

Vascular Factors and Markers of Inflammation in Offspring With a Parental History of Late-Onset Alzheimer Disease

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Context: Alzheimer disease (AD) is a complex disorder with a strong heritable component. Amyloid pathology, vascular factors, and inflammation are postulated to be involved in its pathogenesis, but causality has not been established unequivocally.

Objective: To identify heritable traits in middle age that contribute to AD.

Design: We used a proven family design, comparing middle-aged offspring with and without a parental history of AD. In such a design, the offspring under study are enriched for risk factors of AD but do not yet have the disease.

Setting: The Netherlands.

Participants: Two hundred six offspring of 92 families with a parental history of late-onset AD and 200 offspring of 97 families without a parental history of AD.

Main Outcome Measures: The *APOE* $\epsilon 4$ genotype, vascular factors, production capacity of pro- and anti-inflammatory cytokines upon stimulation with lipopolysaccharide, and circulating markers of inflammation. All

outcome measures were assessed in the offspring only and not in the parental generation.

Results: More offspring with a parental history of AD carried *APOE* $\epsilon 4$ than those without a parental history of the disease (47% vs 21%, $P < .001$). Those with a parental history of AD also had higher systolic blood pressures ($P = .006$), higher diastolic blood pressures ($P < .001$), and lower ankle brachial indices ($P = .005$) when compared with offspring without a family history of dementia. Production capacity of pro-inflammatory cytokines in offspring with a parental history of AD was also different, with higher levels of IL-1 β (interleukin 1 β) ($P < .001$), IL-1 β to IL-1ra ratio ($P < .001$), tumor necrosis factor α ($P = .008$), IL-6 ($P = .04$), and interferon γ ($P = .01$). All of these positive associations were independent of *APOE* $\epsilon 4$ genotype.

Conclusions: Hypertension and the expression of an innate pro-inflammatory cytokine profile in middle age are early risk factors of AD in old age. For the offspring of affected families, it provides clues for screening and preventive strategies, of which blood pressure control can be implemented directly.

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LATE-ONSET ALZHEIMER DISEASE (AD) is a complex disorder.¹ Accumulating evidence suggests that vascular factors and markers of inflammation in middle age are associated with risk of late-onset AD,²⁻⁶ suggesting that the neurodegenerative process leading to AD begins as early as in midlife. However, the association between diabetes, plasma cholesterol, and AD is inconsistent.⁷⁻⁹ Other risk factors such as midlife hypertension have shown a more consistent association with risk of AD.^{2,6} In addition to vascular factors, pathological studies suggest that inflammation is a driving force in neurodegeneration, as markers of inflammation are found abundantly in and around amyloid plaques,¹⁰⁻¹⁵ where there is upregulation of chemo-

kines and pro-inflammatory cytokines by microglia cells and deposits of C-reactive protein. Only 1 study has investigated whether markers of inflammation at middle age confer risk of AD. In the Honolulu Asia Aging Study, subjects with high C-reactive protein levels at baseline were found to be at an increased risk of cognitive deterioration in old age.⁵

Heritable factors make an important contribution to late-onset AD. Estimates from twin studies suggest that 60% of the variation in cognitive decline is under genetic control.¹⁶ However, it remains unclear which genes contribute to AD owing to its heterogeneity. With a few exceptions (like with the *APOE* $\epsilon 4$ genotype), the current strategy of using genome association studies has not been able to identify candidate loci effectively.^{11,12,17}

A likely explanation is the complexity of the biomolecular pathways that contribute to late-onset AD. Additionally, vascular pathologies and inflammation are highly polygenic, making the identification of specific factors in AD a difficult task.^{12,17}

Given that late-onset AD is a complex but heritable polygenic disorder and that vascular and inflammatory factors in midlife are associated with an increased risk of AD later in life, we designed a study to identify and quantify early risk factors that contribute to AD. We used a proven family design comparing middle-aged offspring with and without a parental history of AD.^{18,19} In such a family design, the offspring under study are enriched for risk factors of AD but do not yet have the disease. Differences between offspring dependent on their ancestry therefore lend an argument for causality. We also characterized all offspring for *APOE* $\epsilon 4$ genotype, to determine if the effect of vascular and inflammatory factors on AD is dependent or independent of *APOE* $\epsilon 4$ genotype.

