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Original research

# LDL cholesterol is associated with higher AD neuropathology burden independent of APOE

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## ABSTRACT

**Objective** *APOE* is a strong risk factor for Alzheimer's disease (AD) and associated with higher low-density lipoprotein cholesterol (LDL-C) levels. Moreover, LDL-C is associated with the development of clinically ascertained AD; however, whether this association is present with the underlying neuropathological manifestations of AD or whether it is independent of the effect of *APOE* is unknown and is the focus of this paper.

**Methods** Individuals in the Religious Orders Study/Memory and Ageing Project cohorts with longitudinal measures of blood lipids and detailed autopsies were studied. We modelled the relationship between blood lipids and 12 age-related brain pathologies using a linear mixed model adjusted for potential confounding factors and stratified by *APOE* genotype with overall significance determined by meta-analysis. Blood lipids considered were LDL-C, high-density lipoprotein cholesterol and triglycerides. Brain pathologies included AD pathology measured by silver staining (Braak stage, a modified Consortium to Establish a Registry for Alzheimer's Disease [CERAD] score and global AD pathology) and immunohistochemistry (beta-amyloid and neurofibrillary tangles) as well as cerebral microinfarct, cerebral macroinfarct, cerebral amyloid angiopathy, cerebral atherosclerosis, hippocampal sclerosis, TDP-43 cytoplasmic inclusions and Lewy bodies.

**Results** 559 participants (69.1% female) had complete data for analysis. They were followed for a median of 7 years and a median of 3 years prior to dementia onset. LDL-C was associated with all measures of AD neuropathology (neurofibrillary tangles, beta-amyloid, Braak stage, modified CERAD score and global AD pathology) and cerebral amyloid angiopathy independent of *APOE* after adjusting for age, sex, cholesterol-lowering medication use, body mass index, smoking and education at false discovery rate (FDR)  $p$ -value  $<0.05$ .

**Conclusions** These findings implicate LDL-C in the pathophysiology of AD independent of *APOE* and suggest LDL-C is a modifiable risk factor for AD.

## INTRODUCTION

Blood lipids are routinely used to estimate the risk of heart attack and stroke in clinical care. They have three commonly measured constituents that guide risk assessments: high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). Blood lipids are also associated with dementia, of which Alzheimer's disease (AD) is the most prevalent type.<sup>1–4</sup> Specifically, a recent large retrospective study of

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ While the preponderance of the evidence suggests an association between high low-density lipoprotein cholesterol (LDL-C) in mid-life and increased dementia risk, it is unknown whether this is simply the result of the pleiotropic effect of *APOE*, whose variants (*E2* and *E4*) are strongly associated with both LDL-C and Alzheimer's disease, or whether LDL-C is an independent risk factor for dementia. Additionally, while there are many studies investigating blood lipids and clinically ascertained dementia or Alzheimer's disease, the relationship between premorbid blood lipids and brain neuropathology has not been comprehensively investigated.

## WHAT THIS STUDY ADDS

⇒ We examined premorbid blood lipids (LDL-C, high-density lipoprotein cholesterol and triglyceride) and 12 age-related brain pathologies and rigorously account for the effect of *APOE* and several relevant clinical covariates. We found higher LDL-C was associated with more Alzheimer's disease (AD) hallmark neuropathology (ie, neurofibrillary tangles, beta-amyloid, Braak staging, CERAD score and global AD pathology) and cerebral amyloid angiopathy after multiple testing correction.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ Our study implies that high LDL-C is a risk for AD independently of *APOE*. It provides information to guide recommendations on LDL-C levels to reduce AD risk and suggests that new biomarker-informed trials of lipid lowering strategies to reduce AD ought to be considered. Finally, it should spur new mechanistic investigation to elucidate the pathophysiology of LDL-C in AD.

