Plasma Clusterin and the Risk of Alzheimer Disease

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Several genome-wide association studies have identified the CLU gene, which encodes for clusterin, as a genetic locus involved in Alzheimer disease (AD). The protein clusterin, also known as apolipoprotein J, has been suggested to be involved in the pathogenesis of AD. Clusterin has been found in the frontal cortex and hippocampus of postmortem AD brains and is increased in the cerebrospinal fluid of patients with AD. Plasma clusterin was reported to be associated with brain atrophy, baseline disease severity, and rapid clinical progression in AD, suggesting its possible use as a biomarker of AD.

Data from a large population-based cohort study was used to examine the associations between plasma levels of clusterin and the prevalence, severity, and risk of AD in participants.

Methods
Study Population
This study was based on participants of the Rotterdam Study, a large prospective population-based cohort study that is conducted among all inhabitants aged 55 years or older of Ommoord, a district of Rotterdam, the Netherlands. Baseline examinations were conducted between 1990 and 1993, with follow-up examinations conducted in 1993-1994, 1997-1999, and 2002-2004. At each survey, the diagnosis of dementia was made following a 3-step procedure.

Main Outcome Measures
Prevalent AD, severity of AD measured by the Mini-Mental State Examination (MMSE) score, and the risk of developing AD during follow-up.

Results
The likelihood of prevalent AD increased with increasing plasma levels of clusterin (odds ratio [OR] per SD increase of plasma clusterin level, 1.63; 95% confidence interval [CI], 1.21-2.20; adjusted for age, sex, education level, apolipoprotein E status, diabetes, smoking, coronary heart disease, and hypertension). Among patients with AD, higher clusterin levels were associated with more severe disease (adjusted difference in MMSE score per SD increase in clusterin levels, −1.36; 95% CI, −2.70 to −0.02; P = 0.047). Plasma clusterin levels were not related to the risk of incident AD during total follow-up (adjusted HR, 1.00; 95% CI, 0.85-1.17; P for trend = .77) or within 3 years of baseline (adjusted HR, 1.09; 95% CI, 0.84-1.42; P for trend = .65).

Conclusion
Plasma clusterin levels were significantly associated with baseline prevalence and severity of AD, but not with incidence of AD.

Context
Variants in the clusterin gene are associated with the risk of Alzheimer disease (AD) and clusterin levels have been found to be increased in brain and cerebrospinal fluid of patients with AD. Plasma clusterin was reported to be associated with brain atrophy, baseline disease severity, and rapid clinical progression in patients with AD.

Objective
To evaluate the potential of plasma clusterin as a biomarker of the presence, severity, and risk of AD.

Design, Setting, and Participants
A case-cohort study nested within the Rotterdam Study, a prospective population-based cohort study conducted in Rotterdam, the Netherlands. Plasma levels of clusterin were measured at baseline (1997-1999) in 60 individuals with prevalent AD, a random subcohort of 926 participants, and an additional 156 participants diagnosed with AD during follow-up until January 1, 2007 (mean [SD], 7.2 [2.3] years).

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teria for dementia (Diagnostic and Statis
tical Manual of Mental Disorders
[Third Edition Revised]). Alzheimer
disease (NINCDS-ADRDA),13 and vas-
cular dementia (NINDS-AIREN)16 by a
panel of a neurologist, neuropsycholo-
gist, and research physician. Fol-
low-up for incident dementia was vir-
tually complete (>98%) through
January 1, 2007. The medical ethics
committee at Erasmus University of
Rotterdam, Rotterdam, the Nether-
lands, approved the study, and writ-
ten informed consent was obtained
from all participants.

Study Design
We used a case-cohort study design,
which is an established method that in-
creases efficiency, especially when
costly measurements are required.17 In
this study design, a random subcohort
is drawn from the total cohort at risk. Par-
ticipants from the total cohort who de-
velop the disease outside the subco-
hort are added to the analyses; how-
ever, only persons from the subcohort
contribute follow-up time.

