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CLINICAL RESEARCH
Prevention and epidemiology

Plasma levels of apolipoprotein E, *APOE* genotype, and all-cause and cause-specific mortality in 105 949 individuals from a white general population cohort

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Aims To determine whether plasma apoE levels and *APOE* genotype are associated with all-cause and cause-specific mortality.

Methods and results Using a prospective cohort design with 105 949 white individuals from the general population, we tested the association between plasma apoE at study enrolment and death during follow-up, and whether this was independent of *APOE* genotype. We confirmed the well-known association between *APOE* genotypes and mortality. For all-cause, cardiovascular, and cancer mortality, high levels of apoE were associated with increased risk, while for dementia-associated mortality low levels were associated with increased risk. For the highest vs. the fifth septile of plasma apoE, hazard ratios (HRs) were 1.20 (95% confidence interval 1.12–1.28) for all-cause mortality, 1.28 (1.13–1.44) for cardiovascular mortality, and 1.18 (1.05–1.32) for cancer mortality. Conversely, for the lowest vs. the fifth septile the HR was 1.44 (1.01–2.05) for dementia-associated mortality. Results were similar in analyses restricted to *APOE* ε33 carriers. Examining genetically determined plasma apoE, a 1 mg/dL increase conferred risk ratios of 0.97 (0.92–1.03) for cardiovascular mortality and 1.01 (0.95–1.06) for cancer mortality, while a 1 mg/dL decrease conferred a risk ratio of 1.70 (1.36–2.12) for dementia-associated mortality.

Conclusion High plasma levels of apoE were associated with increased all-cause, cardiovascular, and cancer mortality, however of a non-causal nature, while low levels were causally associated with increased dementia-associated mortality.

Keywords APOE • Apolipoprotein E • Cardiovascular • Dementia • Mortality • Survival

Introduction

The basis of human longevity and healthy ageing remains among the principal questions in biology and medicine. Life expectancy is challenged by major diseases in old age—among these, dementia and cardiovascular disease. Alzheimer disease, the most common form of dementia, is a major cause of disability in later life with an increasing global prevalence, and this presently untreatable disease deteriorates into an inevitable terminal stage.[1–5] Cardiovascular disease is the leading cause of death worldwide,[6] and only in Central and Western Europe has the annual number of deaths from cardiovascular disease declined.[7,8] Biomarkers of lipid metabolism have been associated

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with increased mortality, however, the relationship of plasma levels of a crucial lipid transport protein, apolipoprotein E (apoE), with mortality is presently unknown.

ApoE plays a pivotal role in both peripheral and cerebral cholesterol metabolism. In plasma, apoE is mainly carried by triglyceride-rich lipoproteins, and serves as a ligand for members of the low-density lipoprotein (LDL) receptor family.[9] In the brain, astrocyte-derived apoE is crucial for cerebral cholesterol metabolism and clearance of β-amyloid, an important pathological hallmark of Alzheimer disease.[10,11] Plasma levels of apoE and other lipids and lipoproteins are under strong genetic influence by the well-known *APOE* polymorphism—a combination of two genetic variants (rs429358 and rs7412) giving rise to six common *APOE* genotypes, ε22, ε32, ε33, ε42, ε43, and ε44. Both ε2 and ε4 alleles are associated with unfavourable lipid profiles,[12–19] and the ε4 allele is a strong genetic risk factor for Alzheimer disease,[20] and by far the strongest hit in genome-wide association studies of longevity.[21,22] Interestingly, low levels of plasma apoE were recently reported to be associated with increased risk of dementia,[17,23,24] whereas high levels were associated with increased risk of ischaemic heart disease.[25] Hence both a quantitative importance of plasma apoE levels, and a qualitative genetically determined effect, appears to be important for dementia and cardiovascular disease—two important determinants of life expectancy. A few studies have addressed the association between plasma apoE and cardiovascular mortality.[26,27] Beyond that, the relationship of plasma apoE levels with all-cause and cause-specific mortality is unknown. Such associations are of substantial interest, because they may generate therapeutic and clinical considerations.

We studied the association of plasma apoE levels with all-cause and cause-specific mortality and assessed whether this was independent of isoform differences due to *APOE* genotype, by evaluating both *APOE* genotype adjusted and ε33 genotype stratified analyses. Using a Mendelian randomization design, we also studied genetically determined plasma apoE levels to examine if plasma apoE was causally associated with cause-specific mortality. For this purpose, we studied two large prospective cohorts of the white Danish general population, the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS), totalling 105 949 individuals.

Methods

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

Participants

We included individuals from two similar studies of the Danish general population: The Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS). Individuals were randomly selected from the national Danish Civil Registration System to reflect the adult white population aged 20 to 100+ years. The studies combined included a total of 105 949 individuals, of whom 13 693 died during the follow-up period.

The Copenhagen General Population Study

This prospective study of the Danish general population was initiated in 2003 with the first enrolment period from 2003 to 2015 and with follow-up examinations ongoing.[17,24,28,29] Data collection included a questionnaire, a physical examination, and blood sampling for biochemical and DNA analyses. We included 95 583 individuals; among these, 8321 died during follow-up.

The Copenhagen City Heart Study

This prospective study of the Danish general population was initiated in 1976–78 with follow-up examinations in 1981–83, 1991–94, and 2001–03.[17,24,28,29] Participants were recruited and examined as in the CGPS. We included 10 366 individuals who gave blood for biochemical and DNA analyses at the 1991–94 or 2001–03 examinations; among these, 5372 died during follow-up.

Endpoints

Endpoints are described in detail in the Supplementary material online.

Follow-up began at the time of blood sampling (2003–13 and onwards for CGPS and 1991–94 or 2001–03 and onwards for CCHS). For all-cause mortality, follow-up ended at occurrence of death, emigration, or on 22 March 2017 (last update of the registries), whichever came first. Median follow-up was 8.5 years (range 0–25 years) for all-cause mortality, with no individuals lost to follow-up. End of follow-up for the national Danish Causes of Death Registry (31 December 2015) lags the Danish Civil Registration System (22 March 2017) by more than 1 year: as a consequence some deaths could not be classified by cause (1839 of 13 693 deaths), and cause-specific mortality follow-up was truncated accordingly on 31 December 2015.

Biochemical and genetic analyses

Apolipoprotein E was measured using nephelometry or turbidimetry (Dade Behring, Deerfield, Illinois, USA, or Dako, Glostrup, Denmark) as previously described.[17,25] An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) and Taqman based assays were used to genotype for p.Cys130Arg (rs429358, legacy name Cys112Arg, c.388T>C) defining the ε4 allele and p.Arg176Cys (rs7412, legacy name Arg158Cys, c.526C>T) defining the ε2 allele. A total of 105 949 individuals (with 13 693 deaths) were genotyped, and for 104 153 of these individuals (with 13 312 deaths) plasma apoE measurements were obtained. Genotypes for three *APOE* promotor variants (rs449647, rs769446, and rs405509) in 74 560 individuals were determined similarly, as previously reported.[24]

Other covariates

Body mass index was measured weight in kilograms divided by measured height in metres squared. All other covariates were self-reported and described in detail in the legend to *Table 1*.

