Plasma Levels of Apolipoprotein E and Risk of Dementia in the General Population

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Objective: The apolipoprotein E (APOE) ε4 allele is a major genetic risk factor for Alzheimer disease and dementia. However, it remains unclear whether plasma levels of apoE confer additional risk. We tested this hypothesis.

Methods: Using 75,708 participants from the general population, we tested whether low plasma levels of apoE at study enrollment were associated with increased risk of future Alzheimer disease and all dementia, and whether this association was independent of ε2/ε3/ε4 APOE genotype.

Results: Multifactorially adjusted hazard ratios (HRs) for Alzheimer disease and all dementia increased from the highest to the lowest apoE tertile (p for trends < 1.610^-26). Multifactorially adjusted HRs for lowest versus highest tertile were 2.68 (95% confidence interval [CI] = 2.04–3.52) and 1.80 (95% CI = 1.52–2.13) for Alzheimer disease and all dementia, respectively. After further adjustment for ε2/ε3/ε4 APOE genotype, plasma apoE tertiles remained associated with Alzheimer disease (p for trend = 0.007) and all dementia (p for trend = 0.04). Plasma apoE tertiles did not interact with ε2/ε3/ε4 APOE genotype on risk of Alzheimer disease (p = 0.53) or all dementia (p = 0.79). In a subanalysis, the −219G>T GT promoter genotype, associated with low plasma apoE levels, remained significantly associated with increased risk of Alzheimer disease after adjustment for ε2/ε3/ε4 APOE genotype (HR = 1.56, 95% CI = 1.05–2.30).

Interpretation: Low plasma levels of apoE are associated with increased risk of future Alzheimer disease and all dementia in the general population, independent of ε2/ε3/ε4 APOE genotype. This is clinically relevant, because no plasma biomarkers are currently implemented. Hence, plasma levels of apoE may be a new, easily accessible preclinical biomarker.

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of the receptor-defective ε2 allele on plasma levels of apoE and other lipids, lipoproteins, and apolipoproteins, the biological mechanisms underlying the association between ε4 and plasma levels of apoE and other measures of lipid metabolism, and between ε4 and risk of dementia, are not fully understood.3,16

Alterations in CSF biomarkers for Alzheimer disease are suggested to occur in a temporal order, in which amyloid markers first become abnormal, followed by markers of neurodegeneration, and finally clinical symptoms.10 Because brain apoE is involved in the clearance of β-amyloid, low levels of apoE in the CSF may be an early marker of preclinical Alzheimer disease, as recently suggested by cross-sectional observations between low CSF apoE levels and decreased levels of CSF β42-amyloid, a proxy for preclinical Alzheimer disease.17 Whether plasma levels of apoE associate with future risk of Alzheimer disease and all dementia in the general population, and whether a given association is independent of ε2/ε3/ε4 APOE genotype, is unknown. We tested this hypothesis in 2 prospective studies of the general population, the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS), totalling 75,708 individuals.

Subjects and Methods

Studies were approved by institutional review boards and Danish ethical committees, and conducted according to the Declaration of Helsinki. Written informed consent was obtained from participants. All participants were white and of Danish descent. There was no overlap of individuals between studies.

Participants

We included participants in 2 similar studies of the Danish general population: the CGPS and CCHS. Combining these studies yielded a total of 75,708 participants, of whom 1,060 developed dementia.

CGPS

This prospective study of the Danish general population was initiated in 2003, with ongoing enrollment. Data were obtained from a questionnaire, a physical examination, and blood samples including DNA extraction. We included 65,803 participants from this study in the present analysis. Of these, 397 had dementia.

CCHS


Dementia Endpoints

Information on diagnoses of dementia (World Health Organization International Classification of Diseases, 8th revision [ICD8] 290; and 10th revision [ICD10] F00, F01, F03, G30) was collected from the National Danish Patient Registry and National Danish Causes of Death Registry. Alzheimer disease was ICD8 290.10 and ICD10 F00 and G30, and all dementia further included vascular dementia (ICD10 F01) and unspecified dementia (ICD8 290.18; ICD10 F03). The National Danish Patient Registry has information on all patient contacts with all clinical hospital departments in Denmark since 1977, including emergency wards and outpatient clinics from 1994. The National Danish Causes of Death Registry contains data on the causes of all deaths in Denmark, as reported by hospitals and general practitioners.