Assessment of Clusterin
At the third survey, fasting blood
samples were obtained at the research
center. Citrate plasma (5 mL) was col-
clected and stored at −80°C. In July
2008, 200 µL of citrate plasma from
each participant was sent to Rules-
Based Medicine, Austin, Texas (http://
www.rulesbasedmedicine.com), where
clusterin levels were analyzed via mul-
tiplex immunoassay on a human multi-
analyte profile. The least detectable
dose was 1.3 µg/mL. The intra-assay vari-
ability was less than 4% and the interassay
variability was less than 13%.

Covariates
Educational level was assessed during the
first interview, which took place be-
tween 1990 and 1993, and was dichoto-
mized into primary education (with or
without an unfinished higher educa-
tion) vs lower vocational to university
education. APOE (apolipoprotein E) gene-
type (RefSeq NG_007084) was as-
sessed on coded DNA samples using
polymerase chain reaction without
knowledge of the dementia diagnosis.
APOE ε4 status was defined as carrier-
ship of 1 or 2 ε4 alleles. If APOE geno-
type was missing (n=42, 4.3%), APOE
e4 status was imputed as 0.28 (the pro-
portion with an APOE ε4 allele in the
total population with APOE genotyp-
ing). The MMSE score11 was assessed at
the research center during the third sur-
vey. In addition, a dedicated neuropsy-
chological test battery was used to as-
sess executive function, attention, and
information processing speed. The test
battery included the Letter-Digit Substi-
tution Task,18 the Word Fluency Test,19
and the abbreviated Stroop test.20

Hypertension was defined as a blood
pressure of at least 140/90 mm Hg or
use of antihypertensive medication,
prescribed for the indication of hyper-
tension. Coronary heart disease was
defined as a previous myocardial in-
farction, percutaneous transluminal
coronary angiography, or coronary ar-
tery bypass graft surgery. Smoking
habits were assessed at the home in-
terview. Diabetes was defined as a self-
reported history of diabetes, registra-
tion by a general practitioner as hav-
ing diabetes, or a fasting glucose level of
at least 126 mg/dL (to convert to milli-
moles per liter, multiply by 0.055). Miss-
ings values in covariates (<5%) were
imputed as the mean.

Statistical Analyses
We used linear regression analyses to
investigate the associations between the
baseline characteristics and plasma clus-
terin levels. Analyses were adjusted for
age and sex when applicable. All analy-
yses were performed using SPSS statis-
tical package 15.0 (SPSS Inc, Chicago,
Illinois) or SAS version 9.2 (SAS Insti-
tute Inc, Cary, North Carolina). A priori
level of significance was set at P≤.05
for all analyses.

First, we investigated the cross-
sectional association between plasma
levels of clusterin and prevalent AD and
dementia using logistic regression mod-
els. After establishing that clusterin fol-
lowed a normal distribution, clusterin
was entered continuously per SD in-
crease into the models and per quar-
tile of its distribution. All analyses were
adjusted for age and sex, and addi-
tional adjustments were made for edu-
cational level, APOE ε4 status, and vas-
cular risk factors.

Second, to test whether plasma clus-
terin levels are associated with sever-
ity of AD within individuals with prev-
lent AD, we performed linear regression
analyses of clusterin levels with the
MMSE score and other cognitive test
scores as the dependent variable.

Third, we investigated the associa-
tion between plasma clusterin and the
risk of developing incident AD during
follow-up using Cox proportional haz-
ards regression models with modifica-
tion of the standard errors based on ro-
 bust variance estimates. We used the
method according to Barlow in which the
random subcohort is weighted by the
inverse of the sampling fraction
from the total cohort at risk.21 All analy-
 ses were adjusted for age (used as the
time scale) and sex, and additional ad-
justments were made for the above-
mentioned covariates.

In addition, to see whether plasma clus-
terin levels might have changed due to
subclinical AD, subsequent analyses were
performed on incident cases identified
within and after 3 years of follow-up.