1.8 million individuals found a significant association between midlife LDL-C and dementia risk 10 years later.<sup>4</sup> The biology underlying the association between blood cholesterol and AD risk is not straightforward because the *APOE* variants *E2* and *E4* are strongly associated with both blood lipids and AD risk in opposite directions.<sup>5</sup> On the one hand, the *APOE E4* allele is not only the strongest

genetic risk factor for AD but is also well known to raise total cholesterol (TC), which is a mixture of LDL-C and HDL-C.<sup>6</sup> On the other hand, *APOE E2* is associated with reduced AD risk and lower LDL-C. Thus, the association between LDL-C and AD may be causal or simply the result of the pleiotropic effect of *APOE* variants on both phenotypes. Insight into the nature of the association between cholesterol and AD risk is important because it may provide another tool to lower AD risk since blood lipid levels are potentially modified by diet, exercise and pharmacological means.

Results of studies on the relationship between blood cholesterol and AD risk were mixed, and the latest, largest study suggests an association between mid-life cholesterol and AD risk.<sup>3–4</sup> Inconsistencies in the findings may be explained by several technical challenges. First, TC is composed of multiple cholesterol species with distinct physiological roles—LDL-C, very LDL-C (VLDL-C) and HDL-C. The genetic polymorphisms of *APOE* influence specific cholesterol species (eg, LDL-C and HDL-C) and TC. Many earlier studies were limited to TC<sup>7–12</sup> rather than a more granular assessment of blood lipids, possibly limiting the resolution to detect associations with *APOE* variants. Second, the clinical diagnostic criteria for AD does not necessarily reflect the extent of the underlying AD neuropathology. For example, postmortem pathological examination found that 75% of cognitively normal older adults had amyloid pathology<sup>13</sup> and about 30% of these individuals met pathology-based National Institute on Aging-Reagan criteria for AD.<sup>14</sup> Many previous studies on the relationship between blood lipids and AD inferred the presence of dementia or AD based on clinical criteria or a cognitive screening tool and not on neuropathology. Using clinical diagnosis of AD instead of neuropathological outcomes likely results in heterogeneity of the underlying cause of cognitive impairment or dementia, which reduces the power to observe relationships that vary across neuropathologies. Third, blood cholesterol levels and dementia are both dynamic phenotypes that change over time. Thus, when considering the hypothesis that blood lipids influence AD risk, conclusions must allow for the possibility of reverse causality, that is the possibility that dementia status affects blood cholesterol. Not all studies have the means to discriminate between these situations, which may make them potentially susceptible to confounding. Fourth, statins and other medications are commonly used to lower blood cholesterol and their use is strongly associated with LDL-C. Thus, to determine the effect of LDL-C, it is critical to account for use of these medications, but not all studies could account for these effects. While there have been some studies that have addressed some of the aforementioned challenges individually, we know of no study that account for all of them simultaneously and aimed to do so in this study.

Here, our primary goal was to determine whether blood LDL-C, HDL-C or TG prior to the manifestation of dementia is associated with AD neuropathology (ie, measures of beta-amyloid and neurofibrillary tangles) independent of *APOE* genotype. To this end, we examined data from community-based participants recruited by two prospective studies of memory and ageing, the Religious Orders Study (ROS) and Memory and Ageing Project (MAP). These studies recruit cognitively normal older individuals who agree to annual detailed medical and cognitive assessments, annual blood donation and detailed neuropathological assessments of their brain at the end of life. We used these data to overcome the above technical challenges by combining the longitudinal annual measures of blood lipids, annual assessment of cognitive status, use of lipid lowering medication (including statins) and other comorbidities with 12 measured age-related

neuropathological outcomes stratified by *APOE* genotype. Importantly, our main analysis only considers blood lipid measures obtained before the participants were diagnosed with dementia to avoid potential reverse effect of dementia on blood lipids.

## METHODS

### Participants

Participants are from the ROS and MAP cohorts. These are longitudinal clinical-pathological studies of ageing and AD dementia. The ROS study began in 1994 and has enrolled 1452 individuals and MAP began in 1997 and has 2058 individuals enrolled. All ROS/MAP participants undergo a structured annual clinical evaluation including a cognitive and general health assessment as well as blood draw and a detailed standardised neuropathological assessment at death.<sup>15–16</sup> ROS recruits older Catholic priests, nuns and monks throughout the USA. MAP recruits older lay persons from the greater Chicago area. Both studies perform the same detailed annual cognitive and clinical evaluations and brain autopsy.