Follow-up time began at the time of blood sampling (2003 and onward for CGPS and 1991–1994 or 2001–2003 for CCHS). Follow-up ended at occurrence of event, death, emigration, or on May 10, 2011 (last update of the registry), whichever came first. Median follow-up was 4 years (range = 0–20 years), with no individuals lost to follow-up.

Biochemical and Genetic Analyses

Plasma total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured using colorimetric assays (Boehringer Mannheim, Mannheim, Germany; Konelab, Thermofisher Scientific, Waltham, MA). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation when plasma triglycerides were ≤4.0mmol/L (≤352mg/dl) and otherwise measured directly (Konelab). ApoE was measured in 9,905 individuals from the CCHS 1991–1994 and 2001–2003 examinations, and in 65,803 individuals from the CGPS 2003–2011 examination using nephelometry or turbidimetry (Dade Behring, Deerfield, IL or Dako, Glostrup, Denmark). In the 9,905 individuals from the CCHS and in the first 24,000 individuals from the CGPS, apoE was measured nephelometrically with a BNII autoanalyzer using goat antihuman apoE polyclonal antibodies (OQDLG09, Dade Behring). In the subsequent 41,803 individuals from the CGPS, apoE was measured turbidimetrically with a Kone autoanalyzer (Konelab) using rabbit antihuman apoE polyclonal antibodies (A0077, Dako). A human serum apoE calibrator (Apolipoprotein Standard Serum, OUPGG07; Siemens Healthcare Diagnostics, Ballerup, Denmark) was used for both nephelometry and turbidimetry. Associations between APOE genotype and levels of apoE were similar among the 2 assays; decrease of apoE relative to ε22 for ε32, ε42, ε33, ε43, and ε44 was 37%, 41%, 53%, 58%, 65% and 35%, 39%, 52%, 58%, 65%, respectively. The coefficient of variance for apoE was 5.4% at the level of 1.4mg/dL. To test whether the polyclonal antihuman apoE antibody used in the present assay measured the 3 apoE isoforms similarly, we spiked plasma from an ε22 carrier, an ε33 carrier, and an ε44 carrier (validated by sequencing and with apoE levels of 8.21, 4.04, and 2.85mg/dL, respectively) with equal amounts of recombinant apoE2, apoE3, and apoE4 (2.5mg/dL; Leinco Technologies, St Louis, MO), and measured
Each of the 9 combinations 3 times. Furthermore, we measured equal amounts of recombinant apoE2, apoE3, and apoE4 (5 mg/dL; Leinco Technologies) alone. An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) and Taqman-based assays were used to genotype for Cys130Arg (rs429358, legacy name Cys112Arg) defining the e4 allele and Arg176Cys (rs7412, legacy name Arg158Cys) defining the e2 allele. Variation at these 2 sites defines 6 common APOE genotypes (e2e2, e3e2, e4e2, e3e3, e4e3, e4e4); −491A>T (rs449647), −427T>C (rs769446), and −219G>T (rs405509) were determined in 9,842 participants in the CCHS and genotyped as previously described.22

**Other Covariates**

Body mass index was measured weight in kilograms divided by measured height in meters squared. All other covariates were self-reported, dichotomized, and defined in detail in the legend to the Table.

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**TABLE. Characteristics of Study Participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No Dementia</th>
<th>Alzheimer Disease</th>
<th>All Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals (%)</td>
<td>74,648 (99)</td>
<td>443 (1)</td>
<td>1,060 (1)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>57 ± 0.05</td>
<td>73 ± 0.4a</td>
<td>73 ± 0.3a</td>
</tr>
<tr>
<td>Female, %</td>
<td>55</td>
<td>62b</td>
<td>60b</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.7 ± 0.004</td>
<td>6.2 ± 0.06a</td>
<td>6.2 ± 0.04a</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.3 ± 0.004</td>
<td>3.7 ± 0.05a</td>
<td>3.7 ± 0.04a</td>
</tr>
<tr>
<td>Triglycerides, mmol/Lc</td>
<td>1.5 ± 0.006</td>
<td>1.5 ± 0.008b</td>
<td>1.6 ± 0.05a</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.6 ± 0.002</td>
<td>1.7 ± 0.03a</td>
<td>1.2 ± 0.02a</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
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<td>25 ± 0.2a, d</td>
<td>26 ± 0.1d</td>
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<tr>
<td>Hypertension, %</td>
<td>56</td>
<td>75a</td>
<td>76a</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>4</td>
<td>5</td>
<td>7a, d</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>24</td>
<td>28b</td>
<td>33a, d</td>
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<tr>
<td>Alcohol consumption, %</td>
<td>18</td>
<td>11a, d</td>
<td>14a</td>
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<tr>
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<td>52</td>
<td>62a</td>
<td>69a, d</td>
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<td>Postmenopausal, %e</td>
<td>66</td>
<td>98a</td>
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<td>Hormonal replacement therapy, %e</td>
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<td>13a</td>
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<td>Lipid-lowering therapy, %</td>
<td>9</td>
<td>9</td>
<td>9</td>
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<tr>
<td>Education &lt; 8 years, %</td>
<td>14</td>
<td>45a, d</td>
<td>45a, d</td>
</tr>
</tbody>
</table>