RESULTS
Of the 5990 individuals alive at the time
of the third Rotterdam survey (1997-
1999), 4797 participated in the sur-
vey. A total of 3795 participants had
fasting blood samples drawn that could
be used for clusterin assessment. Among
these participants, 79 were di-
agnosed with prevalent dementia and
7 did not undergo dementia screen-
ing, resulting in a cohort of 3709 par-
ticipants at risk for incident dementia.

From this cohort, we drew a ran-
dom subcohort of 952 participants in
2008, of whom 926 had sufficient
plasma remaining for clusterin mea-
asurement. As of follow-up through
January 1, 2007, we identified 61 par-
ticipants who developed dementia in
this subcohort (of whom 52 were di-
agnosed with AD) with clusterin mea-

Table 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total Cohort at Risk for Incident Dementia (n = 3709)</th>
<th>Subcohort at Risk for Incident Dementia (n = 926)</th>
<th>Prevalent AD Cases (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>72.1 (6.8)</td>
<td>72.8 (7.3)</td>
<td>83.4 (7.3)</td>
</tr>
<tr>
<td>Women</td>
<td>2133 (58)</td>
<td>508 (55)</td>
<td>41 (68)</td>
</tr>
<tr>
<td>APOE ε4 allele present</td>
<td>980 (28)</td>
<td>253 (28)</td>
<td>32 (58)</td>
</tr>
<tr>
<td>Education, only primary</td>
<td>1097 (30)</td>
<td>259 (28)</td>
<td>39 (65)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>589 (16)</td>
<td>130 (14)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>386 (10)</td>
<td>96 (10)</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>514 (14)</td>
<td>118 (13)</td>
<td>15 (25)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2772 (76)</td>
<td>705 (78)</td>
<td>48 (86)</td>
</tr>
<tr>
<td>MMSE score, mean (SD), points</td>
<td>27.7 (1.9)</td>
<td>27.7 (1.9)</td>
<td>18.7 (5.2)</td>
</tr>
<tr>
<td>Clusterin level, mean (SD), µg/mL</td>
<td>NA</td>
<td>115 (25)</td>
<td>129 (29)</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; APOE, apolipoprotein E; CI, confidence interval. aCovariates included education level, APOE ε4 allele present, diabetes, smoking, coronary heart disease, and hypertension. bDefined as a blood pressure of at least 140/90 mm Hg or use of antihypertensive medication prescribed for the indication of hypertension.

Table 2. Associations of Baseline Characteristics With Plasma Clusterin Levels

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Difference in Plasma Clusterin Levels (95% CI), µg/mL</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per y</td>
<td>−0.15 (−0.37 to 0.07)</td>
<td>.18</td>
</tr>
<tr>
<td>Female sex, yes vs no</td>
<td>11.12 (8.00 to 14.23)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>APOE ε4 allele present, yes vs no</td>
<td>0.57 (−2.94 to 4.09)</td>
<td>.75</td>
</tr>
<tr>
<td>Education, only primary, yes vs no</td>
<td>−0.72 (−4.28 to 2.83)</td>
<td>.69</td>
</tr>
<tr>
<td>Current smoking, yes vs no</td>
<td>−0.70 (−5.21 to 3.82)</td>
<td>.76</td>
</tr>
<tr>
<td>Coronary heart disease, yes vs no</td>
<td>−1.94 (−7.11 to 3.24)</td>
<td>.46</td>
</tr>
<tr>
<td>Diabetes, yes vs no</td>
<td>3.77 (−0.89 to 8.43)</td>
<td>.11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5.29 (1.43 to 9.15)</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviations: APOE, apolipoprotein E; CI, confidence interval. aLinear regression analyses were performed in the random subcohort (n = 926) and are adjusted for age and sex, when applicable.