Statistical analysis

We used Stata/S.E. version 13.1 (Stata Corp., College Station, TX, USA). *P*-values were two-sided and values <0.0001 are given as powers of 10. Statistical analyses are described in detail in the Supplementary material online.

Results

During up to 25 years of follow-up, 13 693 individuals in the CGPS and CCHS died. Baseline characteristics of individuals with low

Table 1 Baseline characteristics of individuals in the general population with low and high plasma apolipoprotein E

Characteristic	Plasma apolipoprotein E		
	≤4.5 mg/dL	>4.5 mg/dL	*P*-value[a]
Individuals, *N*	67 023	37 130	—
Women (%)	52	60	5×10^{-128}
Age (years)	56.4 ± 0.1	60.0 ± 0.1	$<1 \times 10^{-300}$
Body mass index (kg/m^2)	25.7 ± 0.0	26.9 ± 0.0	$<1 \times 10^{-300}$
Hypertension (%)	56	66	6×10^{-254}
Diabetes mellitus (%)	4	4	0.01
Smoking (%)	21	21	0.65
High alcohol consumption (%)	16	19	8×10^{-28}
Physical inactivity (%)	48	54	1×10^{-69}
Postmenopausal (%)[c]	59	80	$<1 \times 10^{-300}$
Hormonal replacement therapy (%)[b]	11	10	0.01
Lipid-lowering therapy (%)	12	8	6×10^{-119}
Education<8 years (%)	11	15	5×10^{-61}
APOE genotype (%)			$<1 \times 10^{-300}$
ε22	0	2	
ε32	4	28	
ε33	58	51	
ε42	1	6	
ε43	32	13	
ε44	4	1	

Values are presented as number, mean ± standard error of the mean, or percentage and are from the day of enrolment (2003–13 for the Copenhagen General Population Study and 1991–94 or 2001–03 for the Copenhagen City Heart Study). Missing data on categorical and continuous covariates (<0.3%) were imputed from age, sex, and population. 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 hypoglycaemic agents, and/or non-fasting plasma glucose level of >11 mmol/L (>198 mg/dL). Smoking was defined as current smoking. High 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 spirit or 1 beer (33 cl)]. Physical inactivity was defined as ≤4 h 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 cut-off for low education was 8 years.

[a]*P* for differences by the Kruskal–Wallis equality-of-populations rank test or by the Pearson's χ^2 test, as appropriate.

[b]In women only.

(≤4.5 mg/dL) and high (>4.5 mg/dL) plasma apoE are shown in *Table 1*, and sex stratified results are shown in *Table 2*. The overall mean value of plasma apoE for the population was 4.3 mg/dL (Supplementary material online, *Figure S1*). We found no interaction between seven groups of plasma apoE and study (*P* for interaction = 0.26). Consequently, all further analyses were performed on the studies combined.

APOE genotype and all-cause and cause-specific mortality

The Kaplan–Meier survival curves for overall survival for the six common *APOE* genotypes showed an increased mortality risk for individuals carrying the ε44 genotype relative to ε33 individuals (Supplementary material online, *Figure S2*), and with similar patterns in women and men separately (Supplementary material online, *Figure S3*). Median survival decreased from 86.9 years in ε32 carriers to 86.4 in ε33 carriers to 86.3 in ε22 carriers to 86.1 in ε42 carriers to 85.9 in ε43 carriers through to 83.7 years in ε44 carriers. All-cause, cardiovascular, and dementia-associated mortality increased correspondingly from ε32 to ε33/ε22 to ε42/ε43 to ε44 (*Figure 1*). Hazard ratios (HRs) for all-cause mortality were 1.10 (1.06–1.14) for ε43 carriers and 1.43 (1.29–1.58) for ε44 carriers vs. ε33 carriers (*Figure 1*), with similar patterns in women and men separately (Supplementary material online, *Figure S4*). For the six *APOE* genotypes, total cholesterol, LDL cholesterol, and apolipoprotein B increased from ε22 to ε32 to ε42 to ε33 to ε43 to ε44 (*P* for trends $< 1 \times 10^{-300}$), whereas plasma apoE, high-density lipoprotein (HDL) cholesterol, and apolipoprotein AI levels decreased from ε22 to ε32 to ε42 to ε33 to ε43 to ε44 (*P* for trends $\leq 2 \times 10^{-76}$) (*Figure 2*). For triglycerides and remnant cholesterol *U*-shaped curves were observed with the highest levels in ε22 and ε44 carriers ($P = 1 \times 10^{-65}$ and 7×10^{-62}), whereas the highest lipoprotein(a) level was observed in ε33 carriers ($P = 2 \times 10^{-40}$) (*Figure 2*).

Plasma levels of apolipoprotein E and all-cause and cause-specific mortality

Multifactorially adjusted (including LDL cholesterol, HDL cholesterol, and triglycerides) restricted cubic spline Cox regression models evaluated risk of all-cause mortality by plasma apoE levels, further adjusted for *APOE* genotype (*Figure 3*, upper panel) or in ε33 carriers only (*Figure 3*, bottom panel). All-cause mortality increased with increasing levels of apoE relative to the reference of 4.5 mg/dL. There

Table 2 Baseline characteristics for women and men in the general population with low and high plasma apolipoprotein E

Characteristic	Plasma apolipoprotein E					
	Women			Men		
	≤4.5 mg/dL	>4.5 mg/dL	*P*-value[a]	≤4.5 mg/dL	>4.5 mg/dL	*P*-value[a]
Individuals, *N*	35 087	22 314	—[b]	31 936	14 816	5×10^{-128} [b]
Age (years)	55.0 ± 0.1	61.2 ± 0.1	$<1 \times 10^{-300}$	57.9 ± 0.1	58.3 ± 0.1	0.13
Body mass index (kg/m^2)	25.0 ± 0.0	26.4 ± 0.0	8×10^{-282}	26.4 ± 0.0	27.6 ± 0.0	3×10^{-240}
Hypertension (%)	48	63	5×10^{-270}	64	72	3×10^{-60}
Diabetes mellitus (%)	3	3	0.01	5	5	1×10^{-4}
Smoking (%)	20	19	0.02	21	23	0.003
High alcohol consumption (%)	13	16	2×10^{-13}	19	24	9×10^{-29}
Physical inactivity (%)	53	57	3×10^{-22}	43	50	2×10^{-36}
Postmenopausal (%)[c]	59	80	$<1 \times 10^{-300}$	—	—	—
Hormonal replacement therapy (%)[c]	11	10	0.01	—	—	—
Lipid-lowering therapy (%)	10	8	6×10^{-20}	15	8	4×10^{-106}
Education<8 years (%)	10	15	9×10^{-88}	13	14	0.001
APOE genotype (%)			$<1 \times 10^{-300}$			$<1 \times 10^{-300}$
ε22	0	2		0	2	
ε32	4	26		4	30	
ε33	57	53		60	47	
ε42	1	6		1	6	
ε43	34	13		31	14	
ε44	5	1		3	1	

Values are presented as number, mean ± standard error of the mean, or percentage and are from the day of enrolment (2003–13 for the Copenhagen General Population Study and 1991–94 or 2001–03 for the Copenhagen City Heart Study). Missing data and definitions of variables were identical to the descriptions given in the legend to *Table 1*.
[a]*P* for differences by the Kruskal–Wallis equality-of-populations rank test or by the Pearson's χ^2 test, as appropriate.
[b]One test for men and women with low and high apoE.
[c]In women only.

was no sign of non-linearity for all-cause and cause-specific mortality ($P \geq 0.30$). We found interaction between seven groups of plasma apoE and sex on all-cause and cardiovascular mortality (*P* for interaction = 0.02 and 0.006), but no interaction for seven groups of plasma apoE and sex on dementia-associated and cancer mortality (*P* for interaction = 0.10 and 0.12). The associations between high levels of apoE and all-cause, cardiovascular, and cancer mortality appeared augmented in men (Supplementary material online, *Figures S5–S7*); otherwise largely similar patterns were observed in women and men, although overall with attenuation in women compared with in men.

High plasma levels of apoE were associated with increased risk of cardiovascular and cancer mortality in the *APOE* adjusted model and in ε33 carriers only (*Figure 4*, left and right panels). Conversely, low plasma levels of apoE were associated with increased dementia-associated mortality, in the *APOE* adjusted model and in ε33 carriers only (*Figure 4*, middle panel).

For the highest vs. the fifth septile of apoE, HRs were 1.20 (95% confidence interval 1.12–1.28) for all-cause mortality, 1.28 (1.13–1.44) for cardiovascular mortality, and 1.18 (1.05–1.32) for cancer mortality (*Figure 5*). Conversely, for the lowest vs. the fifth septile the HR was 1.44 (1.01–2.05) for dementia-associated mortality (*Figure 5*). Analyses solely in ε33 carriers showed similar patterns (*Figure 5*, lower panels).

Causal estimates

Estimates from instrumental variable analyses for plasma apoE *per se* indicated causality for dementia-associated mortality but not for cardiovascular and cancer mortality (*Figure 6*); because of the observed opposite association observationally between plasma apoE and respectively cardiovascular and dementia-associated mortality, we did not perform instrumental variable analysis for all-cause mortality. Examining genetically determined plasma apoE, a 1 mg/dL increase conferred risk ratios of 0.97 (0.92–1.03) for cardiovascular mortality and 1.01 (0.95–1.06) for cancer mortality, while a 1 mg/dL decrease conferred a risk ratio of 1.70 (1.36–2.12) for dementia-associated mortality for univariable analyses (*Figure 6*). Plasma levels of apoE and HDL cholesterol decreased, while LDL cholesterol increased across apoE decreasing weighted and simple allele score groups (Supplementary material online, *Figure S8*).

Sensitivity analyses

As apoE plays a central part in lipoprotein metabolism and plasma level and isoform specific differences of apoE are associated with levels of lipids and lipoproteins in peripheral lipid metabolism (*Figure 2*), we also evaluated a multifactorially adjusted model without adjustment for LDL cholesterol, HDL cholesterol, and triglycerides.

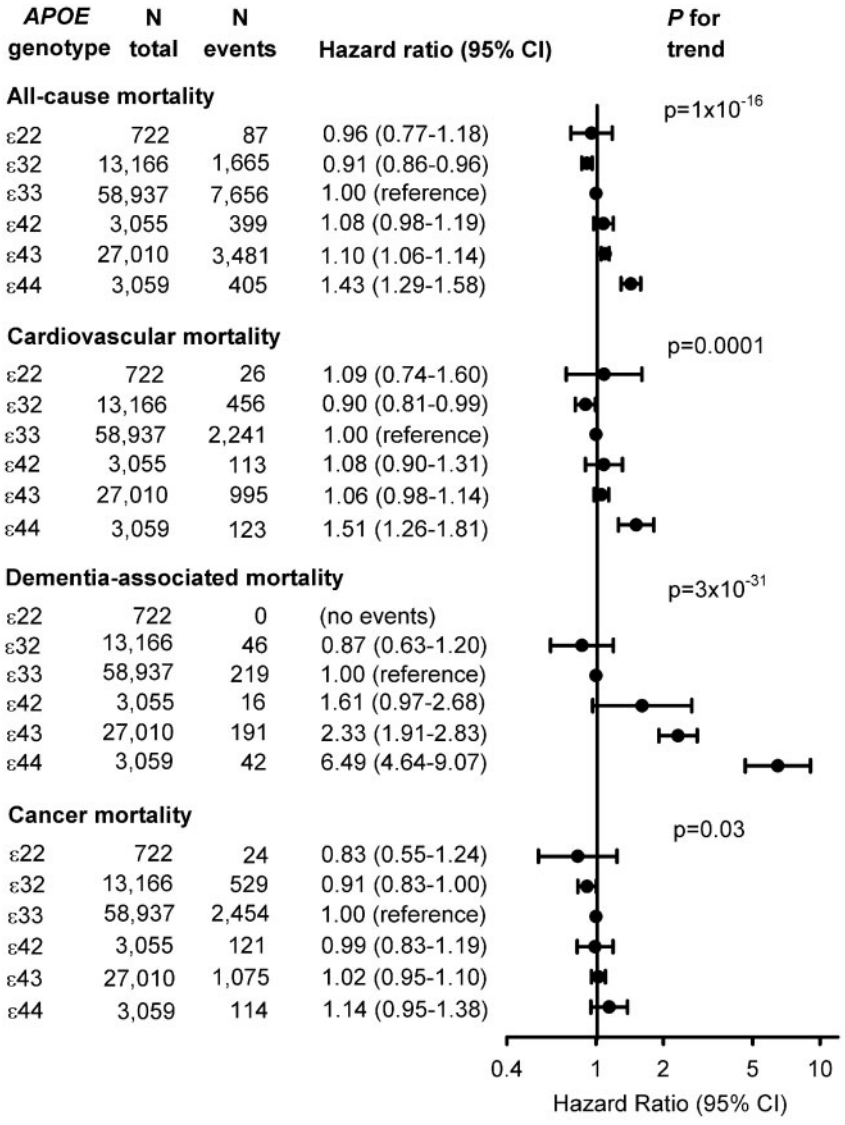


Figure 1 Risk of all-cause, cardiovascular, dementia-associated, and cancer mortality as a function of *APOE* genotype. Cox regression models were adjusted for age (time scale), body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, education, postmenopausal status, hormonal replacement therapy, LDL cholesterol, HDL cholesterol, and plasma triglycerides. *APOE*, *APOE* ε2/ε3/ε4 genotype; CI, confidence interval.

