Parental history of Alzheimer disease associated with lower plasma apolipoprotein E levels

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ABSTRACT

Background: Variation in *APOE* genotype is a determinant of Alzheimer disease (AD), but the risk associated with variation in plasma apoE levels has yet to be determined. Here, we studied offspring with and without a parental history of AD to identify the effect of plasma apoE levels at middle age on the risk of late-onset AD.

Methods: Some 203 offspring from 92 families with a parental history of AD were compared with 197 offspring from 97 families without a parental history of AD. *APOE* genotypes and plasma apoE levels were assessed in all offspring. Difference in plasma apoE level between subjects with and without a parental history of AD was calculated using robust linear regression, both stratified and adjusted for *APOE* genotype.

Results: Offspring with a parental history of AD were more likely to be an *APOE* ε4 allele carrier (46% vs 21%, $p < 0.001$) than offspring without such a parental history. Mean plasma apoE levels strongly decreased from ε2 to ε3ε3 to ε4 carriers ($p < 0.001$). Offspring with a parental history of AD had lower plasma apoE levels than subjects without such a history, both in analyses adjusted for *APOE* genotype (difference: −0.21 mg/dL, $p = 0.02$) and when using standardized *Z* scores, when stratified for *APOE* genotype (difference: −0.22, $p = 0.009$).

Conclusions: Our findings suggest that lower plasma apoE levels in middle age could be a risk factor for Alzheimer disease in old age, independent of *APOE* genotype. ***Neurology*® 2009;73:681–687**

GLOSSARY

AD = Alzheimer disease; **BMI** = body mass index; **CI** = confidence interval; **CVD** = cardiovascular disease; **EOAD** = early-onset Alzheimer disease; **HDL** = high-density lipoprotein; **LDL** = low-density lipoprotein; **LOAD** = late-onset Alzheimer disease; **MMSE** = Mini-Mental State Examination; **VaD** = vascular dementia.

Variation in the apolipoprotein E (*APOE*) gene is the strongest genetic risk factor of late-onset Alzheimer disease (AD). Two common nucleotide polymorphisms constitute the ε2/ε3/ε4 alleles that encode the functionally and structurally different apoE2, apoE3, and apoE4 isoforms. Carriers of the ε4 allele are at an increased risk of AD.[1] Although the exact biologic mechanism explaining the risk differences is unclear, structural variation in the apoE protein has been shown to play a role in numerous processes contributing to AD, such as cholesterol transport and cellular uptake, clearance of oxidative products, and inflammation.[2-4]

Several studies have reported on the association between plasma apoE levels and AD or cognitive impairment, but with opposing results (table 1).[5-10] In some cross-sectional studies, plasma apoE levels were similar in patients with AD and controls,[6-8] whereas others found lower plasma apoE levels in patients with AD.[9] On the contrary, an earlier study reported higher plasma apoE levels in patients with AD.[5] In the Leiden 85-Plus Study, we recently found that ε3ε3 and ε3ε4 carriers with high plasma apoE levels had worse cognitive function

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Table 1 Overview on studies investigating the association between plasma apoE levels and dementia or cognitive function