Table 3. Odds of Prevalent AD and Risk of Incident AD During Follow-up per SD Increase and per Quartile in Plasma Clusterin Levels

<table>
<thead>
<tr>
<th>Plasma Clusterin Level, µg/mL</th>
<th>Per SD Increase</th>
<th>Per Quartile of Distribution</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>47.2-99.5</td>
<td>99.6-115.4</td>
<td>115.5-132.4</td>
</tr>
<tr>
<td>Odds of prevalent AD at baseline (n = 60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>1.49 (1.12-1.98)</td>
<td>1 [Reference]</td>
<td>1.73 (0.70-4.25)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariatesa</td>
<td>1.63 (1.21-2.20)</td>
<td>1 [Reference]</td>
<td>2.17 (0.83-5.68)</td>
</tr>
<tr>
<td>Risk of incident AD during total follow-up (n = 208)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>0.98 (0.84-1.15)</td>
<td>1 [Reference]</td>
<td>1.07 (0.69-1.68)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariatesa</td>
<td>1.00 (0.85-1.17)</td>
<td>1 [Reference]</td>
<td>1.07 (0.67-1.71)</td>
</tr>
<tr>
<td>Risk of incident AD within 3 years (n = 76)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>1.06 (0.82-1.37)</td>
<td>1 [Reference]</td>
<td>0.82 (0.39-1.71)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariatesa</td>
<td>1.09 (0.84-1.42)</td>
<td>1 [Reference]</td>
<td>0.85 (0.39-1.85)</td>
</tr>
<tr>
<td>Risk of incident AD after 3 years (n = 132)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>0.97 (0.90-1.17)</td>
<td>1 [Reference]</td>
<td>1.24 (0.73-2.12)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariatesa</td>
<td>0.99 (0.81-1.20)</td>
<td>1 [Reference]</td>
<td>1.23 (0.70-2.15)</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; CI, confidence interval; HR, hazard ratio; OR, odds ratio.

aCovariates included education level, APOE ε4 allele present, diabetes, smoking, coronary heart disease, and hypertension.
Table 3 shows the associations of plasma clusterin levels with prevalent AD and with the risk of incident AD during follow-up. The odds that a participant had prevalent AD significantly increased by 49% for every SD increase in clusterin levels. This association became even stronger after further adjustments for educational level, APOE ε4 status, and vascular factors. There was no statistically significant association of plasma clusterin levels with incident AD during total follow-up or with incident AD within or after 3 years of baseline. Results for all-cause dementia and vascular dementia were similar and are shown in Table 4.

After adjusting for age and sex, clusterin levels were associated with the MMSE score in patients with prevalent AD (difference in MMSE score per SD increase in clusterin levels, −0.13; 95% CI, −2.54 to −0.13; P = .03), but not in controls without dementia (difference in MMSE score per SD increase in clusterin levels, −0.004; 95% CI, −0.128 to 0.120; P = .95). Adjusting for education level, APOE ε4 status, smoking, diabetes, coronary heart disease, and hypertension did not change the results (difference in MMSE score per SD increase in clusterin levels, −1.36; 95% CI, −2.70 to −0.02; P = .047 for patients with prevalent AD and −0.005; 95% CI, −0.126 to 0.116; P = .93 for controls without dementia). A smaller subset underwent additional cognitive tests (Letter-Digit Substitution Task, Word Fluency Test, and Stroop test), which largely showed the same pattern but did not reach statistical significance (Table 5).

**COMMENT**

In our population-based cohort study, plasma levels of clusterin were associated with the prevalence and severity of AD, but not with the development of incident AD during follow-up. Major strengths of our study were the population-based design and the long and virtually complete follow-up for incident dementia. Therefore, we were able not only to investigate the associations of plasma clusterin with the presence and severity of AD, but also to investigate whether plasma clusterin might be a preclinical marker of AD. However, magnetic resonance imaging was not routinely performed in the third survey; therefore, we were not able to investigate the relationship of plasma clusterin with brain or hippocampal atrophy. The relationship between plasma clusterin and progression of AD was also not investigated. We did explore the associations of clusterin with vascular dementia and all-cause dementia, which were similar to the associations with AD, suggesting that clusterin cannot be used to distinguish AD.