Values are number (%), mean ± standard error of the mean, or percentage, and are from the day of enrollment (2003 and onward for the Copenhagen General Population Study and 1991–1994 or 2001–2003 for the Copenhagen City Heart Study). Missing data on categorical and continuous covariates (0.6%) were imputed from age, sex, and population using multiple imputation. Hypertension was defined as use of antihypertensive medication, systolic blood pressure of ≥140 mmHg, and/or diastolic blood pressure of ≥90 mmHg. Diabetes mellitus was defined as self-reported disease, use of insulin or oral hypoglycemic agents, and/or nonfasting plasma glucose level of >11 mmol/L (>198 mg/dL). Smoking was defined as current smoking. Alcohol consumption was defined as >14/21 U per week for women/men (1 U = 12 g alcohol, equivalent to 1 glass of wine or 1 beer [33 cl]). Physical inactivity was defined as ≤4 hours per week of light physical activity in leisure time. Women reported menopausal status and use of hormonal replacement therapy. Lipid-lowering therapy was primarily statins (yes/no), and the cutoff for education was 8 years.

a p < 0.001
b p < 0.05 by Student t test or Pearson chi-square test for all dementia versus no dementia and for Alzheimer disease versus no Alzheimer disease, respectively.

c Geometric mean ± standard error of the mean for unimputed triglycerides is shown.
d Multifactorially adjusted hazard ratios for Alzheimer disease were significantly decreased for high versus low alcohol consumption. Multifactorially adjusted hazard ratios for all dementia were significantly increased for diabetes versus no diabetes, for smoking versus no smoking, and for physical activity ≤4 hours per week versus physical activity >4 hours per week. Multifactorially adjusted hazard ratios for both Alzheimer disease and all dementia were significantly increased for low versus high body mass index and for education <8 years versus education ≥8 years.
e In women only.

HDL = high-density lipoprotein; LDL = low-density lipoprotein.
Statistical Analysis

We used Stata/S.E. v12.0 (Stata Corp, College Station, TX). Probability values < 0.0001 are given as powers of 10. The t test and Pearson chi-square test were used in 2-group comparisons of continuous and categorical variables, respectively. To account for age and sex differences, plasma apoE percentiles were generated for groups stratified for cohort, sex, and age (using the age groups 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, ≥80 years) and combined into 3 percentile groups (1–33%, 34–66%, 67–100%), named “tertiles” for simplicity. For trend tests subjects were coded according to apoE tertiles as 1, 2, and 3 and according to APOE genotypes as 1, 2, 3, 4, 5, and 6 for e22, e32, e42, e33, e43, and e44. Cuzick nonparametric test for trend or Kruskal–Wallis analysis of variance was used to test for differences in apoE levels between e2/e3/e4 APOE genotypes and age groups. Linear regression estimated the interindividual variation in plasma levels of apoE (logarithmically transformed) explained by e2/e3/e4 APOE genotype. Missing data on categorical and continuous covariates (0.6%) were imputed from age, sex, and population using multiple imputation with 10 imputations. Multinomial logistic regression was applied for categorical variables and linear regression for continuous variables, and were performed using the “mi impute mlogit” and “mi impute chained” commands in Stata. Pairwise linkage disequilibrium (LD) for the 3 promoter SNPs (−491A>T [rs449647], −427T>C [rs769446], −219G>T [rs605509]), and the 2 exonic SNPs (e2/e3/e4 SNPs [rs429358 and rs7412]) were estimated using the software Haplovie 4.2.23 Power was calculated using the “power logrank” command in Stata.