In these analyses, risk of all-cause mortality increased with both increasing and decreasing levels of apoE relative to the reference value of 4.5 mg/dL, which defined the overall lowest all-cause mortality (Supplementary material online, *Figures S9* and *S10*; compare with *Figures 3 and 5*). We found results similar to those in *Figure 5*, when excluding individuals contributing with more than one cause-specific event (Supplementary material online, *Figure S11*), or when using tertiles, quartiles, quintiles, or deciles instead of septiles (Supplementary material online, *Figure S12*). We found no interaction between seven groups of plasma apoE and use of two different autoanalyzers on all-cause mortality (*P* for interaction = 0.80) and estimates before and after the change were similar (Supplementary material online, *Figure S13*). Sensitivity analyses for risk of narrower cardiovascular mortality endpoints showed similar patterns as for all cardiovascular mortality (compare Supplementary material online, *Figure S14* with *Figure 1*). Results for cause-specific mortality adjusted for multiple hypothesis testing are shown in Supplementary material online, *Figure S15*. Instrumental variable sensitivity analyses showed no major deviations and did not change conclusions from the main findings in *Figure 6* (compare with Supplementary material online, *Table S1* and *Figure S16*).

Discussion

In this study of 105 949 individuals from the white general population, high plasma levels of apoE were associated with high all-cause mortality. For cause-specific mortality, high apoE levels were associated

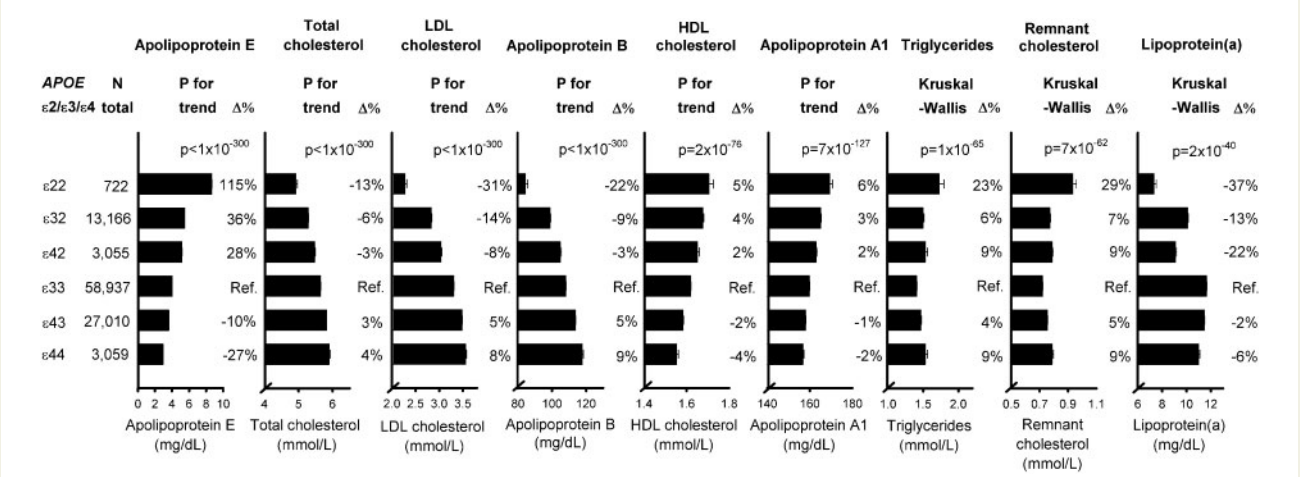


Figure 2 Plasma levels of lipids, lipoproteins, and apolipoproteins as a function of *APOE* genotype. Geometric mean ± standard errors of the mean are given for apolipoprotein E, triglycerides, and lipoprotein(a); arithmetic mean ± standard errors of the mean are given for total cholesterol, LDL cholesterol, apolipoprotein B, HDL cholesterol, apolipoprotein A1, and remnant cholesterol. *APOE* ε2/ε3/ε4 genotypes are ordered according to decreasing plasma apoE levels. Differences in plasma levels of lipids, lipoproteins, and apolipoproteins are given in percent (Δ%); ε33 serves as the reference. *P* for trend (from ε22 to ε32 to ε42 to ε33 to ε43 to ε44) or the Kruskal–Wallis analysis of variance are given. *APOE*, *APOE* ε2/ε3/ε4 genotype; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ref, reference.

with high cardiovascular mortality, however of a non-causal nature, while low apoE levels were causally associated with high dementia-associated mortality (*Take home figure*). These findings are novel.

That both extreme high and low plasma levels of apoE is associated with cause-specific mortality is biologically plausible and is most likely explained by the fact that low apoE levels are associated with risk of dementia[17,23,24] and thus reflected in dementia-associated mortality, whereas high apoE levels are associated with atherogenic lipids and lipoproteins and with risk of ischaemic heart disease[25,30,31] and cardiovascular mortality.[26,27] A previous meta-analysis comprising ∼9500 individuals found an association for fatal coronary heart disease after adjustment for LDL cholesterol, but showed only tendencies for fatal stroke and non-fatal coronary heart disease.[27] In our study, the association between high apoE levels and all-cause and cardiovascular mortality remained after adjustment for lipids, lipoproteins, and *APOE* genotype, and in analyses restricted to ε33 carriers only, most likely reflecting a general correlation between apoE levels and atherogenic triglyceride-rich lipoproteins, independent of *APOE* genotype. Low apoE levels were associated with dementia-associated mortality in *APOE* genotype adjusted analyses and in ε33 carriers only, more pronounced in men than in women. Since low apoE levels repeatedly have been associated with high risk of dementia, independent of the strong per ε4 *APOE* allele effect,[17,23,24] a reflection in dementia-associated mortality makes sense biologically.

Since it is well-established that the *APOE* ε4 allele is associated with increased mortality,[32–40] we included analyses of *APOE* genotype as a positive control and were additionally able to show that the association between apoE levels and cardiovascular mortality, dementia-associated and cancer mortality was independent of the important ε4 allele. Our findings of *APOE* genotype and cause-specific mortality are in agreement with previous findings,[33,35,36,40,41] identifying especially ε32 as associated with decreased and ε43 and ε44 as associated with increased mortality. Further, ε32 may be protective of dementia-associated mortality, in accordance with the higher apoE levels associated with lower dementia-associated mortality. However, apolipoprotein E is a rather complicated lipid risk factor because quantitative as well as qualitative genotype-determined isoform specific effects each influence levels of lipids and lipoproteins; overall, high levels of apoE are associated with high levels of triglycerides, which we have shown previously.[25] ApoE levels are however also influenced by their genotype with the well-established patterns observed in *Figure 2*. When assessed by genotype, those with the lowest apoE levels (ε44) are the ones with the highest risk for both cardiovascular and dementia-associated mortality. This is most likely explained by the fact that genotypes with low apoE in addition are associated with an atherogenic lipid profile, and thus risk of cardiovascular disease and cardiovascular mortality, whereas for dementia and dementia-associated mortality, low apoE in plasma most likely mimic low apoE levels in the brain resulting in decreased β-amyloid clearance.