### Table 3. Odds of Prevalent All-Cause and Vascular Dementia, and Risk of Incident All-Cause and Vascular Dementia During Follow-up per SD Increase in Plasma Clusterin Levels

<table>
<thead>
<tr>
<th>Condition</th>
<th>Odds of Prevalent Dementia</th>
<th>Risk of Incident Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td><strong>All-cause dementia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>1.50 (1.17-1.96)</td>
<td>0.84 (0.61-1.15)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariates</td>
<td>1.47 (0.77-2.83)</td>
<td>0.95 (0.81-1.10)</td>
</tr>
<tr>
<td><strong>Vascular dementia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>1.50 (0.78-2.78)</td>
<td>0.82 (0.60-1.12)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariates</td>
<td>1.47 (0.77-2.83)</td>
<td>0.96 (0.82-1.12)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; OR, odds ratio.

### Table 4. Differences in Cognitive Test Performance at Baseline per SD Increase in Plasma Clusterin Levels

<table>
<thead>
<tr>
<th>Test</th>
<th>Random Subcohort</th>
<th>Prevalent AD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMSE, points</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>−0.004 (−0.128 to 0.120)</td>
<td>−1.34 (−2.54 to −0.13)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariates</td>
<td>−0.005 (−0.126 to 0.116)</td>
<td>−1.36 (−2.70 to −0.02)</td>
</tr>
<tr>
<td><strong>LDST, points</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>−0.010 (−0.437 to 0.417)</td>
<td>−0.95 (−2.85 to 0.94)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariates</td>
<td>0.002 (−0.407 to 0.411)</td>
<td>−1.59 (−3.73 to 0.55)</td>
</tr>
<tr>
<td><strong>WFT, points</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>0.22 (−0.13 to 0.58)</td>
<td>−0.48 (−1.69 to 0.73)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariates</td>
<td>0.22 (−0.13 to 0.58)</td>
<td>−0.85 (−2.16 to 0.47)</td>
</tr>
<tr>
<td><strong>Stroop trial 3, s</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>0.38 (−0.89 to 1.65)</td>
<td>0.06 (−11.01 to 11.13)</td>
</tr>
<tr>
<td>Adjusted for age, sex, and covariates</td>
<td>0.35 (−0.90 to 1.59)</td>
<td>1.72 (−11.56 to 14.99)</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; CI, confidence interval; LDST, Letter-Digit Substitution Task; MMSE, Mini-Mental State Examination; WFT, Word Fluency Test.

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from vascular dementia. Other subtypes of dementia could not be investigated because of small numbers.

Our finding that plasma clusterin was associated with MMSE in patients with prevalent AD was similar to that of Thambisetty et al.," however, unlike their study, our patients with AD had significantly higher levels of plasma clusterin than controls. In addition, our data do not support the suggestion that clusterin is increased, possibly as an etiopathological event, before the development of AD, but fits the hypothesis that the increased expression of clusterin in AD reflects a neuroprotective response. Several protective effects of clusterin on the brain that may play a role in AD have been described in in vitro or in vivo studies, including inhibition of amyloid formation through binding amyloid-beta or enhancing its clearance over the blood-brain barrier, clearance by endocytosis of amyloid-beta aggregates and cell debris to brain phagocytes, and inhibition of complement activation. The neurodegenerative changes that occur in AD may trigger an increased expression of clusterin. This is in line with our finding that plasma clusterin was associated with prevalent AD and severity of AD, but not with the risk of developing incident AD during follow-up. Clusterin was also associated with prevalent allcause dementia and vascular dementia, supporting a reactive rather than a causative role of clusterin and suggesting that clusterin will not be useful in the differential diagnosis of AD vs other subtypes of dementia.

In conclusion, our data from the general population show that increased plasma clusterin levels are associated with prevalent AD and are higher in more severe cases of AD. However, increased levels of clusterin do not precede development of AD and therefore are not a potential early marker of subclinical disease.

Author Contributions: Drs Schrijvers and Breteler had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES