The role of dementia in the association between *APOE4* and all-cause mortality: pooled analyses of two population-based cohort studies

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Summary

Background The ε4 allele of the apolipoprotein E gene (*APOE4*) plays a role in neurodegeneration and in cardiovascular disease, but findings on its association with mortality are inconsistent. We aimed to examine the association between *APOE4* and mortality, and the role of dementia in this association.

Methods In this pooled analysis, data on White participants aged 45–90 years who underwent *APOE* genotyping were drawn from two population-based cohorts: the Whitehall II study (UK), which began in 1985 and is ongoing, and the Three-City study (France), initiated in 1999 and ended in 2012. In the Three-City study, vital status was ascertained through linkage to the national registry of death Institut National de la Statistique des Études économiques, and dementia was ascertained via a neuropsychological evaluation and validation of diagnoses by an independent committee of neurologists and geriatricians. In the Whitehall II study, vital status was ascertained through linkage to the UK national mortality register, and dementia cases were ascertained by linkage to three national registers. Participants with prevalent dementia at baseline and participants missing an *APOE* genotype were excluded from analyses. Cox regression proportional hazard models were used to examine the association of *APOE4* with all-cause, cardiovascular, and cancer mortality. The role of dementia in the association between *APOE4* status and mortality was examined by excluding participants who developed dementia during follow-up from the analyses. An illness-death model was then used to examine the role of incident dementia in these associations.

Findings 14 091 participants (8492 from the Three-City study and 5599 from the Whitehall II study; 6668 [47%] of participants were women and 7423 [53%] were men), with a median follow-up of 15·4 years (IQR 10·6–21·2), were included in the analyses. Of these participants, *APOE4* carriers (3264 [23%] of the cohort carried at least one ε4 allele) had a higher risk of all-cause mortality compared with non-carriers, with hazard ratios (HR) of 1·16 (95% CI 1·07–1·26) for heterozygotes and 1·59 (1·24–2·06) for homozygotes. Compared with *APOE3* homozygotes, higher cardiovascular mortality was observed in *APOE4* carriers, with a HR of 1·23 (1·01–1·50) for heterozygotes, and no association was found between *APOE4* and cancer mortality. Excluding cases of incident dementia over the follow-up resulted in attenuated associations with mortality in homozygotes but not in heterozygotes. The illness-death model indicated that the higher mortality risk in *APOE4* carriers was not solely attributable to dementia.

Interpretation We found a robust association between *APOE4* and all-cause and cardiovascular mortality but not cancer mortality. Dementia explained a significant proportion of the association with all-cause mortality for *APOE4* homozygotes, while non-dementia factors, such as cardiovascular disease mortality, are likely to play a role in shaping mortality outcomes in *APOE4* heterozygotes.

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For the French translation of the abstract see **Online** for appendix 1

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Introduction

The apolipoprotein E (*APOE*) gene, located on chromosome 19 and encoding for the APOE protein, plays a role in lipid metabolism[1] and is also implicated in pathological processes, including neurodegeneration[2,3] and cardiovascular disease.[4,5] The *APOE* gene exhibits three common alleles (ε2, ε3, and ε4), determining distinct APOE isoforms, with the ε4 allele (*APOE4*) associated with a higher risk of Alzheimer's disease[6] and atherosclerosis.[7]

The association of *APOE4* with the risk of dementia in the general population is firmly established,[6,8,9] but findings on mortality are inconsistent[10] and several studies did not find an association between *APOE4* and mortality.[11–13] A study published in 2023 suggested that *APOE4* might be associated with lower mortality risk in people without dementia.[14] It is worth noting that most studies on *APOE4* and mortality compared zero versus one or more ε4 alleles,[15,16] without distinguishing between *APOE4* heterozygotes and homozygotes. This distinction is important because approximately only 2% of the general population are ε4 homozygotes and 20% are ε4 heterozygotes.[17] Moreover, although the association of *APOE4* with the risk of dementia and atherosclerosis[5] is established, no study to date has examined cardiovascular

Research in context

Evidence before this study

We systematically searched PubMed, Web of Science, and Google Scholar for articles published from database inception to Oct 15, 2023, using the search terms ("APOE" OR "APOLIPOPROTEIN E" OR "APOE4") AND ("mortality" OR "life expectancy" OR "life span"), with no language restrictions. Previous research does not separate *APOE4* heterozygotes and homozygotes in analyses of associations with mortality. These two groups vary in size in the general population, and whether the mortality risk is similar between these two groups remains unclear. The extent to which the association between *APOE4* and mortality is explained by dementia also remains unknown.

Added value of this study

This study, based on two large, population-based cohorts, found a higher risk of mortality in both *APOE4* heterozygous and homozygous carriers, with the association being considerably stronger in homozygotes. Incidence of dementia over the follow-up period played an important role in associations with mortality in the homozygous *APOE4* carriers but only a minor role in heterozygotes. A higher risk of cardiovascular mortality was observed in heterozygous *APOE4* carriers, but not in homozygous carriers.

Implications of all the available evidence

This study shows that *APOE* genotype is an important genetic risk factor for mortality in the general population. The risk of mortality in *APOE4* homozygotes is largely caused by dementia, while cardiovascular risk factors play an important role in heterozygotes. These insights might have implications for public health strategies, emphasising the need for the development of targeted interventions to address the risk of mortality in *APOE4* carriers.

mortality. Furthermore, whether dementia explains the association between *APOE4* and all-cause mortality remains unclear.

We used data from two large, population-based studies, the Whitehall II study and the Three-City study, to address these gaps in the literature. These studies provide a wide pooled age distribution at baseline (45–69 years in the Whitehall II study and 65–90 years in the Three-City study) and have long follow-up periods, thereby providing a robust foundation for our investigation. We aimed to examine the association between *APOE4* alleles and mortality and examine (1) whether the association varies by cause of death, including cardiovascular mortality; and (2) the role of incident dementia in this association.

Methods

Study design and participants

In this pooled analysis, White participants were drawn from two large European cohort studies of community-dwelling people: the Whitehall II study (UK) and the Three-City study (France); non-White participants were not included in the analyses due to the small number of these participants, which precluded separate analyses in this group. The Whitehall II study is a longitudinal cohort study initiated in 1985, aimed at investigating the social determinants of health among people who were civil servants working in the London offices of 20 Whitehall departments at baseline.[18] A key feature of this study is the inclusion of middle-aged participants, who were aged 35–55 years at recruitment in 1985–88. This cohort study is ongoing, with a current follow-up period of approximately 40 years. Follow-up includes blood sample collections and regular psychometric testing, and linkage with electronic health records.[19] The Three-City study is a French population-based cohort study that began in 1999 and was done in three French cities, (Bordeaux, Dijon, and Montpellier), and focused on the risk factors for dementia and cardiovascular disease among individuals aged 65 years and older.[20] The follow-up included regular neuropsychological assessments, active detection of incident dementia cases, and linkage with French health registries for tracking vital status.[21] The Three-City Study ended in 2012.

The present study used data taken from the respective waves of each study in which the *APOE* genotype was assayed (baseline in Three-City study and the third wave in the Whitehall II study [1991–93]). At the time of this assay the participants from the Whitehall II study were aged 45–69 years and participants from the Three-City study were aged 65–90 years, and therefore analyses in the present study were done on participants aged 45–90 years at baseline.

In the present study, participants with prevalent cases of dementia at baseline and participants missing an *APOE* genotype were excluded from analyses. Non-White participants were excluded from the Whitehall II study. Due to French legislation, the Three-City study does not provide information on participants' ethnic origin; however, previous genome-wide association research indicated that more than 99% of participants in the Three-City study were of White descent.[22,23]

For the Whitehall II study, written participant consent and research ethics approval were renewed at each contact; the most recent approval was granted by the NHS London Harrow Research Ethics Committee (reference number 85/0938). The Three-City study protocol was approved by the Ethical Committee of the University Hospital of Bicêtre (France) and written informed consent was obtained from each participant.

Procedures

Both cohort studies provided participants' vital status until March 1, 2019, the length of follow-up time, and date of death. Vital status was ascertained through

linkage to the UK national mortality register (National Health Services Central Registry) for the Whitehall II study and the national death registry Institut National de la Statistique des Études économiques for the Three-City study. In one of the three centres (Dijon) of the Three-City study, the monitoring of vital status was restricted up until Dec 31, 2012. We examined all-cause mortality and cause-specific mortality for two main causes of death: cancer and cardiovascular disease. The primary cause of mortality was identified using International Classification of Diseases, Tenth Revision (ICD-10).[24] For cancer mortality the codes C00 and C97 were used, and for cardiovascular mortality codes I00 to I78 (including coronary heart disease, stroke, peripheral vascular disease, and other cardiovascular disease) and code R960 (sudden death) were used.

In the Three-City study, incident dementia was ascertained at baseline and at each follow-up wave using a three-step procedure. The first step was a cognitive evaluation by trained neuropsychologists using a series of psychometric tests. Participants who were suspected of dementia, based on their neuropsychological performance or decline relative to a previous examination were then examined for further medical assessments. Finally, each case was discussed by a validation committee composed of neurologists and geriatricians to classify cause.[21] In the Whitehall II study, dementia cases were ascertained by linkage to three national registers (the national hospital episode statistics database, the Mental Health Services Data Set, and the mortality register) up to March 1, 2019. All-cause dementia was diagnosed based on ICD-10 codes F00-F03, F05.1, G30, and G31.

In Whitehall II and the Three-City study, *APOE* genotyping was performed using Two TaqMan assays (Rs429358 and Rs7412, Assay-On-Demand, Applied Biosystems, Waltham, MA, USA) on a 7900HT analyser (Applied Biosystems, Waltham, MA, USA), using the Sequence Detection Software version 2.0 (Applied Biosystems, Waltham, MA, USA)[25,26]—a widely used method that is recognised for its reliability.[27]

Covariates included age at *APOE* genotyping, Mini-Mental Status Examination (MMSE) score, education, and cardiovascular risk factors. The MMSE score was taken from the date closest to *APOE* genotyping. Education was classified into three categories: (1) no education to primary school, (2) secondary school to high school, and (3) baccalaureate or university degree. Cardiovascular risk factors included hypertension (systolic and diastolic blood pressure ≥140/90 mmHg or use of antihypertensive drugs), smoking (never or former smoker; or current smoker), dyslipidaemia (medical record of dyslipidaemia or use of lipid-lowering drugs), plasma level of LDL-cholesterol, diabetes (medical record of diabetes or use of antidiabetic drugs), and BMI (calculated as weight divided by height squared [kg/m²]).

Statistical analysis

Baseline characteristics of participants included in the analyses were described overall, and as a function of *APOE* genotype (in four groups: ε2/ε2 and ε2/ε3, ε3/ε3, ε2/ε4 and ε3/ε4, and ε4/ε4) and as a function of vital status at the end of follow-up. Comparison of the baseline characteristics between these groups was done using multivariable analyses adjusted for age, sex (sex data were self-reported by participants), education, and cohort study (Whitehall II or Three-City), with general linear regression models used for continuous variables and logistic regression for categorical variables. Baseline characteristics for each cohort separately are shown in appendix 2 (pp 5–6).

See Online for appendix 2

We first examined whether there were differences between the cohort studies (Whitehall II *vs* Three-City) in the association between *APOE* genotype and mortality, using an interaction term in Cox regression analysis that included age, sex, education, *APOE* genotype, a cohort term, and an interaction term between *APOE* genotype and cohort. Because the interaction term to test for cohort differences in associations was not significant (p=0·42), we chose to pool data from both studies for increased statistical power in the main analyses and adjusted for cohort effect. Cohort-specific survival analyses are shown in appendix 2 (p 7). Participants were followed from the date of *APOE* genotyping until their date of death or end of follow-up (March 1, 2019), whichever came first. Age was used as the timescale in all the analyses.

Survival probabilities according to *APOE4* status (zero, one, and two ε4 alleles) were first examined using Kaplan–Meier estimators for all-cause, cancer-specific, and cardiovascular mortality and were compared using the log-rank test. A Cox proportional hazard model was then used to examine the association between *APOE* genotype and mortality over the follow-up period. Proportional hazards assumption was verified using Schoenfeld residuals test (all p>0·20). The reference group in these analyses was homozygous ε3 participants, and the risk was estimated in the ε4 heterozygote and homozygote groups. The analysis was first adjusted for age (as timescale), sex, education, and the cohort study (model 1) and then further adjusted for smoking, diabetes, LDL-cholesterol, hypertension, BMI, and MMSE scores (model 2). In an additional analysis, we explored the relationship between mortality and APOE4 status (carriers *vs* no carriers) and the relationship between mortality and major cardiovascular risk factors (high blood pressure, diabetes, and smoking) using a Cox model adjusted for age on a timescale, sex, education level, and cohort. This analysis was done to compare the effect sizes of these various factors on overall mortality. The role of dementia in the association between *APOE4* status and mortality was examined by excluding participants who developed dementia during follow-up from the analyses (model 3). For analyses of cause-

	Overall (N=14 091)	*APOE* genotype				p value*
		ε2/ε2 and ε2/ε3 (n=1786)	ε3/ε3 (n=9041)	ε2/ε4 and ε3/ε4 (n=3048)	ε4/ε4 (n=216)	
Age, years	66·7 (10·7)	66·9 (10·9)	67·3 (10·6)	65·1 (10·5)	62·1 (10·3)	<0·0001
Sex	..	..	..	..	..	0·072
Women	6668 (47·3%)	817 (45·7%)	4447 (49·2%)	1318 (43·2%)	86 (39·8%)	..
Men	7423 (52·7%)	969 (54·3%)	4594 (50·8%)	1730 (56·8%)	130 (60·2%)	..
Education	..	..	..	..	..	0·025
No education to primary school	3421 (24·3%)	393 (22·0%)	2302 (25·5%)	687 (22·5%)	39 (18·1%)	..
Secondary school to high school	6977 (49·5%)	900 (50·4%)	4449 (49·2%)	1525 (50·0%)	103 (47·7%)	..
Baccalaureate or university degree	3693 (26·2%)	493 (27·6%)	2290 (25·3%)	836 (27·4%)	74 (34·3%)	..
MMSE score	27·9 (1·85)	27·9 (1·77)	27·86 (1·87)	28·02 (1·81)	28·14 (1·94)	0·50
Current smokers	1027 (7·3%)	152 (8·5%)	642 (7·1%)	218 (7·2%)	15 (6·9%)	0·13
Hypertension	9126 (64·8%)	1157 (64·8%)	5979 (66·1%)	1876 (61·6%)	114 (52·8%)	0·26
Total cholesterol, mmol/L	5·89 (1·03)	5·55 (0·99)	5·90 (1·02)	6·02 (1·06)	6·25 (1·01)	<0·0001
LDL cholesterol, mmol/L	3·71 (0·90)	3·28 (0·80)	3·73 (0·87)	3·85 (0·93)	4·09 (0·86)	<0·0001
HDL cholesterol, mmol/L	1·56 (0·41)	1·60 (0·43)	1·57 (0·40)	1·52 (0·41)	1·51 (0·43)	<0·0001
BMI, kg/m²	25·8 (3·9)	25·9 (4·0)	25·8 (4·0)	25·6 (3·9)	25·4 (3·9)	0·0017
Diabetes	988 (7·0%)	136 (7·6%)	636 (7·0%)	211 (6·9%)	5 (2·3%)	0·11
Incident dementia	1377 (9·8%)	133 (7·4%)	823 (9·1%)	377 (12·4%)	44 (20·4%)	<0·0001

Data are Mean (SD) or n (%). MMSE=Mini-Mental Status Examination. *Adjusted on age, sex, level of education and cohort, apart from for sex, age, or education categories.

Table 1: **Baseline characteristics of the study population**

	Death over the follow-up		p value*
	No (n=10 184)	Yes (n=3907)	
Age, years	64·3 (10·2)	73·1 (9·1)	<0·0001
Sex	..	..	0·0020
Women	4901 (48·1%)	1767 (45·2%)	..
Men	5283 (51·9%)	2140 (54·8%)	..
Education	..	..	<0·0001
No education to primary school	2282 (22·4%)	1139 (29·2%)	..
Secondary school to high school	5182 (50·9%)	1795 (45·9%)	..
Baccalaureate or university degree	2720 (26·7%)	973 (24·9%)	..
MMSE score	28·0 (1·8)	27·3 (2·0)	<0·0001
APOE4 carriers	2378 (23·4%)	886 (22·7%)	0·40
Current smokers	681 (6·7%)	346 (8·9%)	<0·0001
Hypertension	6115 (60·1%)	3011 (77·1%)	<0·0001
Total cholesterol, mmol/L	5·92 (1·02)	5·79 (1·05)	<0·0001
LDL cholesterol, mmol/L	3·74 (0·89)	3·62 (0·89)	<0·0001
HDL cholesterol, mmol/L	1·57 (0·41)	1·54 (0·41)	<0·0001
BMI, kg/m²	25·7 (3·9)	26·0 (4·3)	<0·0001
Diabetes	528 (5·2%)	460 (11·8%)	<0·0001

Data are Mean (SD) or n (%). MMSE=Mini-Mental Status Examination. *Student's *t* test for continuous variables and χ^2 test for categorical variables.

Table 2: **Baseline characteristics of the study population according to vital status at the end of follow-up**

specific mortality, participants who died of causes other than cancer or cardiovascular disease during follow-up were censored at their date of death to account for competing risk.[28] In a post-hoc supplementary analyses, we used an alternative approach to assess competing risk using cumulative incidence functions and Fine and Gray sub-distribution hazard models,[29] an approach recommended primarily for clinical prediction models.[28]

We then examined the role of incident dementia over the follow-up period in the association between *APOE4* status and all-cause mortality using an illness-death multistate model with Weibull distribution.[30] These models are an extension of competing risks survival analysis and allow for simultaneous estimation of the risk associated with *APOE4* in (1) the incidence of dementia, (2) the risk of mortality in participants with dementia, and (3) the risk of mortality in participants without dementia. This method has the advantage of taking interval censoring into account as this was the case in the Three-City study, in which dementia was assessed only at clinical examinations. In complementary analysis, we used counterfactual mediation analysis to quantify the extent to which dementia over the follow-up period explained the associations of *APOE4* with mortality (appendix 2 p 2). The counterfactual framework allows for the quantification of the indirect effect (ie, the association of *APOE4* with mortality that is mediated by dementia), the direct effect (ie, the association of *APOE4* with mortality that is not mediated by dementia), the total effect (the direct and indirect effects), and the percentage mediation (reflecting the proportion of the total effect mediated by the indirect effect).[31] Effects were expressed on the hazard ratio scale.

Two-tailed p values of less than 0·05 were considered statistically significant. Statistical analyses were performed using Stata 15 (StataCorp LP, College Station, TX).

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results
14 091 participants from the two cohort studies (8492 from the Three-City study and 5599 from the Whitehall II study) were included in the analysis, with a median follow-up of 15·4 years (IQR 10·6–21·2), corresponding to 214 299 person-years of follow-up; a flow chart showing the selection of participants for inclusion in our analyses can be found in appendix 2 (p 1). The mean age of participants at baseline in the pooled data was 66·7 years (SD 10·7); 6668 (47·3%) of participants were women and 7423 (52·7%) were men; 9041 (64·2%) participants were *APOE* ε3/ε3 homozygous, 3264 (23·2%) carried at least one ε4 allele (3048 [21·6%] heterozygotes and 216 [1·5%] homozygotes), and 1786 (12·7%) were either ε2/ε2 (n=55) or ε2/ε3 (n=1731; table 1). Compared with non-*APOE4* carriers, participants with at least one *APOE4* allele were younger, had lower BMI, higher levels of LDL cholesterol, and lower levels of HDL cholesterol. Characteristics of the Whitehall II and Three-City study populations are in appendix 2 (pp 5–6). During follow-up, 3907 (27·7%) participants died (table 2). Participants who died were older at baseline (73·1 years [SD 9·1] *vs* 64·3 years [10·2], p<0·0001), less likely to be women (45·2% *vs* 48·1%, p=0·0020), and had lower MMSE scores (27·3 [2·0] *vs* 28·0 [1·8], p<0·0001). Participants who died were also more likely to have a history of hypertension, diabetes, smoking, a higher BMI, and lower levels of plasma HDL and LDL cholesterol (all p<0·0001).

Non-*APOE4* carriers had lower mortality risk over the follow-up compared with *APOE4* carriers (log-rank test p<0·0001; figure 1A). The curves started to diverge from the age of 72 years, with an acceleration beginning at 76 years for *APOE4* homozygotes. Compared with non-carriers, *APOE4* carriers had a higher risk of cardiovascular disease mortality (log-rank p=0·034; figure 1B), and there were no differences between non-carriers and *APOE4* carriers for cancer mortality (log-rank p=0·91; figure 1C). The Kaplan–Meier curves for the association between *APOE4* status and risk of all-cause mortality, stratified by cohort, are shown in appendix 2 (p 3). The survival curves were similar across both study populations. The log-rank test was statistically significant in the Three-City cohort (p<0·0001), but not significant in the Whitehall II study (p=0·066).

With homozygous *APOE3* participants as the reference, when adjusted for age, sex, level of education, and cohort study (model 1), both heterozygous *APOE4* (HR 1·16 [95% CI 1·07–1·26], p=0·0002) and homozygous *APOE4* (1·59 [1·24–2·06], p=0·0003) had a higher risk of all-cause mortality (table 3). Further adjustments for the MMSE score and cardiovascular risk factors (model 2) had little impact on these estimates. The exclusion of participants who developed dementia over the follow-up (1377 participants; model 3) did not modify the association with all-cause mortality

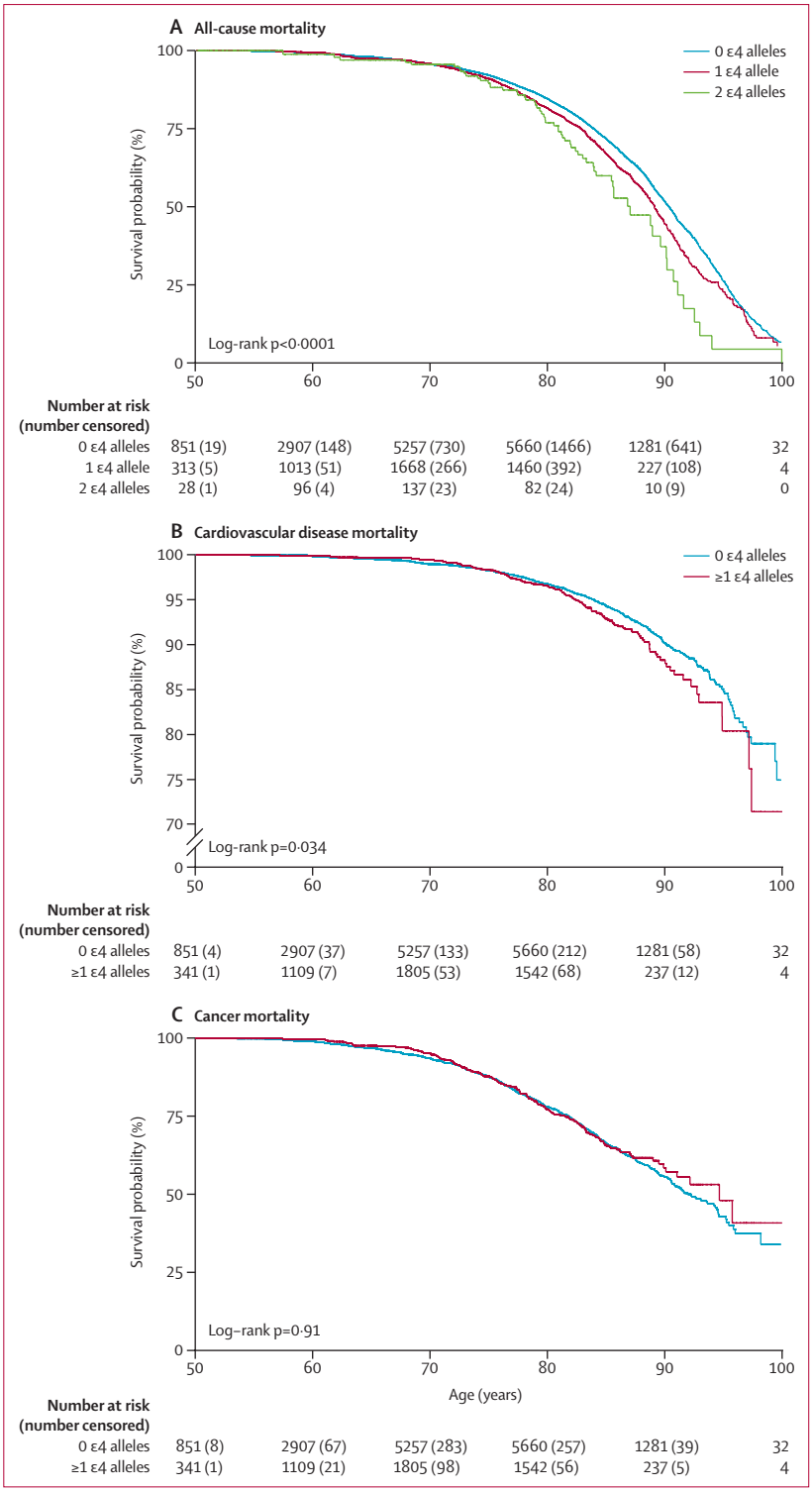

Figure 1: **Kaplan–Meier survival curves according to *APOE4* status in the overall population for all-cause mortality (A), cardiovascular disease mortality (B), and cancer mortality (C)**

	Model 1*			Model 2†		Model 3‡		
	Number of deaths; number at risk	HR (95% CI)	p value	HR (95% CI)	p value	Number of deaths; number at risk	HR (95% CI)	p value
All-cause mortality								
ε2 carriers	505; 1786	0·98 (0·89–1·08)	0·72	0·95 (0·87–1·05)	0·33	440; 1653	0·98 (0·89–1·09)	0·75
Homozygous ε3 carriers	2516; 9041	1	Ref	1	Ref	2099; 8218	1	Ref
Heterozygous ε4 carriers	825; 3048	1·16 (1·07–1·26)	0·0002	1·18 (1·09–1·27)	<0·0001	627; 2671	1·15 (1·05–1·25)	0·0028
Homozygous ε4 carriers	61; 216	1·59 (1·24–2·06)	0·0003	1·63 (1·26–2·10)	0·0002	32; 172	1·17 (0·83–1·66)	0·38
Cardiovascular disease mortality								
ε2 carriers	81; 1631	1·09 (0·85–1·38)	0·50	1·05 (0·82–1·34)	0·70	74; 1528	1·07 (0·83–1·38)	0·61
Homozygous ε3 carriers	364; 8226	1	Ref	1	Ref	324; 7598	1	Ref
Heterozygous ε4 carriers	135; 2838	1·23 (1·01–1·50)	0·041	1·24 (1·02–1·52)	0·031	116; 2533	1·28 (1·03–1·58)	0·026
Homozygous ε4 carriers	6; 198	1·01 (0·45–2·27)	0·98	1·02 (0·45–2·29)	0·96	3; 163	0·61 (0·19–1·90)	0·39
Cancer mortality								
ε2 carriers	113; 1631	1·03 (0·84–1·27)	0·74	1·02 (0·83–1·25)	0·84	109; 1528	1·02 (0·83–1·26)	0·83
Homozygous ε3 carriers	541; 8226	1	Ref	1	Ref	518; 7598	1	Ref
Heterozygous ε4 carriers	169; 2838	0·96 (0·81–1·15)	0·68	0·97 (0·82–1·16)	0·77	160; 2533	1·02 (0·85–1·22)	0·86
Homozygous ε4 carriers	12; 198	1·14 (0·64–2·03)	0·65	1·17 (0·66–2·08)	0·59	9; 163	1·03 (0·53–1·99)	0·94

Risk of mortality with homozygous ε3 carriers as reference. HR=hazard ratio. *Model 1=age (as timescale), sex, education, and cohort. †Model 2=model 1 plus Mini-Mental Status Examination score, smoking status, diabetes, LDL cholesterol, hypertension, and BMI. ‡Model 3=model 2 with exclusion of participants with incident dementia (n=1377).

Table 3: **Association between *APOE* genotype and the risk of mortality over the follow-up period**

for *APOE4* heterozygous, but considerably attenuated the association among ε4 homozygotes, such that it was no longer statistically significant. 29 (48%) of 61 *APOE4* homozygote participants who died had developed dementia over the follow-up period, compared with only 44 (20%) of 216 *APOE4* homozygote participants who did not die during follow-up. Compared with *APOE3* homozygotes, the risk of all-cause mortality in carriers of the *APOE2* allele was not significantly different. A higher risk of cardiovascular mortality was found among *APOE4* heterozygotes (1·23 [1·01–1·50], p=0·041) but not *APOE4* homozygotes (1·01 [0·45–2·27], p=0·98). Cancer mortality was not associated with *APOE4* status.

Results of the Cox regression done separately in each cohort study were broadly similar to those of the pooled analysis and are shown in appendix 2 (p 7). In supplementary analyses, the Fine and Gray subdistribution hazard models yielded results consistent with those in the main analyses. The cumulative incidence functions and subdistribution hazard ratios are shown in appendix 2 (pp 4, 9). Carrying at least one ε4 allele was associated with an overall 20% higher risk of all-cause mortality (HR 1·20 [95% CI 1·11–1·29]; appendix 2 p 8). The increased risk was similar to the increased mortality risk associated with having hypertension at baseline (1·21 [1·12–1·31]) and was slightly less than the risk associated with having diabetes (1·65 [1·50–1·82]) or smoking (1·68 [1·51–1·88]). There was no interaction with sex for the association between *APOE4* and all-cause mortality (p for interaction=0·33; 1·16 [1·05–1·29] for men and 1·23 [1·09–1·38] for women).

Based on the illness-death model for natural transitions across incident dementia and mortality over the follow-up period, the rate of incident dementia in the overall study population was 7·51 per 1000 person-years (figure 2A) and participants who developed dementia had a higher mortality rate (117·9 per 1000 person-years) compared with those who did not develop dementia (18·3 per 1000 person-years). *APOE4* carriers (heterozygous and homozygous) compared with non-carriers had a higher risk of all-cause mortality, HR 1·20 (95% CI 1·11–1·29). This association was not fully explained by incident dementia (figure 2B) as demonstrated by the higher hazard of all-cause mortality in those free of dementia over the follow-up (1·15 [1·05–1·25], p=0·0016). As expected, compared with non-*APOE4* carriers, *APOE4* carriers had a higher risk of dementia over the follow-up (1·89 [1·69–2·13], p<0·0001), but among people who developed dementia over the follow-up, APOE4 was not associated with mortality compared with non-APOE4 carriers (1·09 [0·92–1·29], p=0·30). In complementary analysis, we examined the proportion of the association between *APOE4* and all-cause mortality explained by incident dementia over the follow-up using mediation analysis (appendix 2 p 10). The direct effect of *APOE4* on mortality was 1·16 (1·08–1·24) and the indirect effect was 1·02 (1·01–1·03), with incident dementia mediating 11% of the association between *APOE4* and mortality.

Discussion

We examined the association between *APOE* genotype and all-cause and cause-specific mortality using data from 14 091 community-dwelling individuals, and we

present four key findings. First, the risk of all-cause mortality was higher in *APOE4* carriers compared to non-carriers. Compared with *APOE3* homozygotes, the association with mortality was stronger in *APOE4* homozygous individuals (59% higher risk of mortality) but was also observed in *APOE4* heterozygous individuals (16% higher risk of mortality). Second, we found that *APOE4* heterozygote, but not homozygote, carriers had a higher risk of death due to cardiovascular disease but not cancer. Third, illness-death models allowed us to show that a part of the association between *APOE4* and all-cause mortality was independent of incident dementia over the follow-up period. Fourth, excluding participants with incident dementia in analyses of *APOE4* status and all-cause mortality in multivariable Cox analyses had a considerable effect on associations in *APOE4* homozygotes but only marginally affected associations in *APOE4* heterozygotes. This finding suggests that dementia explains a significant proportion of the association with all-cause mortality for *APOE4* homozygotes, while non-dementia factors, such as cardiovascular disease mortality, are likely to play a role in shaping mortality outcomes in *APOE4* heterozygotes.

Although the relationship between *APOE4* status and dementia appears to be firmly established, the relationship between *APOE4* and all-cause or cause-specific mortality in the general population seems less clear, with several studies showing null findings.[11–13] Most previous studies that found an association between *APOE4* and mortality have not considered *APOE4* heterozygous and homozygous status separately.[10,16,32] Our findings support the association between *APOE4* and all-cause mortality and add to previous studies by showing a gradient between the number of ε4 alleles and the risk of all-cause mortality. The effect of the ε4 allele on mortality risk is subtler than its effect on dementia risk and the effect appears later in life, necessitating larger studies with longer follow-up periods.

Despite the known protective effect of *APOE2* on the risk of dementia, we did not find such an effect on the risk of all-cause mortality, using *APOE3* homozygotes as the reference. Previous studies that have examined this association are discordant, with some studies reporting null findings[33,34] and others showing a lower mortality risk in *APOE2* carriers.[32] A large study done in centenarians showed a small protective effect of the *APOE2* allele on mortality, but these results are not necessarily generalisable because less than 1% of the population live to 100 years.[35]

The primary strengths of our study are the large size of the analytic sample, drawn from two independent, prospective cohorts from two different countries with a large age range at baseline and a long follow-up period. The timeframe and robust sample size allowed us to investigate the effect of the *APOE4* allele on all-cause mortality, as well as on cardiovascular and cancer mortality.

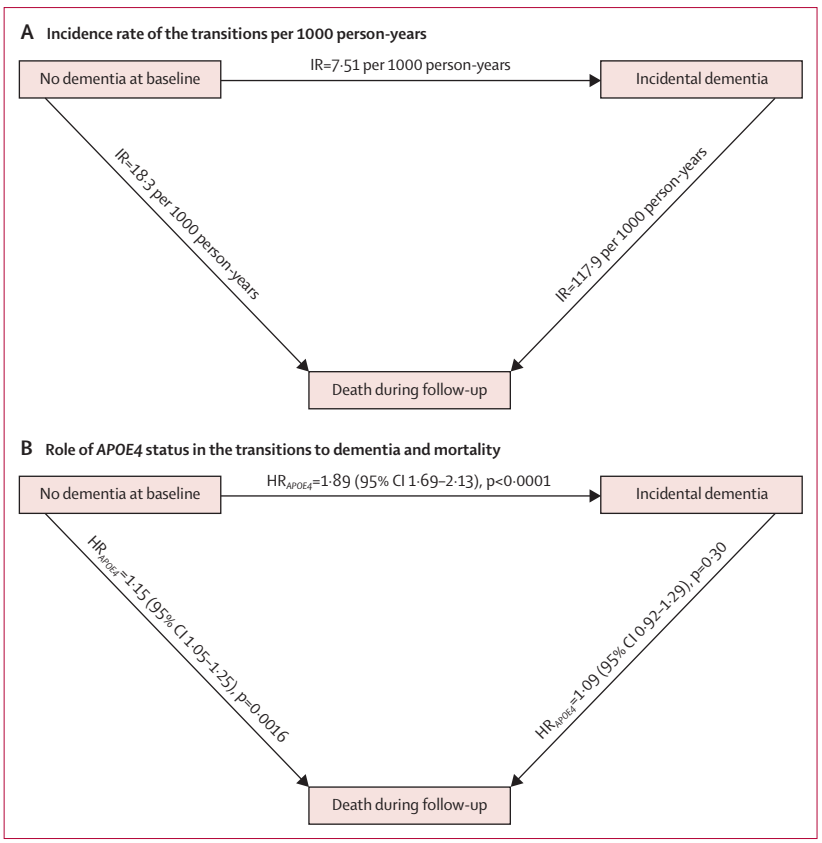

***Figure 2*: Illness-death model for transitions across health states (dementia and all-cause mortality; A) and the role of *APOE4* status (carriers *vs* non-carriers; B) in these transitions**
HR=hazard ratio. IR=incidence rate.

We also used an illness-death model to examine explicitly the role of incident dementia in the association between *APOE4* and all-cause mortality. This method allowed us to consider the role of *APOE4* in all transitions, including transitions to death in participants who did and did not develop dementia over the follow-up. Our study also has some limitations. First, we were unable to explore the potential role of other genetic variants or gene-environment interactions that might influence the association between *APOE4* and mortality. Second, the diagnosis of dementia in the Whitehall II study relied on health records, which might have resulted in milder cases being missed. However, this approach to diagnosing dementia is unlikely to affect the association between *APOE* and dementia because previous meta-analyses on dementia risk factors using data from the Whitehall II study found estimates similar to those from studies using other ways of ascertaining dementia.[36] Moreover, we did not have complete data on dementia subtypes and could therefore not examine the role of specific dementia types in the association between *APOE* and mortality. Third, although our study design allowed the examination of dementia as a potential mediator in the APOE4-mortality association, we could not examine other potential mediators such as

systemic inflammation or oxidative stress. Future studies that examine these pathways might provide further insights into the role of *APOE4* in mortality risk. Fourth, our study considered two main causes of mortality (cancer and cardiovascular diseases) due to limited statistical power to examine other causes of death. Even with the analyses restricted to only two causes of death, the homozygous *APOE4* carrier group was small and did not have sufficient statistical power to draw firm conclusions. Finally, analyses were restricted to participants of White origin. There is increasing evidence of the role of ethnicity in the association between *APOE4* and dementia. Whether ethnicity also plays a role in the association between *APOE* genotype and mortality remains unclear. Our results need to be replicated in other populations to test the generalisability of our results to other ethnic groups.

In conclusion, our findings show that both *APOE4* heterozygotes and homozygotes carry a higher risk of all-cause mortality, extending beyond the known associations with Alzheimer's disease and cardiovascular disease. Dementia played an important role among *APOE4* homozygotes in associations with mortality. In contrast, for *APOE4* heterozygotes, who comprise about a fifth of the study population, other factors, notably cardiovascular mortality, played a major role. Future research should aim to further elucidate the mechanisms underpinning the association between *APOE4* and mortality, and to investigate potential strategies for mitigating this risk.

Contributors

MR, SS, AS-M, and JD designed the study. MR and AD did the analyses. MR, SS, AS-M, and JD wrote the first draft of the Article. All authors contributed to the interpretation of the results, critically revised the Article for important intellectual content, and approved the final version submitted. AD, SS, and JD are the guarantors of this work, had full access to and verified all the data in the study, and took responsibility for data integrity and the accuracy of the data analysis. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

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Data sharing

Data coming from the Whitehall II study can be made freely available to interested researchers upon request at https://www.ucl.ac.uk/epidemiologyhealth-care/research/epidemiology-and-publichealth/research/whitehall-ii/data-sharing. Contact email address: whitehall2@ucl.ac.uk. The Three-City study is managed by the UMR1219, Bordeaux Population Health Research Center, Bordeaux University, France. Data coming from the Three-City study can be made freely available to interested researchers upon request: http://www.three-citystudy.com/the-three-city-study.php. Contact email address: E3C.CoordinatingCenter@u-bordeaux.fr.

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