Mechanisms underlying the association between high apoE levels and increased all-cause and cardiovascular mortality are likely due to the key function of apoE in receptor-mediated endocytosis by binding to triglyceride-rich lipoproteins, and serving as a ligand for the LDL receptor and LDL receptor Related Protein (LRP).[9] In mice, overexpression of apoE has been shown to lead to hypertriglyceridaemia, and *in vitro* increase in apoE expression by hepatoma cells correlates with increased very low-density lipoprotein synthesis and/or secretion irrespective of apoE isoform.[42] Hypertriglyceridaemic patients have higher plasma levels of apoE compared with normal control subjects, a finding probably explained by the mechanism that increased levels of apoE stimulate very low-density lipoprotein triglyceride production in the liver and impairs lipoprotein lipase-mediated lipolysis, thus leading to hypertriglyceridaemia.[42,43] Also, the well-known propensity of LDL receptor affinity defective ε22 carriers to

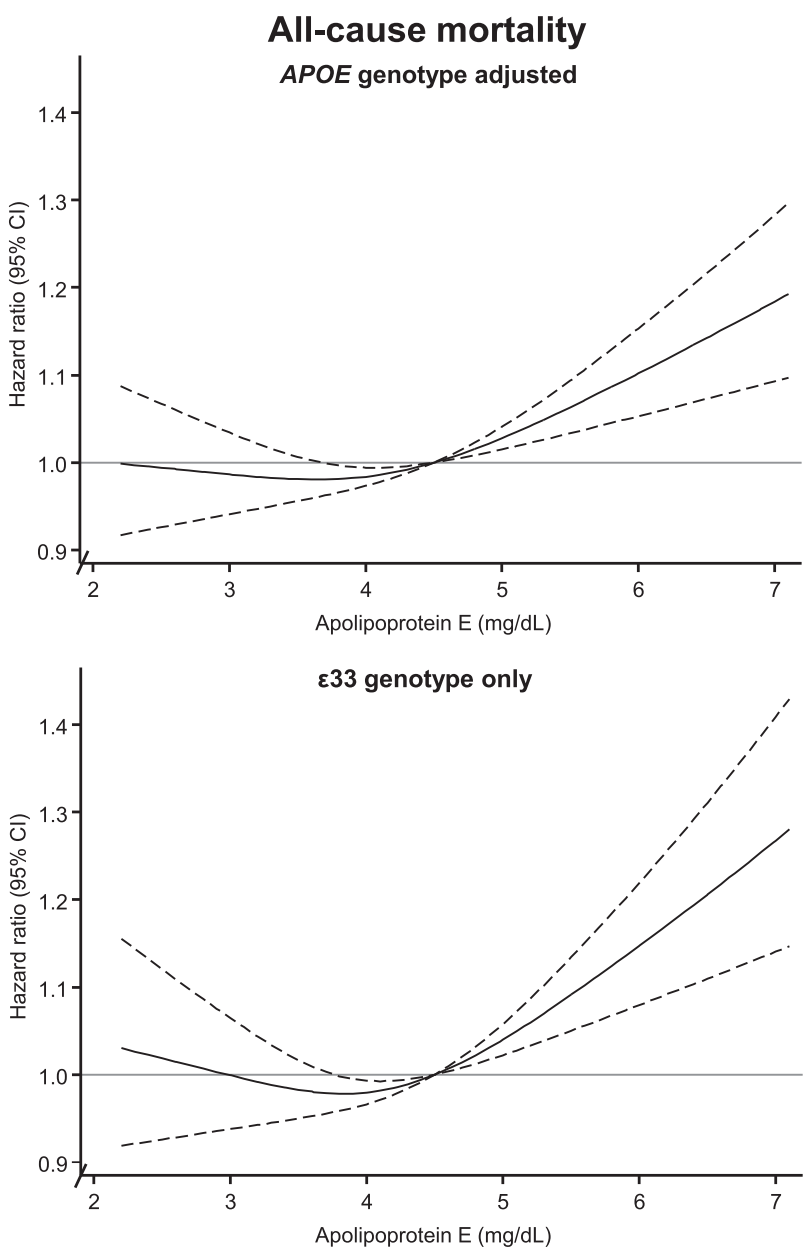


Figure 3 Multifactorially adjusted hazard ratios for all-cause mortality according to plasma levels of apolipoprotein E in individuals in the general population. Solid lines are multifactorially adjusted hazard ratios, whereas dashed lines indicate 95% confidence intervals derived from restricted cubic splines with three knots and with the reference defined as the plasma level of apolipoprotein E with lowest overall mortality (4.5 mg/dL). Graphs are truncated at the level of 2.1 mg/dL and 7.1 mg/dL, due to statistically unstable estimates at extreme low and high levels thus only including 98 350 individuals from the Copenhagen General Population Study and the Copenhagen City Heart Study in these analyses (56 264 in the ε33 genotype stratified analyses). Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, education, postmenopausal status, hormonal replacement therapy, LDL cholesterol, HDL cholesterol, and plasma triglycerides (both panels), and further adjustment for *APOE* genotype in the upper panel while analyses were restricted to ε33 carriers in bottom panels. 95% CI, 95% confidence interval; *APOE*, *APOE* ε2/ε3/ε4 genotype; ε33, *APOE* wildtype carriers.

develop type III hyperlipoproteinaemia support this understanding.[9] Only <1% of the general population are however ε22 carriers and will thus only account for a minor fraction of our overall findings of high plasma apoE and high all-cause and cardiovascular mortality. As triglyceride-rich lipoproteins are atherogenic and causally associated with cardiovascular disease,[44,45] and also associated with mortality,[46,47] our findings of high apoE levels and high cardiovascular mortality may be mediated by triglyceride-rich lipoproteins via remnant cholesterol content, although our results did not change after adjustment for triglycerides, LDL cholesterol, and HDL cholesterol. It is

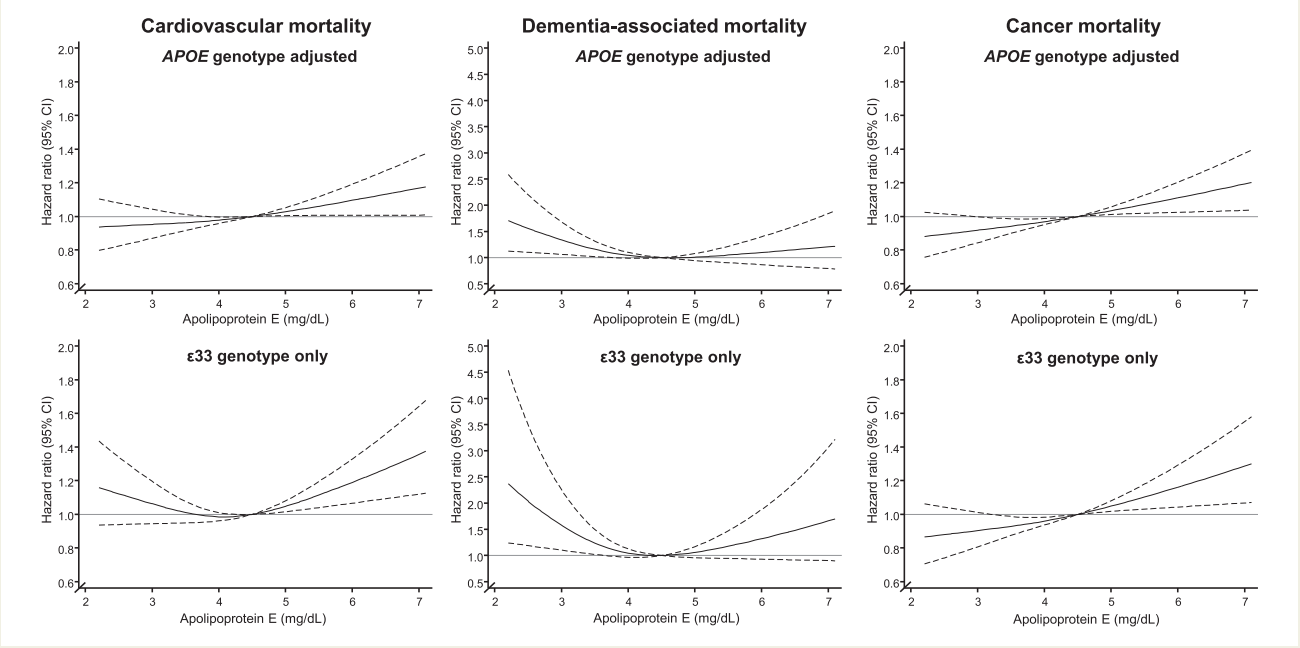


Figure 4 Multifactorially adjusted hazard ratios for cause-specific mortality according to plasma levels of apoE in individuals in the general population. Solid lines are hazard ratios, whereas dashed lines indicate 95% confidence intervals derived from restricted cubic spline regression models similar to *Figure 3* with respect to reference (4.5 mg/dL) and truncation (2.1–7.1 mg/dL). Cox regression models were multifactorially adjusted similar to *Figure 3*, with additional adjustment for *APOE* genotype in upper panels and with analyses restricted to ε33 carriers in bottom panels. 95% CI, 95% confidence interval; *APOE*, *APOE* ε2/ε3/ε4 genotype; ε33, *APOE* wildtype carriers.

puzzling that our results do not attenuate after these lipid adjustments, and fuels speculations on a less lipid dependent effect of plasma apoE on cardiovascular mortality, e.g. involvement in thrombosis and bleeding as suggested in a recent experimental study.[48] These speculations are however purely theoretical and need further experimental and clinical scrutinization. Further, mechanisms underlying the association between high apoE levels and increased cancer mortality could likely be due to apoE involvement in metastasis and angiogenesis.[49–56] The present Mendelian randomization estimates suggest however that the observational findings are not of a causal nature.

Plausible mechanisms underlying the association between low apoE levels and dementia-associated mortality are not explained by peripheral lipid metabolism, but likely by the role of apoE in β-amyloid handling and in neurodegenerative processes.[57,58] The ε4 allele has detrimental effects on production, deposition, and clearance of amyloid-β, in phosphorylation of tau, in neurotoxicity, mitochondrial dysfunction, and in blood–brain barrier permeability.[57,59,60] Also, recently the effect of low apoE levels *per se*, independent of the ε4 allele, was observed to be associated with high risk of dementia in three independent prospective European cohorts,[17,23] and further suggested to mark causal pathways in the brain.[24] These robust findings of low apoE on dementia risk most likely explain the observed associations with dementia-associated mortality.

As outlined above, apoE is involved in different mechanisms in the periphery and in the central nervous system. Human liver transplantation studies clearly showed that apoE in the peripheral circulation and in the central nervous system originates from two separate compartments.[61] Further, previous work suggest a limited blood–brain barrier permeability to circulating lipid-poor apoE,[62] suggesting that apoE in blood and brain are regulated independently.[61,63] Levels of apoE in human cerebrospinal fluid display a similar genotype-dependent pattern as in plasma,[64] and in murine interstitial fluid a similar genotype-dependent pattern as in cerebrospinal fluid was observed.[65] Hence, we suggest that plasma levels of apoE mirror levels in cerebrospinal fluid and brain, despite originating from two different compartments.

We further substantiated our findings by causal genetic estimates from instrumental variable analyses, which indicated a causal association between plasma apoE levels and dementia-associated mortality but not with cardiovascular or cancer mortality. We chose to use an instrument consisting of five variants (three promoter variants and two common exonic variants), because this combination of variants reflects the quantitative effects of apoE as well as the qualitative, isoform specific effects. Because *APOE* variants not only affect plasma levels of apoE but also all other major lipid, lipoprotein, and apolipoprotein classes—a well-established biological fact due to the fundamental role of apoE in lipid metabolism—it is difficult to decipher whether it is plasma levels of apoE *per se* that is responsible for a potential causal association, or whether the other affected lipid, lipoprotein, or apolipoprotein traits are responsible. This phenomenon is referred to as so-called vertical pleiotropy and may be overcome by applying multivariable instrumental variable analysis, which we did together with a series of other relevant MR sensitivity analyses.[66–68] These analyses showed no major deviations and did not change conclusions from the main instrumental variable findings. Mendelian