Kaplan–Meier curves and log-rank trend tests evaluated cumulative incidence, and Cox proportional hazards regression models estimated hazard ratios (HRs) for Alzheimer disease and all dementia as a function of apoE tertiles or APOE genotypes. Age was accounted for by using follow-up time as the time scale with further adjustment for age at blood sampling. For Cox regression models, proportionality of hazards over time was assessed by plotting –ln(–ln(survival)) versus ln(analysis time). There was no suspicion of nonproportionality.

To test whether plasma apoE tertiles were associated with increased risk of dementia, we used Cox regression models adjusted for age, sex, body mass index, hypertension, diabetes mellitus, smoking, alcohol consumption, physical inactivity, menopausal status, hormonal replacement therapy, lipid-lowering therapy, total cholesterol, LDL cholesterol, triglycerides, HDL cholesterol, and e2/e3/e4 APOE genotype. Interaction between apoE tertiles and e2/e3/e4 APOE genotype on risk of Alzheimer disease and all dementia was evaluated by the inclusion of 2-factor interaction terms in the Cox regression model, using a likelihood ratio test between models excluding and including the interaction term. HRs including confidence intervals were corrected for regression dilution bias using a nonparametric method.24 For this correction, we used a subset of individuals (n = 4,303) from the CCHS with replicate measurements of apoE from the 1991–1994 examination and approximately 10 years later at the 2001–2003 examination to compute a regression dilution ratio of 0.93.

Results

Characteristics of the 75,708 study participants by disease status are shown in the Table. APOE genotype frequencies were 0.7%, 12.4%, 2.9%, 55.8%, 25.4%, and 2.9% for e22, e32, e42, e33, e43, and e44, respectively, and did not deviate from those predicted by the Hardy–Weinberg equilibrium (p = 0.82 for Cys130Arg, defining the e4 allele, and p = 0.43 for Arg176Cys, defining the e2 allele). Also no deviations from the Hardy-Weinberg equilibrium were present for the 3 APOE promoter variants (all p ≥ 0.48).

Plasma Levels of apoE

Plasma levels of apoE increased in both women and men with increasing age (probability values < 1 × 10−6; Fig 1) and were higher in women than in men (p < 1 × 10−6). Data were similar in the CGPS and CCHS separately (data not shown). Hence to account for these differences, we corrected plasma levels of apoE for the effect of age and sex using age- and sex-specific percentiles, and combined these into 3 percentile groups (1–33%, 34–66%, 67–100%), named “tertiles” for simplicity. Median plasma levels of apoE were 3.1mg/dL, 4.1mg/dL, and 5.8mg/dL in the tertiles.

e2/e3/e4 APOE Genotype and Plasma Levels of apoE

e2/e3/e4 APOE genotype was associated with stepwise decreases in plasma levels of apoE of up to −65%
Plasma Levels of apoE and Risk of Alzheimer Disease and All Dementia

Kaplan–Meyer cumulative incidences of Alzheimer disease and all dementia increased stepwise from the highest

(5.5mg/dL; p for trend < 1 x 10^-6) from e22 to e32 to e42 to e33 to e43 to e44 (Fig 2, left panel). Mean residuals from a linear regression of logarithmically transformed apoE, adjusted for age and sex, showed a similar pattern (p < 1 x 10^-6; see Fig 2, right panel). e2/e3/e4 APOE genotype explained 25% (age and sex adjusted) of the interindividual variation in plasma levels of apoE.

Findings were similar in the CGPS and CCHS separately (data not shown). We tested the present apoE immunoassay for apoE isoform-dependent sensitivity by spiking plasma from e22, e33, and e44 carriers with equal amounts of recombinant apoE2, apoE3, and apoE4, as well as by measuring the 3 recombinant proteins alone.

Mean recoveries for apoE2, apoE3, and apoE4 were 103%, 99%, and 155% when spiked in plasma from an e22 carrier, 62%, 66%, and 88% when spiked in plasma from an e33 carrier, 69%, 74%, and 87% when spiked in plasma from an e44 carrier, and 63%, 70%, and 78% when recombinant protein was measured alone, respectively. Pairwise LD in the CCHS was estimated for 3 common promoter SNPs (rs449647 > T [rs449647], rs769446 > C [rs769446], rs405509 > T [rs405509]) and the 2 exonic SNPs (e4 [rs429358], e2 [rs7412]). Exact r^2 values were the following: rs449647/rs769446, 0.01; rs449647/rs405509, 0.001; rs449647/rs429358 (e4), 0.03; rs449647/rs7412 (e2), 0.07; rs769446/rs405509, 0.00; rs769446/rs429358 (e4), 0.002; rs769446/rs7412 (e2), 0.16; rs405509/rs429358 (e4), 0.10; rs405509/rs7412 (e2), 0.08; rs429358 (e4)/rs7412 (e2), 0.02. All r^2 values involving e4 were ≤0.10, suggesting that none of the promoter SNPs can sufficiently serve as proxies for the e4 allele (rs429358).

Plasma Levels of apoE and Risk of Alzheimer Disease and All Dementia

Kaplan–Meyer cumulative incidences of Alzheimer disease and all dementia increased stepwise from the highest
to the lowest apoE tertile (log-rank $p$ for trends < $1 \times 10^{-6}$; Fig 3). Multifactorially adjusted HRs increased from the highest through the middle to the lowest apoE tertile for Alzheimer disease and for all dementia ($p$ for trends < $1 \times 10^{-6}$; Fig 4, left panel). Multifactorially adjusted HRs for lowest versus highest apoE tertile were 2.68 (95% confidence interval [CI] = 2.04–3.52) and 1.80 (95% CI = 1.52–2.13) for Alzheimer disease and all dementia, respectively. After further adjustment for $\varepsilon 2$/ $\varepsilon 3$/ $\varepsilon 4$ APOE genotype, plasma apoE levels remained associated with Alzheimer disease ($p$ for trend = 0.007) and all dementia ($p$ for trend = 0.04; see Fig 4, right panel). Data were similar in the CGPS and CCHS separately (data not shown).

**FIGURE 4:** Risk of Alzheimer disease and all dementia as a function of plasma levels of apolipoprotein E in tertiles (lowest and middle vs highest) in the Copenhagen General Population Study and Copenhagen City Heart Study combined. Hazard ratios were multifactorially adjusted for age, sex, body mass index, hypertension, diabetes mellitus, smoking, alcohol consumption, physical inactivity, menopausal status and hormonal replacement therapy (women only), lipid-lowering therapy, education, total cholesterol, low-density lipoprotein cholesterol, triglycerides, and high-density lipoprotein cholesterol (left panel). Right panel additionally includes APOE genotype. APOE genotype = $\varepsilon 2$/ $\varepsilon 3$/ $\varepsilon 4$. ApoE = apolipoprotein E plasma level; CI = confidence interval.

**APOE Promoter Variants, Levels of apoE, and Risk of Alzheimer Disease**

The $-219$G$\rightarrow$T promoter variant was associated with stepwise decreases in plasma levels of apoE of up to $-16$% ($p$ for trend < $1 \times 10^{-6}$) from GG to GT to TT, whereas $-427$T$\rightarrow$C and $-491$A$\rightarrow$T were associated with stepwise increases of 35% and 12% ($p$ for trends < $1 \times 10^{-6}$). Multifactorially adjusted HRs increased from GG to GT to TT for $\varepsilon 2$/ $\varepsilon 3$/ $\varepsilon 4$ APOE genotype on risk of Alzheimer disease (Fig 6, right panel); $\varepsilon 2$/ $\varepsilon 4$ and $-491$A$\rightarrow$T were not associated with risk of Alzheimer disease.

**Discussion**

The principal findings of this study are: (1) low plasma levels of apoE are associated with increased future risk of Alzheimer disease and all dementia in a large analysis of
the general population and (2) this association is independent of $e_2/e_3/e_4$ APOE genotype. These findings are novel, and potentially clinically important, because they suggest that low plasma levels of apoE add information beyond $e_2/e_3/e_4$ APOE genotype on risk of Alzheimer disease and all dementia.

