.
6. Sando SB, Melquist S, Cannon A, et al. APOE epsilon 4 lowers age at onset and is a high risk factor for Alzheimer's disease; a case control study from central Norway. BMC Neurol 2008;8:9.
7. Murman DL, Foster NL, Kilgore SP, et al. Apolipoprotein E and Alzheimer's disease: strength of association is related to age at onset. Dementia 1996;7:251–255.
8. Frisoni GB, Manfredi M, Geroldi C, et al. The prevalence of apoE-epsilon4 in Alzheimer's disease is age dependent. J Neurol Neurosurg Psychiatry 1998;65:103–106.
9. Olarte L, Schupf N, Lee JH, et al. Apolipoprotein E ε4 and age at onset of sporadic and familial Alzheimer disease in Caribbean Hispanics. Arch Neurol 2006;63:1586–1590.
10. Bañares VG, Peterson G, Aguilar D, et al. Association between the APOE*4 allele and atherosclerosis is age dependent among Argentine males. Hum Biol 2005;77:247–256.
11. Ilveskoski E, Perola M, Lehtimaki T, et al. Age-Dependent Association of Apolipoprotein E Genotype With Coronary and Aortic Atherosclerosis in Middle-Aged Men : an Autopsy Study. Circulation 1999;100:608–613.
12. Brainerd CJ, Reyna VF, Petersen RC, et al. The apolipoprotein E genotype predicts longitudinal transitions to mild cognitive impairment but not to Alzheimer's dementia: findings from a nationally representative study. Neuropsychology 2013;27:86–94.
13. Geda YE, Knopman DS, Mrazek DA, et al. Depression, apolipoprotein E genotype, and the incidence of mild cognitive impairment: a prospective cohort study. Arch Neurol 2006;63:435–440.
14. Marquis S, Moore MM, Howieson DB, et al. Independent predictors of cognitive decline in healthy elderly persons. Arch Neurol 2002;59:601–606.
15. Bunce D, Fratiglioni L, Small BJ, et al. APOE and cognitive decline in preclinical Alzheimer disease and non-demented aging. Neurology 2004;63:816–821.
16. Wilson RS, Schneider JA, Barnes LL, et al. The apolipoprotein E epsilon 4 allele and decline in different cognitive systems during a 6-year period. Arch Neurol 2002;59:1154–1160.
17. Beydoun MA, Boueiz A, Abougergi MS, et al. Sex differences in the association of the apolipoprotein E epsilon 4 allele with incidence of dementia, cognitive impairment, and decline. Neurobiol Aging 2012;33:720–731.
18. Altmann A, Tian L, Henderson VW, Greicius MD. Sex modifies the APOE-related risk of developing Alzheimer disease. Ann Neurol 2014;75:563–573.
19. Beekly DL, Ramos EM, van Belle G, et al. The National Alzheimer's Coordinating Center (NACC) Database: an Alzheimer disease database. Alzheimer Dis Assoc Disord 2004;18:270–277.
20. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 1993;43:2412–2414.
21. Mirra SS, Hart MN, Terry RD. Making the diagnosis of Alzheimer's disease: a primer for practicing pathologists. Arch Pathol Lab Med 1993;117:132–144.
22. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–259.
23. Beecham GW, Hamilton K, Naj AC, et al. Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. PLoS Genet 2014;10:e1004606.
24. Grambsch TMT, Grambsch PM. Modeling Survival Data: extending the Cox Model. New York: Springer, 2000.
25. Klein JP, Moeschberger ML. Survival Analysis: techniques for Censored and Truncated Data. New York: Springer, 2003.

26. Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology 2013;80: 1778–1783.
27. Therneau TM (2015). A Package for Survival Analysis in S. version 2.38. http://CRAN.R-project.org/package=survival.
28. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. J Stat Softw 2010;36:1–48.
29. Rockwood K, Strang D, Macknight C, et al. Interrater reliability of the Clinical Dementia Rating in a multicenter trial. J Am Geriatr Soc 2000;48:558–559.
30. Webber D, Collins M, DeFilippis N, Hill F. Reliability of the clinical dementia rating with a traumatic brain injury population: a preliminary study. Appl Neuropsychol Adult 2013;20:145–151.
31. Liu D, Pan X, Zhang J, et al. APOE4 enhances age-dependent decline in cognitive function by down-regulating an NMDA receptor pathway in EFAD-Tg mice. Mol Neurodegener 2015;10:7.
32. Bien-Ly N, Gillespie AK, Walker D, et al. Reducing human apolipoprotein E levels attenuates age-dependent Aβ accumulation in mutant human amyloid precursor protein transgenic mice. J Neurosci 2012;32:4803–4811.
33. Michaelson DM. APOE ε4: the most prevalent yet understudied risk factor for Alzheimer's disease. Alzheimer's Dement 2014;10:861–868.

Supporting Information

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

Figure S1. *APOE* ε4 carrier-associated AD risk over older adulthood.