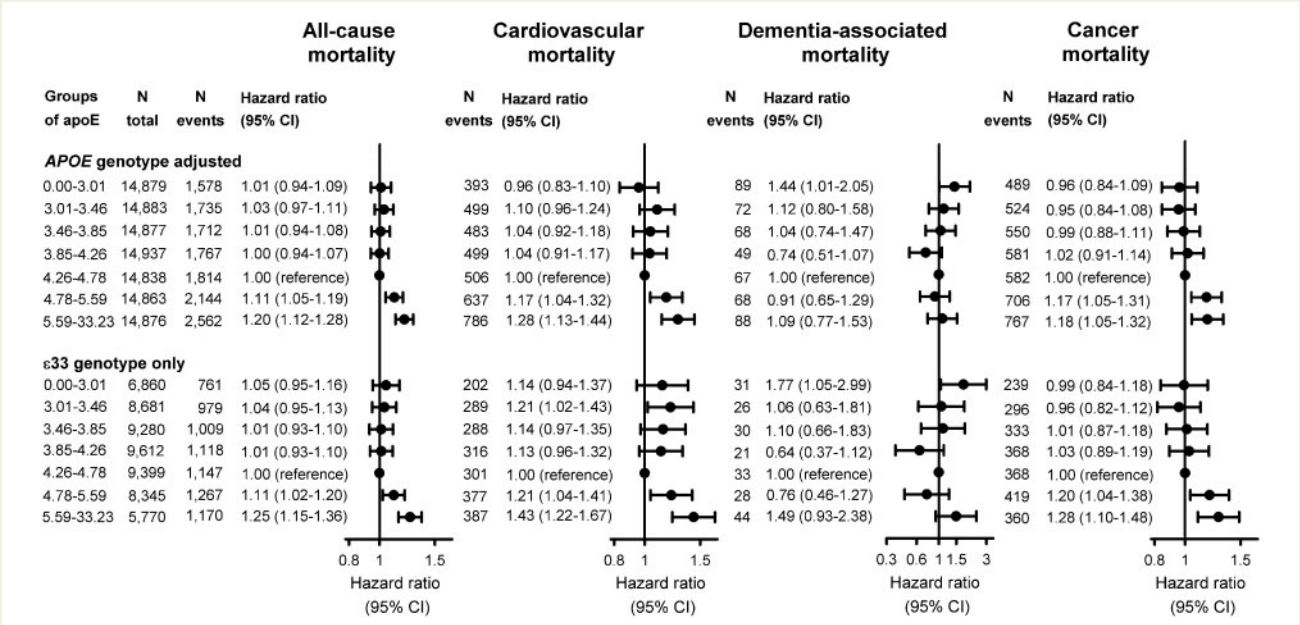


Figure 5 Multifactorially adjusted hazard ratios for all-cause and cause-specific mortality for septiles of apolipoprotein E in individuals in the general population. Cox regression models were multifactorially adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, education, postmenopausal status, hormonal replacement therapy, LDL cholesterol, HDL cholesterol, triglycerides (all panels), with further adjustment for *APOE* genotype (upper panels) and in analyses restricted to ε33 carriers (bottom panels). For cause-specific mortality, there are three parallel analyses: when Bonferroni-correction is applied ($P = 0.05/3 = 0.02$), results for the first septile of apoE for dementia-associated mortality does not adhere to this significance level ($P = 0.04$) and likewise for the second septile for cardiovascular mortality ($P = 0.03$). All other results are unaffected. 95% CI, 95% confidence interval; apoE, plasma apolipoprotein E level; *APOE*, *APOE* ε2/ε3/ε4 genotype; ε33, *APOE* wildtype carriers.

randomization analyses for peripheral effects of apoE should however still be interpreted with caution when pleiotropy is a direct cause of the biology of apoE. In contrast, it seems more straight forward to use the instrument for dementia-associated mortality, because the brain relies almost exclusively on HDL-like apoE-containing lipoprotein particles for its lipid transport and does not have apoB and apoB-containing lipoproteins (LDL and triglyceride-rich lipoproteins). Thus, the lipid and lipoprotein pleiotropic effects in the periphery will most likely not be an issue in the brain. Despite the current limitations, we obtained causal estimates that make biological sense: high plasma levels of apoE are not causally associated with cardiovascular and cancer mortality, whereas low plasma levels of apoE—mimicking brain apoE levels—are suggested to play a causal role in dementia-associated mortality.

Results from the present study may generate therapeutic and clinical considerations. Since both extreme high and low apoE were associated with cause-specific mortality most likely representing two different disease entities, the concern is that a systemic therapeutic increase of apoE—of potential benefit for dementia—could possibly generate harmful hypertriglyceridaemia peripherally due to apoE stimulated very low-density lipoprotein triglyceride production in the liver and apoE inhibited lipoprotein lipase mediated lipolysis as indicated by mechanistic studies.[42] Vice versa, a systemic therapeutic decrease of apoE—of potential benefit for ischaemic heart disease due to decreased levels of atherogenic triglyceride-rich lipoproteins—could possibly generate harmful effects in the brain, as supported by experimental evidence in mice,[69–72] and indirectly by large human population studies.[17,23,24,73] Whether plasma apoE could be a modifiable risk factor for dementia, rather than simply a risk marker, is a crucial question to solve. This would however require that therapeutically modified high plasma apoE level also should be reflected in a high brain apoE level. As brain and periphery are two separate compartments this can only be done by whole body *APOE* overexpression, which may result in potential detrimental hypertriglyceridaemic effects in plasma.[42,43] Alternatively, whole body overexpression of *ABCA1* which experimentally increases lipidation of apoE-containing lipoproteins and decreases brain amyloid burden,[74] may be a way to increase apoE levels in the brain without potential detrimental hypertriglyceridaemic effects in plasma. Also, small molecule inducers of ABCA1 and apoE that act through indirect activation of the LXR pathway may be a future pharmacological possibility for dementia without the known hepatotoxic side-effects caused by LXR ligands.[75] Naturally, these speculations are purely theoretical and can only be confirmed or rebutted in randomized clinical trials. Alternatively, specific therapeutics only targeting brain apoE or liver-derived apoE could be considered.

A major strength of the study is the large prospective general population design with no losses to follow-up: that is, every single individual could be followed to end of follow-up, death, or emigration. Further, due to the large sample size, we were able to perform analyses adjusted for *APOE* genotype as well as analyses for *APOE* ε33 carriers alone. Finally, by performing *APOE* adjusted and stratified analyses we