Study: ref; year	Study design	Study population (mean age)	Findings (plasma apoE levels), *p* value	
5; 1997	Case-control	10 EOAD patients (62 y)	ε4: EOAD: 3.6 μg/mg protein, $p < 0.001$	Non-ε4: EOAD: 3.8 μg/mg protein, $p < 0.01$
		43 LOAD patients (80 y)	LOAD: 3.3 μg/mg protein, $p < 0.001$	LOAD: 3.2 μg/mg protein, $p < 0.001$
		12 control subjects (64 y)	Control: 2.6 μg/mg protein, reference	Control: 2.2 μg/mg protein, reference
7; 1998 (Rotterdam Study)	Nested case-control	129 AD patients (84 y)	AD: 25.5 mg/L, control: 28.2 mg/L, difference: 0.12 mg/L, $p < 0.05$	
		890 control subjects (68 y)	Adjustment for age/sex/protein/albumin/BMI/*APOE* genotype, difference: 0.05 mg/L, $p > 0.05$	
8; 1999	Case-control	85 AD patients (86 y)	AD: 44.5 mg/L, control: 44.3 mg/L, difference: 0.2 mg/L, $p > 0.05$	
		156 control subjects (84 y)	Stratification for *APOE* genotype: no difference between groups	
9; 2000 (ApoEurope Study)	Multicenter case-control	489 AD patients (75 y)	AD: 42.9 mg/L, control: 48.3 mg/L, difference: 5.4 mg/L, $p < 0.001$	
		429 control subjects (71 y)	Adjustment for age/sex/*APOE* genotype, difference: 3.7 mg/L, $p < 0.001$	
6; 2004	Case-control	48 AD patients (85 y)	AD: 45.6 mg/L, VaD: 45.4 mg/L, control: 43.4 mg/L, difference: 2.0–2.2 mg/L, $p > 0.05$	
		30 VaD patients (85 y)	Stratification for *APOE* genotype: no difference between groups	
		30 control subjects (84 y)		
10; 2007 (Leiden 85-Plus Study)	Population-based follow-up	546 subjects aged 85 y at baseline	Median plasma apoE level for whole population: 50.2 mg/L	
			Adjustment for *APOE* genotype, subjects with high plasma apoE levels had 1.79-point lower MMSE scores than subjects with low plasma apoE levels, $p = 0.001$	

EOAD = early-onset Alzheimer disease; LOAD = late-onset Alzheimer disease; AD = Alzheimer disease; BMI = body mass index; VaD = vascular dementia; MMSE = Mini-Mental State Examination.

during a 5-year follow-up period.[10] The mechanisms that underlie these divergent results are unknown, but possibly disease state influences plasma apoE levels.

To overcome the possible distorting effect of disease on the apoE phenotype, we used a family study design comparing middle-aged offspring with and without a parental history of AD to identify and quantify whether variation in plasma apoE levels is associated with an increased risk of AD. Additionally, adjustment for lipid levels was performed to study the association, independent of the lipid profile. All offspring were characterized for *APOE* genotype, and plasma apoE levels were determined.

METHODS

Study design and participants. From 2006 to 2007, we initiated a family study to investigate midlife factors that are associated with an increased risk of late-life AD in subjects with and subjects without a parental history of AD. Ninety-two consecutive patients aged 70 years and older with a diagnosis of probable AD according to National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria (mean age 82 years) were recruited from the memory clinic of the Alzheimer Center of the Vrije Universiteit Medical Center and affiliated nursing homes. Subjects with mixed-type dementia or vascular dementia were excluded. Ninety-seven married couples, aged 70 years and older, who were free from dementia, were also recruited (mean age 82.6 years). At least 1 spouse participated in either the Longitudinal Aging Study Amsterdam or the Leiden 85-Plus Study, 2 Dutch prospective population-based studies. Subjects were classified as free from dementia when having a Mini-Mental State Examination score greater than 27 points. When one of the spouses was deceased (n = 55), a history on cognitive function from the surviving spouse was obtained. Children from the patients with AD (n = 203) and the married couples without AD (n = 197) were invited to participate in the study. All measurements were confined to the offspring of patients with AD and offspring of couples with good cognitive function, hereafter described as "offspring with or without a parental history of AD."

Standard protocol approvals, registrations, and patient consents. We received approval to perform this study from the Medical Ethical Committee for Mental Health Care of The Netherlands. Consent for participation in the study was given by all married couples or the legal guardian of eligible patients with AD.

APOE genotyping. For genotyping, 2 TaqMan assays (Applied Biosystems, Foster City, CA) were used, which has been described in detail elsewhere.[11]

Plasma measurements. Nonfasting blood samples were collected early in the morning. Plasma levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides were ana-

Table 2 Characteristics of offspring with and offspring without a parental history of AD

	Offspring with a parental history of AD		
	Present (n = 203)	Absent (n = 197)	*p* Value
Demographics			
Female, n (%)	100 (49)	103 (52)	0.545
Mean age, y	49.8 (7.6)	51.6 (6.7)	0.079
Cardiovascular status, n (%)			
History of CVD	11 (5)	12 (6)	0.790
Ankle-arm index <1.0	2 (1)	1 (1)	0.580
Plasma measurements			
apoE, mg/dL	2.76 (1.03)	3.33 (1.58)	0.001
Total cholesterol, mmol/L	5.55 (1.03)	5.56 (0.93)	0.925
LDL cholesterol, mmol/L	3.32 (0.92)	3.38 (0.86)	0.576
HDL cholesterol, mmol/L	1.47 (0.41)	1.43 (0.39)	0.441
Triglycerides, mmol/L	1.30 (0.92–2.10)	1.44 (1.03–1.97)	0.817
APOE genotype, n (%)			
ε2ε2	1 (1)	2 (1)	<0.001
ε2ε3	5 (3)	28 (14)	
ε2ε4	12 (6)	2 (1)	
ε3ε3	103 (51)	126 (64)	
ε3ε4	67 (33)	38 (19)	
ε4ε4	15 (7)	1 (1)	

The *p* values represent the significance of the difference in frequencies, calculated using χ^2 testing, or the difference in mean values, calculated using robust linear regression analysis between offspring with and offspring without a parental history of Alzheimer disease (AD). Normally distributed continuous data are presented as means with standard deviation, whereas skewed data are presented as medians with interquartile ranges.
CVD = cardiovascular disease; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

lyzed at baseline on fully automated computerized analyzers (Modular P 800 analyzer, Roche, Almere, The Netherlands). The level of low-density lipoprotein cholesterol was estimated by the Friedewald equation.[12]

Plasma apoE levels were determined using a human apoE-specific sandwich ELISA as described in detail elsewhere.[11]

Cardiovascular status. A history of cardiovascular disease was obtained through a questionnaire, filled out by participating subjects. The ankle–arm index was determined by calculating the ratio between ankle and arm systolic blood pressure. An ankle–arm index <1.0 was considered to represent the presence of peripheral artery disease.

Statistical analysis. First, the difference in distribution of *APOE* genotypes between subjects with and subjects without a parental history of AD was assessed with the χ^2 test.

Second, the difference in mean plasma apoE level between offspring with and offspring without a parental history of AD was analyzed with linear regression analysis, using robust standard errors to correct for familial aggregation. The robust character of the linear regression model adequately handles the multiple observations (subjects) per family. All models were additionally adjusted for sex, age, and plasma lipids. Analyses were stratified for *APOE* ε2/ε3ε3/ε4 carriership, with ε2ε2 and ε2ε4 carriers excluded from the analyses.

Third, the difference in plasma apoE level between offspring with and offspring without a parental history of AD was calculated for all subjects combined, using robust linear regression analysis with *Z* scores of plasma apoE level as determinant. *Z* scores of plasma apoE level were calculated per *APOE* genotype group separately.

Finally, broad heritability of plasma apoE levels was estimated with the following formula: heritability = 2 * (between-families variance)/(between-families variance + within-families variance), under the assumption of shared environment by siblings.

Calculations were performed using SPSS software (version 14.0.1, SPSS Inc., Chicago, IL) and STATA statistical software (version SE 9.0, StataCorp LP, College Station, TX).

RESULTS Plasma apoE measurement failed in 2 subjects. Table 2 shows that among the remaining 398 subjects, without correcting for *APOE* genotype, a parental history of AD was associated with a 0.57-mg/dL lower plasma apoE level ($p = 0.001$). This finding stands out because most other factors, most notably plasma lipid levels, were similar regardless of parental history of AD. The *APOE* allele frequencies for all offspring were ε2, 0.07; ε3, 0.75; and ε4, 0.19, and the genotypes were in Hardy–Weinberg equilibrium. We noted that, expectedly, offspring with a parental history of AD were more likely to carry the *APOE* ε4 allele compared with subjects without such a history (46% vs 21% ε4 allele carriership, $p < 0.001$), giving rise to the possibility of confounding by the *APOE* genotype.

Thus, we accounted for this possibility by comparing plasma apoE levels according to *APOE* genotype. There were large differences in plasma apoE levels according to *APOE* genotype with decreasing levels from ε2 to ε3ε3 to ε4 carriers (table 3; $p < 0.001$). However, for each *APOE* genotype there was approximately a 0.20-mg/dL lower plasma apoE level among offspring with compared with offspring without a parental history of AD. After adjustment for *APOE* genotype, sex, age, and lipid levels in a linear model, overall, offspring with a parental history of AD had a 0.21-mg/dL lower plasma apoE level ($p = 0.005$).

Taking an alternative approach, we standardized plasma apoE levels for each *APOE* genotype, creating *Z* scores as an outcome variable for use in linear models. Taking this approach, a parental history of AD was associated with a 0.22-point lower *Z* score (figure; $p = 0.009$). Adjustment for factors related to cardiovascular disease did not materially alter this estimate (difference in *Z* score: 0.20 points, $p = 0.02$). Broad heritability of plasma apoE levels was calculated as 0.24 when using *Z* scores, whereas it was 0.61 when using nonstandardized plasma apoE levels.

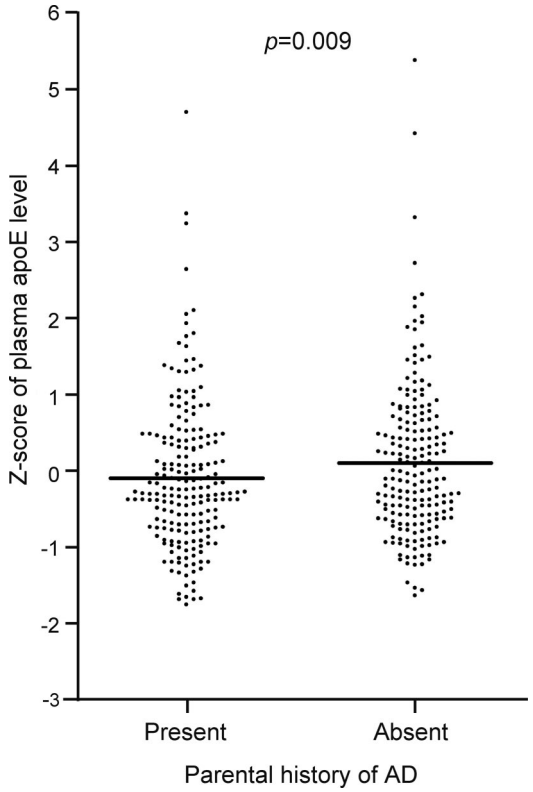
DISCUSSION The main finding of this study is that middle-aged offspring with a parental history of AD

Table 3 Plasma apoE levels dependent on parental history of AD, stratified for *APOE* genotype

APOE genotype	Parental history of AD	n	Plasma apoE level (95% CI), mg/dL	*p* Value
ε2 carriers	Present	5	4.43 (3.28–5.57)	0.624
	Absent	28	4.66 (4.18–5.15)	
ε3ε3	Present	103	2.90 (2.74–3.06)	0.035
	Absent	125	3.14 (2.99–3.28)	
ε4 carriers	Present	82	2.23 (2.07–2.39)	0.370
	Absent	38	2.35 (2.12–2.58)	
All carriers*	Present	190	2.82 (2.70–2.95)	0.018
	Absent	191	3.03 (2.91–3.16)	

ε2 carriers represent offspring with the *APOE* ε2ε3 genotype; ε4 carriers represent offspring with the *APOE* ε3ε4 and ε4ε4 genotype. Plasma apoE levels are presented as means, with their 95% confidence intervals (CIs). The *p* values represent the significance of the difference in mean plasma apoE level between offspring with and offspring without a parental history of Alzheimer disease (AD), calculated using robust linear regression.
*Analysis adjusted for *APOE* ε2/ε3ε3/ε4 carriership.

Figure Association between plasma apolipoprotein E levels and parental history of AD



Dots represent *Z* scores of plasma apoE levels, calculated for each *APOE* genotype separately. The *p* value represents the significance of the difference in mean *Z* score between offspring with and offspring without a parental history of Alzheimer disease (AD), calculated with robust linear regression model, adjusted for sex, age, and levels of plasma total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides.

have lower plasma apoE levels when compared with offspring without such a parental history of AD. This association was also observed after adjustment for *APOE* genotype and when using plasma apoE levels standardized for *APOE* genotype. Our findings suggest that low plasma apoE levels in midlife are associated with an increased risk of late-life AD, independent of *APOE* genotype.

The present study was designed to identify heritable risk factors in midlife that contribute to the development of late-onset AD. From twin studies, heritability of AD has been estimated to be as high as 0.77,[13] in line with the observation that first-degree relatives of patients with AD have an increased risk of developing AD.[14] Therefore, in the present study, we have compared offspring without a parental history of AD with offspring with a parental history of AD who are enriched for risk factors of AD but are not likely to have the disease yet. The increased risk is reflected in an overrepresentation of the *APOE* ε4 allele among offspring with a parental history of AD. Because circulating levels of apoE are under tight genetic control,[15] variation in plasma apoE levels is highly heritable. The lower plasma apoE levels in middle-aged offspring with a parental history of AD might therefore be a risk factor for AD in late life.

Possible pathophysiologic pathways, explaining the association between lower plasma apoE levels and the risk of AD, can be found in the numerous actions of apoE. First, apoE plays an important role in lipid transportation, clearance, and metabolism.[2] Higher levels of plasma apoE may protect against atherosclerosis, because a deficiency in apoE has been shown to result in the accumulation of very-low-density lipoprotein and remnant particles and subsequent atherosclerosis in mice. Second, apoE has been shown to have antioxidant effects, mainly by functioning as a scavenger of lipid peroxidation products.[3] Third, although apoE produced by macrophages has been shown lately to be involved in lipid antigen presentation,[16] numerous studies showed immunosuppressive properties of apoE, which may result in decreased inflammation.[4] A decrease in atherosclerosis burden, the prevention of oxidative stress, and decreased inflammation may all protect against AD. These protective effects are generally weakest for apoE4 and strongest for apoE2. Therefore, ε4 allele carriers may be at an increased risk of AD, because besides the less functional protective properties of the structural variant apoE4, ε4 allele carriers also have the lowest plasma apoE levels.

The presented results are in accordance with the ApoEurope Study, in which lower plasma apoE levels were found in patients with AD.[9] On the contrary, in a prospective population-based study, we found previously that lower plasma apoE levels were

associated with better cognitive function,[10] whereas others found no association between plasma apoE levels and cognitive function.[7,8] A possible explanation for the opposing results could be the difference in study populations. In the current study, we studied middle-aged offspring with and without a parental history of AD. All other studies were performed in subjects who were selected on the presence of dementia, were age-matched controls, or were selected on old age. Possibly, the effect of plasma apoE levels on the risk of dementia is prone to change with increasing age, as has been suggested for other risk factors for dementia, such as blood pressure and cholesterol levels.[17,18] In old age, high plasma apoE levels may be the result of decreased clearance of lipids,[11] or could be the result of an adaptive response to systemic damage, such as cardiovascular disease and dementia, which could up-regulate the expression of apoE. A recently published study showed that an inflammatory response in mice led to a decreased clearance of circulating apoE, which resulted in increased plasma apoE levels, despite the down-regulation of apoE gene expression.[19] Therefore, we hypothesize that high plasma apoE levels in late life may reflect ill health, whereas high plasma apoE levels in midlife may reflect higher innate apoE expression, which subsequently may be protective for AD. Indeed, in another family study, which we performed to study determinants of longevity, plasma apoE levels were higher in the old-aged parental generation compared with the middle-aged offspring generation ($p < 0.001$, unpublished results). Unfortunately, plasma apoE levels were not determined in the parental generation in the current study.

As hypothesized, lower plasma apoE levels may be the result of decreased innate production, which could be explained by variation in gene regulatory regions. Previous studies have indeed shown that polymorphisms in the *APOE* gene regulatory region and the hepatic control region are associated with plasma apoE levels.[20] Three polymorphisms in the promoter region of the *APOE* gene have been studied extensively. The -219 G/T, -427 C/T, and -491 A/T polymorphisms have been shown to be associated with plasma apoE levels.[21-23] These polymorphisms have also been shown to be associated with the risk of AD, although results are not consistent throughout all studies.[22,24,25] Because these polymorphisms are in strong linkage with the common *APOE* polymorphisms, this may explain some part of the inconsistencies between studies. The observed lower plasma apoE levels in $\varepsilon 4$ carriers and the higher plasma apoE levels in $\varepsilon 2$ carriers, which is in accordance with the results from other studies,[5-8,10] are not likely explained by a difference in innate production. Evidence points in the direction of different clearance of the various isoforms as the explanation for the genotype dependent plasma apoE levels. ApoE4 has the highest affinity for the low-density lipoprotein receptor and is cleared fastest, whereas apoE2 has the lowest affinity for the low-density lipoprotein receptor and is cleared at a much lower rate, explaining the variation in plasma apoE levels.[26,27]

Besides the influence of genetic variation on plasma apoE levels, phenotypic variation, such as dietary intake, has also been shown to influence plasma apoE levels. Several studies have reported increasing plasma apoE levels after the administration of diets containing saturated fatty acids, or fish and vegetable oil,[28,29] although others did not report such an association.[30,31] In the current study, the collection of blood samples was performed in nonfasted subjects. Although this could have introduced some increased random variation in the plasma apoE level measurements, this is unlikely to be a confounder in our study, because feeding state is not likely to be different between offspring with and offspring without a parental history of AD. Other studies reporting on plasma apoE levels and dementia or cognitive function could have been confounded by measuring in nonfasted blood samples, because eating patterns of patients with dementia have been shown to be different from healthy subjects, e.g., due to decreased food-seeking behavior. However, the studies reporting on nonfasted plasma apoE levels showed higher plasma apoE levels to be associated with dementia or worse cognitive function, making confounding as an explanation for the findings unlikely.[5,10]

An important strength of this study is that the family study design allowed for the identification and quantification of the effect of plasma apoE levels measured in middle age on an increased risk of AD later in life, without the possible distorting effect of disease on plasma levels of apoE. A limitation of this study is that, although subjects with a parental history of AD are likely to have an increased risk of having AD in late life, it is uncertain which subjects will eventually experience dementia. Therefore, associations could only be made based on a likely increased risk for the group of subjects with a parental history of AD and not on an individual level. Another limitation is that plasma apoE levels represent systemically produced apoE and do not reflect intracerebral apoE, which is locally produced. Brain autopsy studies have shown apoE levels to be lower in hippocampal and frontal cortical regions.[32,33] Moreover, some studies have shown CSF apoE levels to be lower in patients with AD compared with controls,[34,35] although other studies did not find this association[36,37] or even showed an association in the

opposite direction.[38,39] However, all proposed pathophysiologic pathways, explaining the observed association in this study, may play an important part in the development of AD and do not involve intracerebral apoE.

AUTHOR CONTRIBUTIONS

Statistical analyses were performed by P. van Vliet.

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DISCLOSURE

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