METHODS

STUDY DESIGN

We recruited offspring from patients with late-onset AD and offspring without such a parental history between 2006 and 2007. Ninety-two consecutive patients aged 70 years and older with AD (mean age, 82 years) were recruited from the Memory Clinic of the VU University Medical Center and affiliated nursing homes. The Medical Ethical Committee for Mental Health Care of the Netherlands approved the study. All selected patients were diagnosed as having probable AD according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria. Patients with other types of dementia were not eligible. The legal guardians of these patients gave written informed consent. All children of these patients were invited to participate in the study.

The control population consisted of the offspring of 97 married couples aged 70 years and older (mean age, 83 years) who were both free from dementia, ie, had a Mini-Mental State Examination score higher than 27 points.²⁰ All of these participants also gave written informed consent. At least 1 spouse in the control pair had participated in either the Longitudinal Aging Study Amsterdam²¹ or the Leiden 85-Plus Study,²² both prospective population-based studies on cognitive function. Additional information on the cognitive function of the spouse was obtained through the Mini-Mental State Examination. When one of the spouses was deceased (n=55), this information was obtained by taking a history of the deceased spouse from the surviving spouse, using the Informant Questionnaire on Cognitive Decline in the Elderly.²³ When the couple or surviving individual consented, all adult children were invited to participate. Vascular factors and inflammatory markers were assessed in the offspring only and not in the parental generation.

VASCULAR FACTORS AND *APOE* GENOTYPES

Manual blood pressure measurements were obtained with a Doppler stethoscope over both brachial arteries and both posterior tibial arteries. The subjects were first seated for 10 minutes. The same trained research nurse did this in all cases to ensure consistency. For the current analysis, the lowest measured blood pressure readings were used. In addition, we determined the ankle brachial index (the ratio of ankle to arm

systolic blood pressure), pulse pressure (systolic pressure – the diastolic pressure), and mean arterial blood pressure ($[(\text{diastolic} + \text{pulse pressure})/3]$). From the nonfasting venous sample we measured concentrations of hemoglobin A_{1c}, glucose, total cholesterol, high-density lipoprotein cholesterol, total cholesterol to high-density lipoprotein ratio, triglycerides, and homocysteine.²⁴ Low-density lipoprotein cholesterol was calculated using the Friedewald equation. Finally, we determined *APOE* genotypes.²⁵

INFLAMMATION

Innate pro-inflammatory responsiveness was determined by the incubation of lipopolysaccharide-stimulated whole blood samples *ex vivo*, resulting in typical cytokine profiles that are under strong heritable control.^{18,26,27} The methods by which whole blood samples were obtained and stimulated with lipopolysaccharide during 24 hours have been described elsewhere.²⁶ In short, heparinized whole blood was diluted 2-fold with RPMI 1640. Lipopolysaccharide (endotoxin, 10 ng/mL) was used as a primary stimulus. After the addition of lipopolysaccharide, samples were incubated for 24 hours at 37°C and 5% carbon dioxide. After centrifugation, the supernatants were stored at –80°C to determine production capacity of various inflammatory cytokines using standard enzyme-linked immunosorbent assay techniques.

Moreover, we showed in a previous study that sex or age did not explain the differences in cytokine production.²⁶ We used lipopolysaccharide-stimulated whole blood samples rather than peripheral blood mononuclear, because it has been suggested that lipopolysaccharide-stimulated whole blood samples mimic the natural environment more closely than peripheral blood mononuclear.²⁸ The capacity of cytokine production in relation to the number of mononuclear cells is higher in stimulated whole blood samples than in conventional cultures of concentrated peripheral blood mononuclear.²⁸

In addition to the whole blood stimulation assay, circulating levels of C-reactive protein and IL-6 (interleukin 6) were also determined.²¹ All blood samples were collected before 10 AM, and travel time between the sample collection site and the Department of Clinical Chemistry of the Leiden University Medical Center was similar for offspring with and without a parental history of AD.

POSSIBLE CONFOUNDERS

Sociodemographic characteristics, medical history, information on the use of medication, physical activity,²⁹ dietary fat intake during the past week (assessed with a validated modified version of the Food Frequency Questionnaire³⁰), and caregiver stress³¹ were obtained in all offspring. Subjects who used anti-inflammatory medication, such as aspirin and nonsteroidal anti-inflammatory medications, were equally distributed between offspring with (n=10) and without (n=10) a parental history of AD. Physical activity, including strenuous labor, mild labor, and walking, was measured with the Longitudinal Aging Study Amsterdam Physical Activity Questionnaire, a validated questionnaire about typical weekly activity.²⁹ Caregiver stress was measured using a validated questionnaire³¹ based on the Zarit Burden Scale.³² Caregiver stress was considered a possible confounder, as conflicting results on the relation of caregiver stress, blood pressure, and inflammatory response exist.^{33,34}