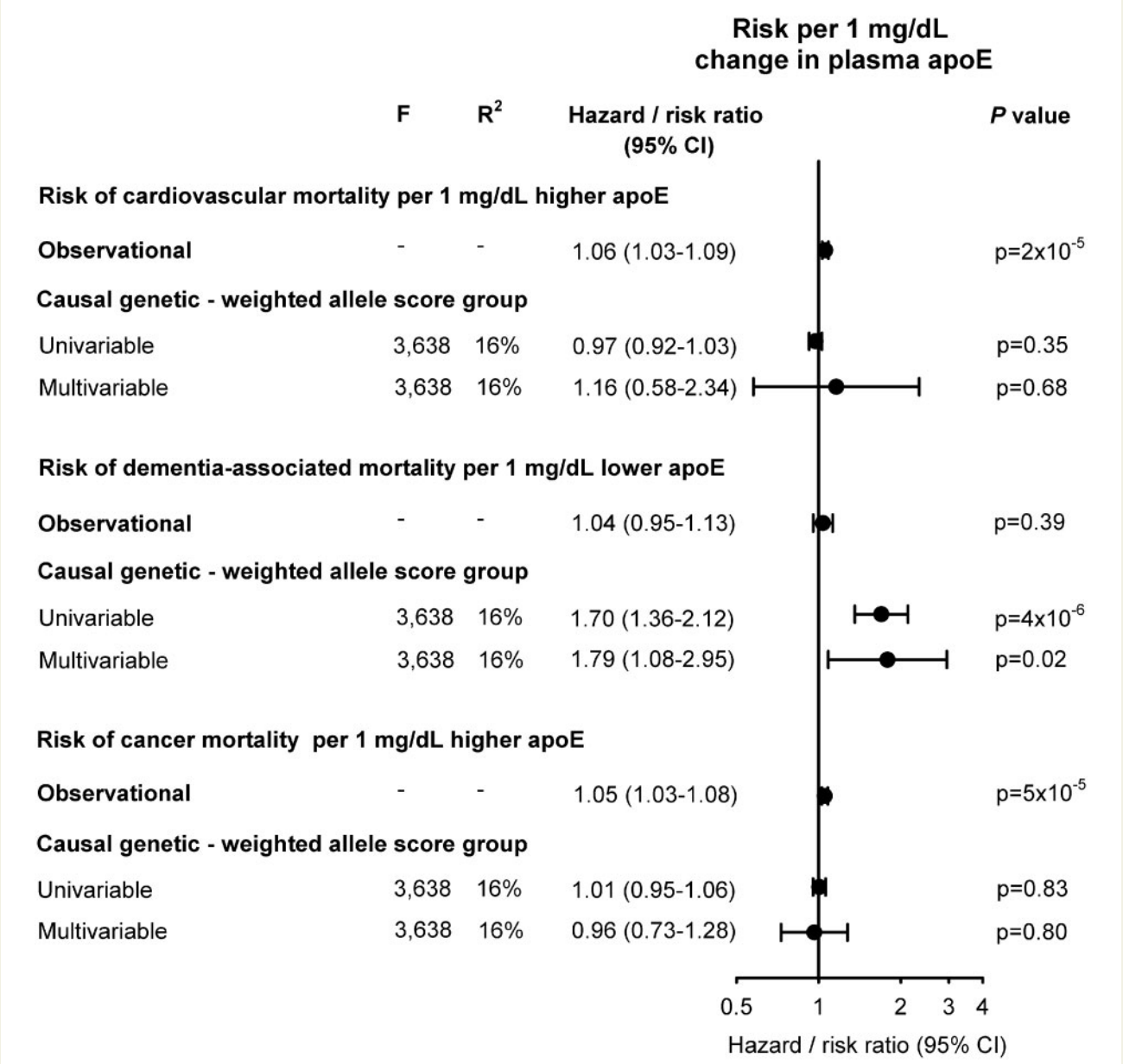
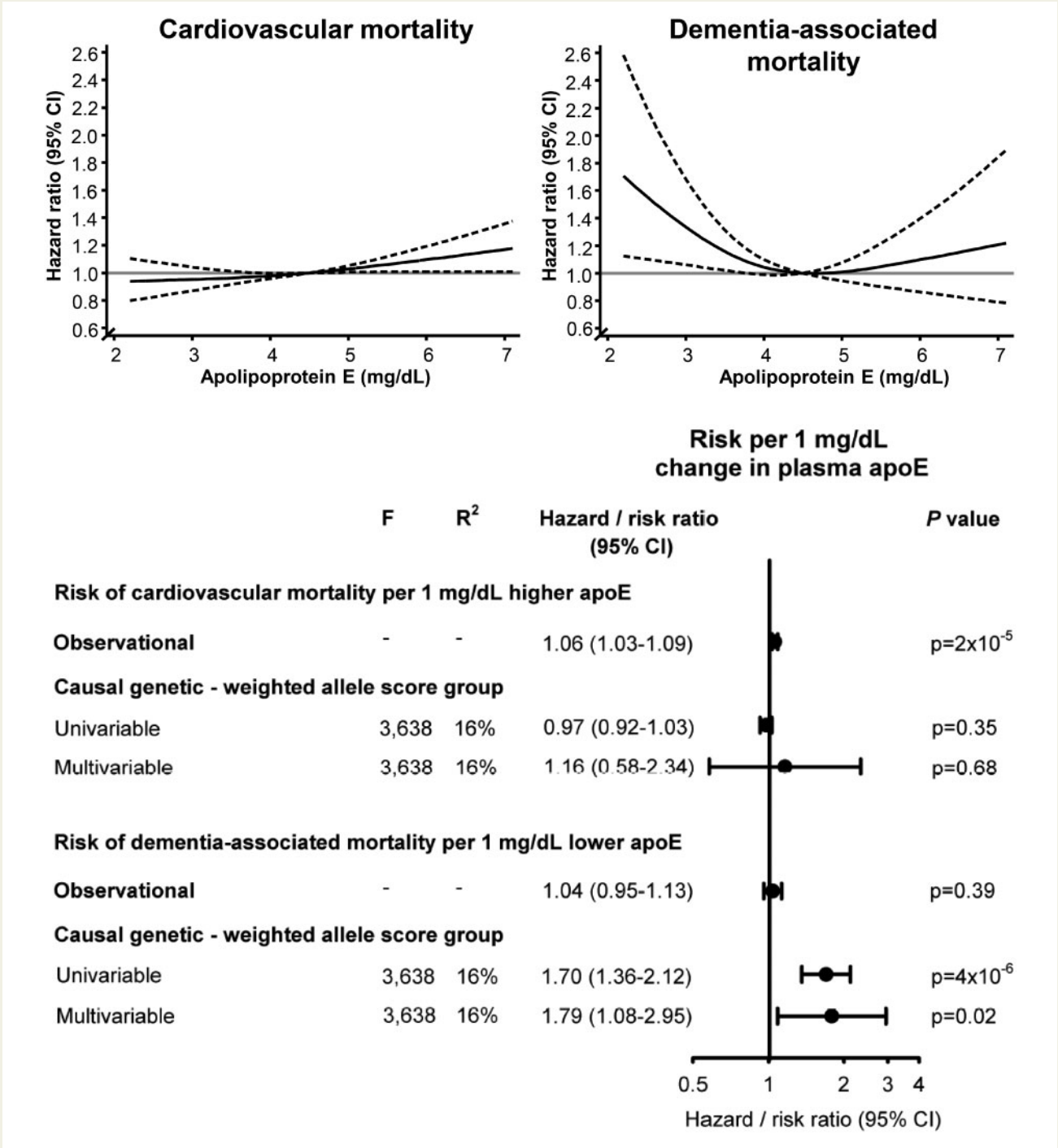


Figure 6 Risk of cause-specific mortality for a 1 mg/dL change in observational and causal, genetically determined plasma apolipoprotein E level. The hazard ratio for a 1 mg/dL change in observational plasma apoE was calculated using Cox regression with adjustment for age (time scale), body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, education, postmenopausal status, hormonal replacement therapy, LDL cholesterol, HDL cholesterol, plasma triglycerides, and *APOE* genotype, whereas the corresponding risk ratios for the genetic change in plasma apoE for the weighted allele score was derived from univariable and multivariable instrumental variable analyses, including LDL cholesterol and triglycerides in multivariable analyses. A total of 74 560 individuals are included in these analyses. apoE, apolipoprotein E; CI, confidence interval; F, strength of the genetic instrument (>10 indicates sufficient statistical strength); *P*-value, significance of hazard ratios or risk ratios; R^2, percent contribution of genetic instrument to the variation in plasma apoE.

were able to differentiate the quantitative effects of apoE levels from the genetically determined isoform specific qualitative effects.

One potential limitation concerns the availability and completeness of the diagnostic information; however, the national Danish Patient Registry includes all hospital visits, as well as out-patient visits. Dementia diagnoses in the Danish registries have high diagnostic validity.[24,76] Further, myocardial infarction and cancer as a primary diagnosis or underlying cause of death has been shown to have high diagnostic validity.[77,78] Unregistered contributing causes of death would only lead to decreased power of our estimate and bias our results towards the null hypothesis. Given the Danish registration practice it is highly unlikely that an event of death would remain



Take home figure Epidemiological and causal relationship of plasma apolipoprotein E levels and cardiovascular and dementia-associated mortality in the Copenhagen General Population Study and the Copenhagen City Heart Study. apoE, apolipoprotein E; CI, confidence interval.

unregistered. Another potential limitation is that blood samples were not drawn in the fasting state, which could add to random measurement error. However, plasma levels of apoE vary only slightly between the fasting and non-fasting state.[60,79] Finally, another limitation could be that the generalizability of our study results is limited by the fact that we studied only white individuals. Our results may therefore not necessarily apply to other ethnicities, although we are not aware of data to suggest that our results should not be applicable to all humans.

Conclusion

In conclusion, we found that high plasma levels of apoE were associated with all-cause, cardiovascular, and cancer mortality, however of

a non-causal nature, while low apoE levels were causally associated with dementia-associated mortality.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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