An independent association between low plasma apoE levels and increased risk of dementia was supported in 2 different ways: (1) plasma apoE tertiles remained associated with dementia after adjustment for $e_2/e_3/e_4$ APOE genotype with nontrivial HRs of up to 1.53 and (2) the HR remained significant for the common $219G>T$ GT promoter genotype, associated with a 9% reduction in plasma levels of apoE, after adjustment for $e_2/e_3/e_4$ APOE genotype. In the present cohorts, interactions between apoE levels and $e_2/e_3/e_4$ APOE genotype on risk of Alzheimer disease and all dementia were nonsignificant, reflecting that there is no differential risk of plasma apoE levels by $e_2/e_3/e_4$ APOE genotype or no differential risk of genotype by plasma level. Furthermore, it does not seem likely that the $e_4$ risk-increasing effect is explained by its association with lower plasma apoE levels, because $e_{44}$ versus $e_{33}$ is associated with an 8-fold risk of Alzheimer disease but only a $\sim 1$mg/dL lower apoE level, whereas lowest versus highest apoE tertile is associated with a 3-fold risk and a $\sim 3$mg/dL lower apoE level. Collectively, these data provide the strongest evidence to date that low plasma levels of apoE are associated with dementia independently of $e_2/e_3/e_4$ APOE genotype, and that both low levels of apoE and $e_2/e_3/e_4$ APOE genotype contribute to risk of dementia, independently of each other.

Possible mechanisms underlying our finding are unclear, and human studies of CSF with various Alzheimer disease–related outcomes are conflicting.\(^{17,25}\) These conflicting observations may reflect that disease stages differ between studies, so that the association of low CSF apoE levels in a healthy individual at baseline with future development of dementia does not necessarily correspond to the association seen in a severe state of Alzheimer disease with ongoing neuronal degeneration, and perhaps compensatory upregulation of apoE. We speculate that low levels of apoE both in the brain and in plasma may be early preclinical markers, because $\beta$-amyloid markers become abnormal up to 20 years before clinical symptoms,\(^{10}\) and because brain apoE expression mediates clearance of $\beta$-amyloid peptides,\(^{12}\) a clearance that is impaired in Alzheimer disease.\(^{26}\) This is supported by recent case–control studies associating low levels of CSF apoE with decreased levels of CSF amyloid-$\beta_{42}$ (a proxy for preclinical Alzheimer disease\(^{17}\)) and associating low plasma levels of apoE with Alzheimer disease and mild cognitive impairment.\(^{16,27,28}\) The present findings suggest that low plasma levels of apoE might be a useful

FIGURE 5: Risk of Alzheimer disease and all dementia as a function of APOE genotype ($e_{22}$, $e_{32}$, $e_{42}$, $e_{43}$, and $e_{44}$ vs $e_{33}$) in the Copenhagen General Population Study and Copenhagen City Heart Study combined. Hazard ratios were multifactorially adjusted for age, sex, body mass index, hypertension, diabetes mellitus, smoking, alcohol consumption, physical inactivity, menopausal status and hormonal replacement therapy (women only), lipid-lowering therapy, education, total cholesterol, low-density lipoprotein cholesterol, triglycerides, and high-density lipoprotein cholesterol (left panel). Right panel additionally includes plasma apolipoprotein E levels in tertiles. APOE genotype = $e_2/e_3/e_4$ APOE genotype. apoE = apolipoprotein E plasma level; CI = confidence interval.
biomarker for dementia, representing an obvious benefit for easy access compared with any other body fluid. Current biomarkers include measures of brain \(\beta\)-amyloid deposition (CSF \(\beta\)-amyloid\textsubscript{42} and positron emission tomography [PET] amyloid imaging) and measures of neurodegeneration (CSF total tau, CSF phosphorylated tau, hypometabolism by fluorodeoxyglucose PET, and atrophy on structural magnetic resonance imaging), whereas no plasma biomarkers for dementia have been demonstrated convincingly or implemented clinically.\(^{10,29}\)

Whether plasma apoE levels mirror apoE levels in CSF and brain tissue remains to be determined. Previous work suggests a limited blood–brain barrier permeability to circulating lipid-poor apoE2, apoE3, and apoE4,\(^{30}\) supporting that apoE in blood and brain are regulated independently.\(^{31,32}\) Levels of apoE in human CSF do however display a similar genotype-dependent pattern as in plasma,\(^{17}\) and recently murine interstitial fluid was observed to have similar genotype-dependent apoE levels as in CSF.\(^{33}\) We therefore speculate that at least in a preclinical baseline state, as in the present large human study, plasma levels of apoE may reflect CSF and brain apoE levels, although this reflection happens in parallel, in 2 metabolically different compartments. Interestingly, brain apoE protein levels were reported to be modulated by an extended haplotype structure.\(^{34}\) In this study, chromosome phase–separated haplotypes of the proximal APOE region were constructed,\(^{34–36}\) and apoE protein expression was found to differ among Alzheimer disease brain regions and to differ between Alzheimer disease and control hippocampus. Plasma levels of apoE are thus likely to mark a causal pathway for \(\beta\)-amyloid metabolism in dementia, and adding plasma levels of apoE to the panel of currently recommended biomarkers may have the potential to increase the sensitivity of preclinical markers.

A number of experimental studies have demonstrated that apoE critically regulates the fate of \(\beta\)-amyloid in the brain. In \(A\beta\) precursor protein–expressing transgenic mice, deletion of mouse apoE gene inhibits development of fibrillar amyloid plaques.\(^{37,38}\) Conversely, expression of apoE3 results in a dose-dependent decrease
in amyloid burden. Recently, studies in Apoe-/- mice have spread new light on mechanistic roles for both apoE levels and the ε4 allele. Targeted replacement of murine Apoe with human apoE4, as well as lack of any murine Apoe proteins, leads to blood–brain barrier breakdown by activating a proinflammatory cyclophilin A–nuclear factor κB–matrix metalloproteinase 9 pathway in pericytes, cells that are important for the formation and maintenance of the blood–brain barrier. Pericyte inflammation in turn leads to neuronal uptake of blood-borne neurotoxic proteins, and microvascular and cerebral blood flow reductions, a process that subsequently leads to impaired β-amyloid clearance. In further support of a mechanistic function of apoE levels, induction of murine Apoe expression improves β-amyloid clearance and cognition. Because defective clearance from brain and across the blood–brain barrier appears to be the primary determinant of β-amyloid load in the human brain, β-amyloid clearance pathways may be important therapeutic targets for the treatment of Alzheimer disease and related disorders. Taken together, these recent experimental data suggest that both apoE levels and APOE genotype mechanistically are involved in cognitive decline via impaired cerebral β-amyloid clearance. However, whether lack of murine Apoe is beneficial or detrimental for Alzheimer disease seems to be influenced by the mouse model studied, which weakens the translation of results from such models to the human situation.

Strengths of the present findings include a very large study and prospective design with apoE measurements preceding a clinical diagnosis of Alzheimer disease and all dementia, making reverse causation unlikely. Furthermore, we studied white individuals from an ethnically homogeneous population, and although our results may therefore not necessarily apply to other ethnicities, we are not aware of data to suggest that our results should not be applicable to all humans. The presence of proportionality of hazards over time, meaning that the ratio of the hazards comparing different exposure groups remained constant over time, ensured that the observed associations were not dependent on specific periods of time or different ages of onset. Finally, a critical point is whether the present immunoassay is equivalently sensitive to different apoE isoforms, because a previous mass spectrometry analysis showed no association of apoE plasma levels with Alzheimer disease, and suggested that immune-based methods may be biased. However, our apoE immunoassay measured recombinant apoE2, apoE3, and apoE4 isoform in a similar manner when spiked in plasma from ε22, ε33, and ε44 carriers or as recombinant protein alone, in accordance with other independent results. If anything, our assay possibly measured the apoE4 isoform slightly better than the apoE2 and apoE3 isoforms, which would only bias our results toward the null hypothesis. Thus, different isoform sensitivity of our assay cannot explain the present findings of low plasma levels of apoE and risk of dementia, but rather suggests that the present overall findings represent conservative estimates. Finally, our data are in agreement with results from mass spectrometry concerning the association between plasma levels of apoE and ε2/ε3/ε4 APOE genotype, as well as with an observed higher plasma apoE level in women compared with men.

In conclusion, low plasma levels of apoE are associated with increased risk of future Alzheimer disease and all dementia in the general population, and may be a new, easily accessible preclinical biomarker. These associations were independent of ε2/ε3/ε4 APOE genotype.

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Authorship
K.L.R.: study concept and design, acquisition of data, statistical analysis, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content; A.T.-H.: study concept and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, obtained funding, administrative, technical, and material support, study supervision; B.G.N.: acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, obtained funding, administrative, technical, and material support. R.F.-S.: study concept and design, acquisition of data, statistical analysis, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for
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Potential Conflicts of Interest
Nothing to report.

References