STUDY SIZE

Calculations to determine study size were based on the number of participating families. We used a 90% probability of de-

tecting differences in inflammatory responsiveness and vascular factors at a 5% significance level.³⁵ The data on inflammatory responsiveness on cognitive function and AD came from 2 earlier studies.^{36,37} The calculations showed that it was sufficient to have 85 married couples, of whom 1 had AD. The number was the same for married couples without dementia. Furthermore, we assumed that the average number of offspring was 2.0 per couple. We therefore needed 170 offspring with a parental history of AD and 170 offspring without a parental history of AD.

Finally, our earlier experience with cytokine production measurements in families showed that 50 case families and 50 control families were sufficient to detect differences in inflammatory responsiveness between subjects with brain disorders, such as multiple sclerosis¹⁹ and fatal meningococcal disease.¹⁸

STATISTICAL ANALYSIS

The parental generation, ie, the patients with AD and elderly couples without a history of AD, was not included in the analyses. Differences in vascular factors and inflammatory markers between offspring with and without a parental history of AD were analyzed using robust linear regression. This was done because the robust character of the linear regression model adequately manages multiple observations per family, ie, the model adjusts for familial aggregation. All models were therefore adjusted for age, sex, and familial aggregation.

In additional analysis we also determined the effect of vascular and inflammatory factors on AD in relation to *APOE* ε4 genotype. To do this we adjusted for *APOE* ε4 genotype in the regression models and tested for possible interaction between it and vascular or inflammatory factors. In the second additional analysis we adjusted for caregiver stress. The analysis presented herein was preplanned to determine if an innate pro-inflammatory response and early vascular risk factors contribute to AD.

In these analyses we have not made Bonferroni corrections, as there is only a small chance (1 of 20) that we found some spurious findings due to multiple comparisons.³⁸ Moreover, if one tests for the significance of an association using variables that are mutually correlated, the Bonferroni correction is too conservative.³⁸ Finally, data were also analyzed after averaging outcomes of all the siblings for each family separately, ie, the sibship being the unit of observation. Calculations were performed using SPSS software, version 12.0.1 (SPSS Inc, Chicago, Illinois), and Stata statistical software, version 9.0 (Stata Corp, College Station, Texas).

RESULTS

SUBJECT CHARACTERISTICS

Demographic and clinical characteristics of the middle-aged offspring with and without a parental history of AD are listed in **Table 1**. Offspring with a positive family history were on average 2.7 years younger and had significantly less education compared with offspring without a parental history of AD. We found no differences in lifestyle characteristics, ie, smoking, dietary fat intake, or physical activity, between the 2 groups. However, we did find that the offspring with a parental history of AD had significantly higher scores on the caregiver stress inventory than subjects without a parental history of AD. We also found that prevalence of the *APOE* ε4 genotype was higher in offspring with a parental history of AD (46.5% vs 21%, $P < .001$).

Table 1. Demographic and Clinical Characteristics of Offspring With and Without a Parental History of Late-Onset AD

Characteristic	Mean (SD) by Parental History of AD		P Value
	Present (n = 206)	Absent (n = 200)	
Demographics			
Female sex, No. (%)	102 (50)	105 (53)	.5
Age, y	48.9 (0.75)	51.6 (0.48)	.03
Education, y	11.9 (0.24)	12.9 (0.25)	.004
Lifestyle factors			
Current smoker, No. (%)	58 (27)	53 (27)	.7
Fat intake score ^a	43.2 (0.49)	42.8 (0.49)	.5
Physical activity score ^b	15.0 (1.17)	14.9 (1.19)	>.99
Caregiver burden stress score, mean (SD)	4.7 (0.37)	2.7 (0.33)	<.001
<i>APOE</i> ε4 carrier, %			
ε2 ε2	46.5	21.0	<.001
ε2 ε3	0.5	1.0	
ε2 ε4	2.5	14.0	
ε3 ε3	6.0	1.0	
ε3 ε4	50.5	64.0	
ε4 ε4	33.0	19.5	
	7.5	0.5	

Abbreviation: AD, Alzheimer disease.

^aEstimates total fat intake in a week.

^bTotal score of strenuous labor, mild labor, and walking during the past week.

Finally, we found that *APOE* ε4 genotype was associated with a lower mean level of education in offspring with and without a parental history of AD: 11.8 years in *APOE* ε4 carriers vs 12.7 years in non-*APOE* ε4 carriers ($P = .03$, after adjustment for family size). When we restricted the analysis to offspring with a parental history of AD, results were similar: 11.5 years in *APOE* ε4 carriers vs 12.2 years in non-*APOE* ε4 carriers ($P = .1$).

VASCULAR FACTORS

The middle-aged offspring with a parental history of AD had higher arterial blood pressure, both systolic and diastolic (**Table 2**). About 40% of those with a parental history of AD were classified as having hypertension (either a systolic blood pressure >139 mm Hg or a diastolic blood pressure >89 mm Hg) compared with 29% of the offspring without a parental history of AD ($P = .02$). The ankle brachial index was lower in offspring with a parental history of AD, reflecting a higher atherosclerotic burden ($P = .005$) (Table 2). Other vascular factors, including levels of lipids, glucose, and homocysteine, were not significantly different between offspring with and without a parental history of AD (Table 2).

MARKERS OF INFLAMMATION

The production capacity of the pro-inflammatory cytokines IL-1β (interleukin 1β), IL-6, tumor necrosis factor α, and interferon γ was higher in offspring with a parental history of AD upon stimulation of whole blood samples with lipopolysaccharide (all $P < .05$) (**Table 3**). Similar results were found for the IL-1β to IL-1ra ratio

Table 2. Vascular Factors in Offspring With and Without a Parental History of Late-Onset AD

Characteristic	Mean (SE) ^a by Parental History of AD		P Value
	Present (n = 206)	Absent (n = 200)	
Blood pressure, mm Hg			
Mean arterial pressure	102 (0.91)	97 (0.91)	<.001
Systolic	131 (1.29)	126 (1.29)	.006
Diastolic	87 (0.82)	83 (0.79)	<.001
Pulse pressure	44 (0.71)	43 (0.78)	.3
Ankle arm index	1.27 (0.01)	1.31 (0.01)	.005
Circulating markers			
Total cholesterol, mg/dL	214.3 (3.5)	214.3 (2.7)	.7
HDL cholesterol, mg/dL	56.8 (1.2)	54.8 (1.2)	.1
Cholesterol to HDL ratio	4.10 (0.12)	4.21 (0.12)	.3
LDL cholesterol, mg/dL	128.6 (3.1)	130.5 (2.7)	.9
Triglycerides, mg/dL	147.8 (8.0)	145.1 (6.2)	>.99
Glucose, mg/dL	100.5 (2.5)	103.1 (2.0)	.4
HbA _{1c} %	4.87 (0.08)	4.91 (0.07)	.8
Homocysteine, mg/L	1.65 (0.04)	1.65 (0.04)	.8

Abbreviations: AD, Alzheimer disease; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SI conversion factors: To convert glucose to millimoles per liter, multiply by 0.0555; HDL, LDL, and total cholesterol to millimoles per liter, multiply by 0.0259; homocysteine to micromoles per liter, multiply by 7.397; and triglycerides to millimoles per liter, multiply by 0.0113.

^aObtained from linear regression analysis adjusting for age, sex, and family relations.

($P < .001$), which we calculated because it is known that IL-1ra is the natural antagonist of the pro-inflammatory acute-phase cytokine IL-1 β . Therefore, higher ratios reflect a pro-inflammatory host response. There were no significant differences in circulating markers of inflammation in contrast to the whole blood stimulation assays (Table 3).

ADDITIONAL ANALYSIS

First, we tested whether the associations between the vascular factors, the production capacity of cytokines, and a parental history of AD could be distorted by differences in caregiver stress and/or years of education. When we adjusted for caregiver stress and years of education using robust linear regression, the estimates and significance levels as presented in Table 2 and Table 3 were unaffected (data not shown). The estimates and significance levels as presented were also unaffected when we further explored the effect of lifestyle factors, such as current smoking, fat intake, physical activity, and caregiver stress, on blood pressure and the ankle brachial index (data not shown).

Second, we explored whether the associations could be explained by the different frequencies of the APOE $\epsilon 4$ allele between the 2 groups of offspring. The associations remained unaltered after adjustment for APOE $\epsilon 4$ allele frequencies (data not shown). We also tested for interaction, ie, if the associations between vascular factors and markers of inflammation and a parental history of AD differed between APOE $\epsilon 4$ carriers and noncarriers. No such interaction was found (all $P \geq .10$).

Table 3. Inflammation in Offspring With and Without a Parental History of Late-Onset AD

Characteristic	Mean (SE) ^a by Parental History of AD		P Value
	Present (n = 206)	Absent (n = 200)	
Production capacity of inflammatory cytokines ^b			
IL-1 β , pg/mL	13 091 (380)	10 548 (580)	<.001
IL-1ra, pg/mL	10 695 (426)	11 030 (448)	.5
IL-1 β :IL-1ra	1.38 (0.06)	1.10 (0.05)	<.001
Tumor necrosis factor α , pg/mL	8551 (466)	7147 (357)	.008
IL-6, pg/mL	96 031 (2809)	88 226 (2827)	.04
IL-8, pg/mL	26 888 (1972)	23 523 (2557)	.2
IL-10, pg/mL	5526 (165)	5022 (189)	.06
Interferon γ , pg/mL	6369 (616)	4402 (463)	.01
Circulating markers			
C-reactive protein, mg/L ^c	1.36 (0.1)	1.33 (0.1)	.7
IL-6, pg/mL	1.20 (0.1)	1.26 (0.1)	.7

Abbreviations: AD, Alzheimer disease; IL, interleukin.

^aObtained from linear regression analysis adjusting for age, sex, and family relations.

^bThe mean production capacity of cytokines was determined after stimulation with 10-ng/mL lipopolysaccharide.

^cExpressed as logarithmically transformed values of high-sensitivity C-reactive protein.

Third, we excluded offspring who used nonsteroidal anti-inflammatory drugs or aspirin from the analyses. There were 10 of these subjects in each group. The results as shown in Table 2 and Table 3 remained similar (data not shown).

Finally, we analyzed the data with sibship as the unit of observation by averaging outcomes of all the siblings of each family separately. **Figure 1** shows cumulative distribution of blood pressure and ankle brachial index for the 2 groups of offspring. The distribution of these vascular factors was significantly more adverse in sibships with a parental history of AD (all $P < .05$). **Figure 2** shows the cumulative distribution of IL-1 β production capacity and IL-1 β to IL-1ra ratio according to status of the parental generation. The graphs show that distributions in sibships with a parental history of AD are shifted toward a pro-inflammatory profile (both $P < .01$).

COMMENT

In this family study comparing offspring with and without a parental history of late-onset AD, APOE $\epsilon 4$ genotype, arterial blood pressure, indices of vascular disease, and pro-inflammatory cytokine production were all significantly higher among offspring with a positive parental history. The higher percentage of APOE $\epsilon 4$ genotype in offspring with a parental history of AD underscores the heritability of the familial clustering of late-onset AD. This is reinforced by the finding that lifestyle factors were not different between the 2 groups. As the middle-aged offspring did not yet have AD, the higher blood pressure and lower ankle brachial index may also represent a cluster of heritable risk factors that confers risk of dementia. From a similar point of view, the higher produc-

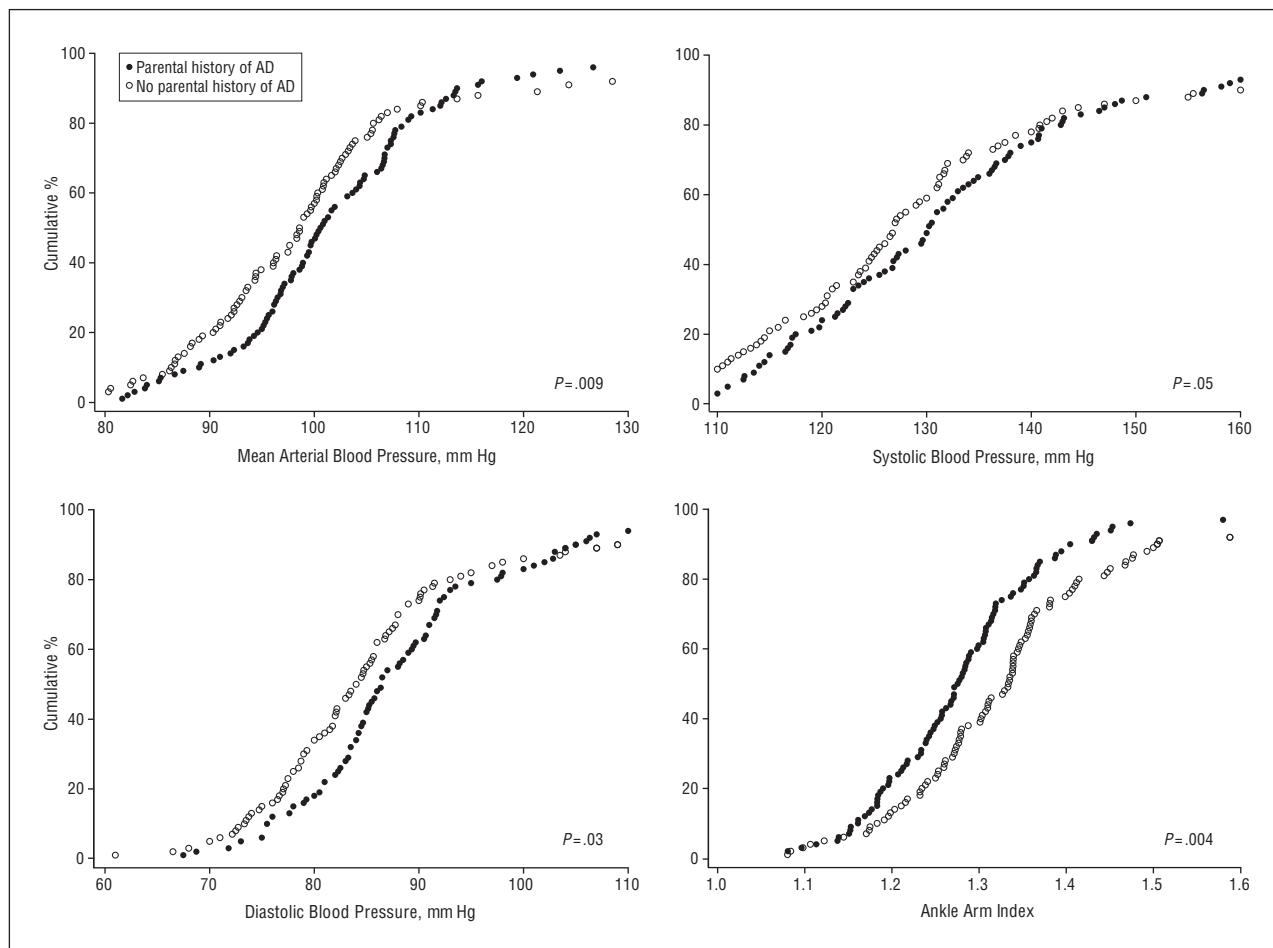


Figure 1. Cumulative percentage of blood pressure and ankle brachial index in the offspring of families with and without a parental history of Alzheimer disease (AD). Each dot represents the family mean, clustering an average of 2.1 offspring with or without a parental history of AD.

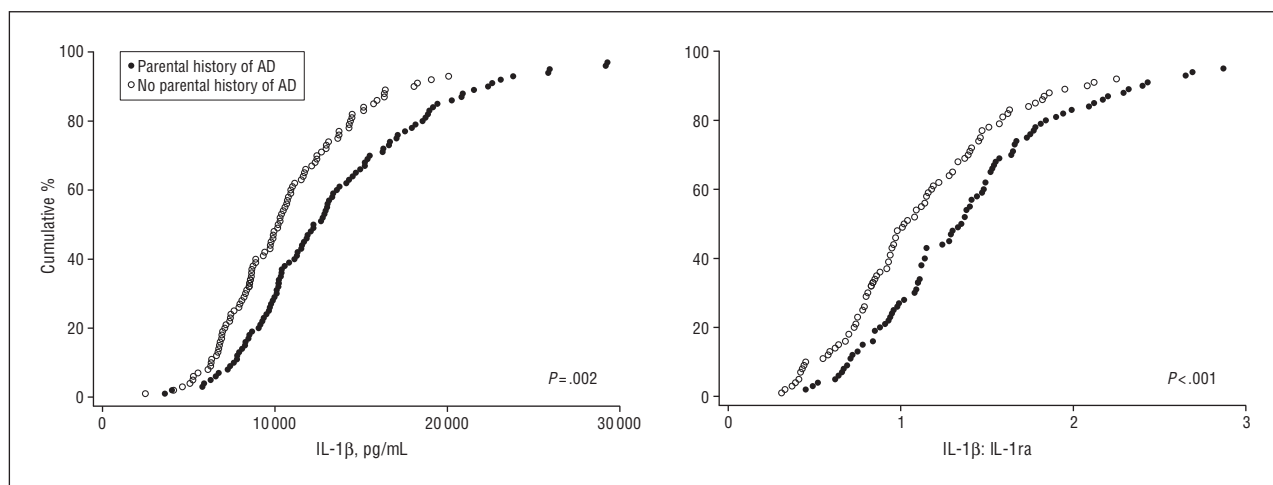


Figure 2. Cumulative percentage of interleukin (IL)-1 β and IL-1 β to IL-1ra ratio in the offspring of families with and without a parental history of Alzheimer disease (AD). Each dot represents the family mean of IL-1 β and IL-1 β to IL-1ra ratio upon stimulation with lipopolysaccharide in whole blood samples, clustering an average of 2.1 offspring with or without a parental history of AD.

tion capacity of the pro-inflammatory cytokines IL-1 β , IL-1 β to IL-1ra ratio, IL-6, tumor necrosis factor α , and interferon γ are likely to be part of this risk profile. No consistent associations were found between circulating inflammatory markers IL-6 and C-reactive protein, fur-

ther emphasizing that the subjects under study have as yet only a subclinical effect from the disease. All the reported associations between vascular factors, inflammatory markers, and cognitive outcome were independent of APOE $\epsilon 4$ genotype. This lends further weight to the

argument that late-onset AD is a complex disorder with various heritable components.

VASCULAR FACTORS AND AD

Blood pressure and atherosclerosis have been postulated to play a role in the pathogenesis of AD for many years, but the magnitude of the effect is disputed.^{1,2,6,39} In this study we found familial clustering of high blood pressure and vascular disease in middle-aged offspring with a parental history of AD in old age. Our study confirms the notion that hypertension in midlife is associated with the development of AD later in life,^{2,6} but the underlying genetics have yet to be explored. The Honolulu Asian Aging Study showed that atherosclerosis in middle age, as estimated with lower ankle brachial indices, was modestly associated with AD later in life.³⁹ The different ankle brachial indices that we have described herein cannot be explained by lifestyle-related factors, such as smoking, dietary fat intake, or physical activity, because these factors were similar in offspring with and without a parental history of AD. Moreover, our regression analyses made it less likely that these phenotypes were merely a reflection of higher stress in those who provide care for their demented parents.

Other known cardiovascular risk factors—high levels of lipids, lipoproteins, glucose, hemoglobin A_{1c}, and homocysteine—were not associated with a parental history of AD in our study; it is tempting to speculate why. Our findings are in line with several prospective studies that report that lipids, lipoproteins, glucose, hemoglobin A_{1c}, and homocysteine do not carry a risk of AD.⁷⁻⁹ In addition, 2 randomized controlled trials using statins in subjects at risk for AD showed no benefit against cognitive decline.^{40,41} Results from randomized controlled trials on the effect of lowering homocysteine concentrations with vitamin B₁₂ or folic acid showed no improvement on cognitive function in subjects with cognitive impairment.^{42,43}

On the other hand, some prospective studies have reported a positive association between lipids, lipoproteins, glucose, hemoglobin A_{1c}, homocysteine, and the risk of AD.^{2-4,6} Such associations may have been absent in our study because the blood samples were collected under nonfasting conditions.

INFLAMMATORY MARKERS AND AD

Cytokines are key players in the inflammatory process. However, their contribution to the development of AD is yet unknown. One of the reasons for this lack of knowledge is that serum levels of circulating cytokines are typically very low. Moreover, it is unlikely that the amount of circulating cytokines is causally linked to the development of AD. In this study, associations between circulating inflammatory markers C-reactive protein and IL-6 and parental history of AD were absent. A possible explanation for this finding is that the heritability of circulating inflammatory markers is modest, ranging between 17% and 20%,⁴² which suggests that increased levels of circulating markers^{21,44} in AD are symptomatic of frailty rather than being a causal factor. It has been known for

more than 15 years that the production of pro- and anti-inflammatory cytokines by white blood cells upon standardized stimulation differs considerably between individuals. We have previously demonstrated that this cytokine response is under strong genetic control.^{2,3} This innate responsiveness is determined by incubation of lipopolysaccharide-stimulated whole blood samples *ex vivo*, resulting in innate production capacity of inflammatory cytokines. Heritability estimates of the production capacity of the various cytokines range from 53% to 86% in nondiseased populations.^{18,27}

There is a strong message when offspring of patients with late-onset AD and patients with late-onset AD^{37,45} have a skewed cytokine profile; it is a potentially malleable risk factor. Nonsteroidal anti-inflammatory drugs have long been thought to be beneficial. However, clinical trials have indicated that this is not necessarily the case.⁴⁶ Despite these disappointing outcomes, the search for means to modulate the pro-inflammatory host response of middle-aged subjects at risk of dementia is further strengthened by the findings of this study.

The identified clustering of vascular factors and inflammatory markers in affected families are independent of APOE $\epsilon 4$. At first glance this is an unexpected finding, since APOE is recognized for its importance in lipoprotein metabolism, cardiovascular disease, and AD. The mechanism whereby APOE $\epsilon 4$ influences the development of AD is not known. APOE $\epsilon 4$ affects plasma lipid levels and is involved in cholesterol uptake and transport in the brain.⁴⁷ However, in a prospective cohort study, APOE $\epsilon 4$ was a risk factor for AD, independent of lipids and lipoproteins, suggesting that these vascular factors are not the primary mechanism by which APOE $\epsilon 4$ influences the development of AD.⁴⁸ Another mechanism by which APOE $\epsilon 4$ could influence AD is inflammation.¹¹ It has been suggested that the association between inflammatory markers and cognitive decline is stronger when an APOE $\epsilon 4$ allele is present.⁴³ However, another prospective study did not find a possible interaction between inflammatory markers, APOE $\epsilon 4$, and cognitive decline.²¹ Clearly, the possible interaction between inflammation, APOE $\epsilon 4$, and AD needs further study.

STRENGTHS AND WEAKNESSES

The current study has several strengths. First, it is a family study, which makes it possible to identify and quantify the association between the innate inflammatory markers and the risk of AD, using *ex vivo* differences in the production capacity of cytokines that resemble hereditary interindividual differences in the host response. A family study design could allow for causal inference⁴⁹ without the need for a long follow-up, and it circumvents studying inflammatory polymorphisms involved in AD, which have given disappointing results.¹³ Moreover, this study design has successfully been used in other studies in which we were able to relate the inflammatory responsiveness to the susceptibility of brain diseases such as meningococcal disease² and multiple sclerosis.¹⁶

Second, the diagnosis of AD in the parental generation was made according to National Institute of Neu-

rological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria. Patients with other types of dementia, including mixed-type dementia, were not eligible.

Third, it is well known that outcome-based sampling in family studies is more informative and increases power.⁴⁹ However, all family-based studies can be biased.⁴⁹ In our study, there were no differences in physical activity, smoking, or dietary intake in the offspring with and without a parental history of AD, suggesting that the environmental conditions from which the families originated were similar. Adjusting for these possible confounders and differences in age, years of education, or caregiver stress did not alter the data, which suggests that distribution of possible confounders did not influence our results. This is underscored by the remarkable coherence and consistency in our findings with our hypothesis that innate inflammation and innate or early vascular factors contribute to AD. However, our findings can be further strengthened when we prospectively follow up our study sample to characterize the nature of cognitive change to further identify underlying preclinical neurobiological changes.

This study has limitations. One drawback is that the blood samples were collected under nonfasting conditions, which could have explained the absent association between lipids, lipoproteins, glucose, and AD. Second, the whole blood assay may not reflect the secretion of cytokines by astroglia in the brain. Nonetheless, we know from earlier studies that this *ex vivo* assay is a valid instrument to phenotype interindividual capacities of immune responsiveness. It has been used successfully to predict the effect of systemic inflammation on brain disorders, such as multiple sclerosis,¹⁹ fatal meningococcal disease,¹⁸ and stroke.²² It has also been suggested that the cytokine response in whole blood induces the same effects across the blood-brain barrier.⁵⁰ Moreover, a recent study showed that intravenous delivery of IL-1ra penetrates the human brain at experimentally therapeutic concentrations,⁵¹ which again suggests that innate immunity measured in plasma also influences the inflammatory response in the brain. Finally, it is possible that some offspring with and without a parental history of AD had dementia. However, the chance that this occurred in our study is small since the estimated overall annual incidence of dementia (0.033%) is extremely low in subjects younger than 60 years.⁵²

IMPLICATIONS

Our study shows that high blood pressure and an innate pro-inflammatory cytokine response in middle age significantly contribute to AD. As these risk factors cluster in families, it is important to realize that early interventions could prevent late-onset AD. One could argue for a high-risk-prevention strategy by identifying the offspring of patients with AD, screening them for hypertension and vascular factors, and implementing various (non)pharmacological health measures. The final proof for the effectiveness of such a health strategy can only come from longitudinal randomized clinical trials.

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