### Clinical dementia diagnosis, blood lipids and covariates

A clinical diagnosis of cognitive status is rendered annually and at the time of death by a neurologist using all available clinical data, but blinded to postmortem data, using the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association guidelines.<sup>17</sup> Case conferences including one or more neurologists and a neuropsychologist were used for consensus, as necessary. Clinical diagnoses of cognitive status can include no cognitive impairment (NCI), mild cognitive impairment (MCI), dementia due to AD or dementia not due to AD.

A subset of ROS and MAP participants had annual lipid measures performed by Laboratory Corporation of America Holdings (Labcorp, Burlington, North Carolina) to determine TC, HDL-C, VLDL-C and TG. The Friedewald formula<sup>18</sup> was used to estimate LDL-C for samples with TG below 400 mg/dL to account for blood lipids from non-fasting participants. Use of cholesterol-lowering medications was collected and available in these participants.<sup>19</sup>

### Neuropathology

Brain autopsy was performed by examiners who were unaware of deceased participants' clinical information and autopsy methods have been described in detail before.<sup>20–21</sup> Nine brain regions of interest (ie, mid-frontal, mid-temporal, inferior parietal, anterior cingulate, entorhinal and hippocampal cortices, basal ganglia, thalamus and midbrain) were dissected and stained for assessment of pathology. All brains were systematically characterised for AD pathology (ie, beta-amyloid, neurofibrillary tangles, global AD pathology, Braak staging and CERAD score), cerebral microinfarct, cerebral macroinfarct, cerebral amyloid angiopathy (CAA), cerebral atherosclerosis, hippocampal sclerosis, TDP-43 cytoplasmic inclusions and Lewy bodies.

Global AD pathology (ie, neuritic plaques, diffuse plaques and neurofibrillary tangles) was visualised in five cortical regions using a modified Bielschowsky silver stain. Counts of silver-stained neuritic plaques, diffuse plaques and neurofibrillary tangles were used to create a continuous measure of AD global pathology. The square root of this global pathology measure was used in our analyses to better approximate a normal distribution. To assess presence of amyloid-beta in the brain, immunohistochemistry with MMO0972 (DAKO, 1:100) was

**Table 1** Demographics for individuals with NCI or MCI at baseline

	Overall (N=559)
Sex	
Female	386 (69.1%)
Male	173 (30.9%)
Years of formal education	
Mean (SD)	15.4 (3.3)
Median (min, max)	16.0 (5.0, 28.0)
Age at first visit	
Mean (SD)	83.7 (5.88)
Median (min, max)	83.5 (66.7, 102)
Age at death	
Mean (SD)	89.8 (6.28)
Median (min, max)	90.1 (71.3, 106)
Clinical diagnosis at first lipid measure	
NCI	380 (68.0%)
MCI	179 (32.0%)
AD	0 (0%)
Other dementia	0 (0%)
Clinical diagnosis at death	
NCI	212 (37.9%)
MCI	160 (28.6%)
AD	178 (31.8%)
Other dementia	9 (1.6%)
No of predementia annual lipid measures	
Mean (SD)	4.0 (2.7)
Median (min, max)	3.0 (1.0, 12.0)

AD, Alzheimer's disease; MCI, mild cognitive impairment; NCI, no cognitive impairment.

performed in six regions (entorhinal, CA1/subiculum, dorsolateral prefrontal, inferior temporal, angular gyrus/supramarginal, calcarine cortices) and a composite measure was generated for each subject using computer-assisted sampling.<sup>22</sup> Neurofibrillary tangles were assessed using a similar approach with AT8 (Innogenetics, 1:1000) to label paired helical filament-tau.<sup>22</sup> A modified CERAD score of no, possible, probable or definite AD was assigned based on CERAD criteria modified so that the diagnosis was blinded to age and clinical data. Braak scores were based on staging of neurofibrillary tangle pathology assessed by Bielschowsky silver stain.

Chronic gross infarcts were identified visually by examining slabs and pictures from both hemispheres and confirmed histologically and was treated as a dichotomous variable (present vs absent) in our analyses. Microinfarcts were those that were not visible to the naked eye but were identified under microscope using H&E stain in a minimum of nine regions, including six cortical regions, two subcortical regions and midbrain. Microinfarcts were treated as present or absent in our analyses. CAA was assessed using amyloid- $\beta$  immunostaining in four regions (midfrontal, inferior temporal, angular and calcarine). In each region, meningeal and parenchymal vessels were assessed for amyloid deposition and scored from 0 to 4, where 0 indicates no deposition, 1 refers to scattered segmental but no circumferential deposition, 2 means circumferential deposition up to 10 vessels, 3 reflects circumferential deposition in >10 vessels and up to 75% of the vessels and 4 indicates circumferential deposition in over 75% of the vessels.<sup>23</sup> The score for each region was the maximum of the meningeal and parenchymal scores, and a continuous summary score was created by averaging scores across the regions.<sup>23</sup> Hippocampal sclerosis was identified as severe neuronal loss and gliosis in hippocampus or subiculum using H&E stain and treated as present or absent in analyses.<sup>24</sup> Lewy

body pathology was assessed using antibodies to  $\alpha$ -synuclein in six regions including substantia nigra, limbic and neocortices and treated as present or absent in our analyses. TDP-43 cytoplasmic inclusions were assessed in six regions using antibodies to phosphorylated TDP-43. Inclusions in each region were rated on a six-point scale and the mean of the regional scores was created.<sup>25</sup> TDP-43 was dichotomised into absent (ie, mean score of 0) or present (mean score >0) in our analyses. Cerebral atherosclerosis was assessed by visual inspection of vessels in the circle of Willis and rated as absent, mild, moderate or severe and treated as a semiquantitative variable in our analyses.<sup>26</sup>

### Statistical analysis

We examined the relationship between circulating cholesterol measures (ie, LDL-C, HDL-C and TG) and each neuropathology using a linear mixed model that accounts for the repeated measurements of premorbid circulating cholesterol (R lme4 package V.1.1–26).<sup>27</sup> Importantly, only cholesterol measures during the timepoints that an individual did not have a diagnosis of dementia were included in the analysis to avoid a potential effect of dementia diagnosis on circulating cholesterol levels. Pathologies tested were the modified CERAD score, Braak staging, global AD pathology, quantitative amyloid-beta, quantitative neurofibrillary tangles, Lewy bodies, TDP-43, cerebral atherosclerosis, gross infarcts, chronic microinfarcts, CAA and hippocampal sclerosis. To estimate the effect of circulating cholesterol on AD pathologies taking into consideration the effects of APOE genotype, we performed a mixed linear regression among individuals of a given APOE genotype (ie, E23, E33 or E34), provided there were >10 individuals with that genotype. In the mixed linear regression model, the outcome was a specific blood cholesterol (ie, HDL-C, LDL-C or TG) and the predictor was a neuropathology measure adjusting for sex, age at recruitment, years of formal educational, smoking status, body mass index (BMI), use of cholesterol lowering medications and study (ROS vs MAP). A meta-analysis of results of the association between blood lipids and neuropathology stratified by APOE was performed with METAL<sup>28</sup> using a fixed-effect model with effect size estimates and SEs. The rationale for using a fixed-effect model for the meta-analysis is that assessments were identical for all individuals and our goal was to estimate a common effect of blood lipids on the 12 measured neuropathologies with the ROS/MAP cohorts. The CI of the pooled effect was estimated using the Knapp-Hartung adjustment by the R meta package (V.4.18–2), and the heterogeneity variance tau was estimated using a restricted maximum likelihood estimator by the R meta package (V.4.18–2). Multiple testing was addressed using Benjamini-Hochberg false discovery rate (FDR) applied to all meta-analyses performed. Meta-analysis results are shown as forest plots generated by the R meta package (V.4.18–2).<sup>29</sup> Two sensitivity analyses were performed. The first, restricted the analysis to only individuals with NCI at baseline to understand the effect that any degree of cognitive impairment or likely presence of significant brain pathology may have on the relationship between blood lipids and the 12 measured brain pathologies. The second, included all individuals with blood lipids without censoring for cognitive status to understand the effect censoring for dementia may have on the relationship between blood lipids and 12 measured brain pathologies.

**Table 2** Study characteristics by final cognitive diagnosis for individuals with NCI or MCI at baseline

	NCI (N=212)	MCI (N=160)	AD (N=178)	Other dementia (N=9)
<b>Gender</b>				
Female	148 (69.8%)	100 (62.5%)	133 (74.7%)	5 (55.6%)
Male	64 (30.2%)	60 (37.5%)	45 (25.3%)	4 (44.4%)
<b>Education (years)</b>				
Mean (SD)	15.5 (3.6)	15.5 (3.1)	15.3 (3.2)	16.0 (3.8)
Median (min, max)	16.0 (7.0, 27.0)	16.0 (5.0, 25.0)	16.0 (8.0, 28.0)	16.0 (12.0, 24.0)
<b>Age at diagnosis</b>				
Mean (SD)	–	–	88.8 (6.1)	84.0 (5.9)
Median (min, max)	–	–	88.9 (71.4, 106)	84.4 (74.6, 91.7)
Missing	–	–	12 (6.7%)	3 (33.3%)
<b>Age at death</b>				
Mean (SD)	88.1 (6.5)	89.8 (5.9)	91.8 (5.7)	86.9 (5.9)
Median (min, max)	88.5 (71.3, 103)	90.2 (72.1, 104)	92.3 (76.0, 106)	85.9 (77.3, 95.9)
<b>APOE genotype</b>				
<i>E23</i>	43 (20.3%)	14 (8.8%)	20 (11.2%)	2 (22.2%)
<i>E33</i>	143 (67.5%)	114 (71.3%)	115 (64.6%)	3 (33.3%)
<i>E34</i>	26 (12.3%)	32 (20.0%)	43 (24.2%)	4 (44.4%)
<b>Global AD pathology score</b>				
Mean (SD)	0.5 (0.5)	0.7 (0.6)	0.9 (0.6)	0.7 (0.4)
Median (min, max)	0.3(0, 2.6)	0.6(0.0, 2.3)	0.8(0.0, 2.8)	0.8(0.0, 1.4)
<b>Beta-amyloid score</b>				
Mean (SD)	3.4 (4.0)	4.7 (4.6)	6.5 (4.8)	7.4 (6.9)
Median (min, max)	1.5(0, 22.9)	3.5(0, 17.9)	6.0(0, 19.9)	8.4(0.0, 17.0)
Missing	2 (0.9%)	0 (0%)	0 (0%)	0 (0%)
<b>Neurofibrillary tangle score</b>				
Mean (SD)	3.5 (4.0)	5.6 (5.9)	9.3 (8.5)	2.6 (1.9)
Median (min, max)	2.6 (0.0, 27.3)	4.1 (0.0, 38.2)	6.9 (0.0, 42.8)	2.5 (0.5, 5.9)
Missing	2 (0.9%)	0 (0%)	1 (0.6%)	0 (0%)
<b>Modified CERAD</b>				
Definite AD	37 (17.5%)	44 (27.5%)	74 (41.6%)	2 (22.2%)
Probable AD	62 (29.2%)	64 (40.0%)	79 (44.4%)	5 (55.6%)
Possible AD	26 (12.3%)	17 (10.6%)	6 (3.4%)	0 (0%)
No AD	87 (41.0%)	35 (21.9%)	19 (10.7%)	2 (22.2%)
<b>Braak stage</b>				
0–II	49 (23.1%)	27 (16.9%)	13 (7.3%)	0 (0%)
III–IV	147 (69.3%)	99 (61.9%)	90 (50.6%)	9 (100%)
V–VI	16 (7.5%)	34 (21.3%)	75 (42.1%)	0 (0%)
<b>Cerebral amyloid angiopathy</b>				
0	66 (31.1%)	39 (24.4%)	31 (17.4%)	3 (33.3%)
1	96 (45.3%)	74 (46.3%)	79 (44.4%)	4 (44.4%)
2	33 (15.6%)	33 (20.6%)	47 (26.4%)	1 (11.1%)
3	16 (7.5%)	14 (8.8%)	21 (11.8%)	1 (11.1%)
Missing	1 (0.5%)	0 (0%)	0 (0%)	0 (0%)
<b>Cerebral atherosclerosis stage</b>				
0	66 (31.1%)	43 (26.9%)	27 (15.2%)	1 (11.1%)
1	96 (45.3%)	72 (45.0%)	86 (48.3%)	2 (22.2%)
2	38 (17.9%)	36 (22.5%)	50 (28.1%)	5 (55.6%)
3	12 (5.7%)	9 (5.6%)	15 (8.4%)	1 (11.1%)
<b>Gross cerebral infarctions</b>				
Not present	132 (62.3%)	96 (60.0%)	86 (48.3%)	3 (33.3%)
Present	80 (37.7%)	64 (40.0%)	92 (51.7%)	6 (66.7%)
<b>Hippocampal sclerosis</b>				
Not present	208 (98.1%)	155 (96.9%)	151 (84.8%)	8 (88.9%)
Present	4 (1.9%)	5 (3.1%)	27 (15.2%)	1 (11.1%)
<b>Lewy bodies</b>				
Not present	173 (81.6%)	124 (77.5%)	115 (64.6%)	5 (55.6%)

Continued

Table 2 Continued

	NCI (N=212)	MCI (N=160)	AD (N=178)	Other dementia (N=9)
Present	34 (16.0%)	29 (18.1%)	56 (31.5%)	4 (44.4%)
Missing	5 (2.4%)	7 (4.4%)	7 (3.9%)	0 (0%)
Microinfarcts				
Not present	134 (63.2%)	105 (65.6%)	105 (59.0%)	5 (55.6%)
Present	78 (36.8%)	55 (34.4%)	73 (41.0%)	4 (44.4%)
TDP-43 staging				
0	125 (59.0%)	81 (50.6%)	65 (36.5%)	6 (66.7%)
1	46 (21.7%)	33 (20.6%)	29 (16.3%)	2 (22.2%)
2	29 (13.7%)	31 (19.4%)	46 (25.8%)	1 (11.1%)
3	7 (3.3%)	14 (8.8%)	38 (21.3%)	0 (0%)
Missing	5 (2.4%)	1 (0.6%)	0 (0%)	0 (0%)
HDL-C (mmol/L)				
Mean (SD)	1.47 (0.442)	1.48 (0.462)	1.46 (0.418)	1.18 (0.270)
Median (Min, Max)	1.40 (0.518, 2.88)	1.42 (0.674, 2.67)	1.40 (0.725, 3.73)	1.30 (0.725, 1.50)
LDL-C (mmol/L)				
Mean (SD)	2.65 (0.833)	2.73 (0.850)	2.80 (0.871)	2.99 (0.972)
Median (min, max)	2.59 (0.648, 4.87)	2.66 (1.06, 6.06)	2.73 (0.933, 5.41)	3.01 (1.37, 4.77)
TG (mmol/L)				
Mean (SD)	1.67 (0.800)	1.58 (0.774)	1.56 (0.755)	1.88 (0.700)
Median (min, max)	1.51 (0.542, 4.22)	1.37 (0.440, 4.28)	1.37 (0.474, 4.28)	1.91 (0.926, 3.09)
Lipid lowering treatment				
Not present	130 (61.3%)	108 (67.5%)	118 (66.3%)	8 (88.9%)
Present	82 (38.7%)	52 (32.5%)	60 (33.7%)	1 (11.1%)

AD, Alzheimer's disease; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; HDL-C, high-density lipoprotein cholesterol; MCI, mild cognitive impairment; NCI, no cognitive impairment; TG, triglycerides.

## RESULTS

### Demographics

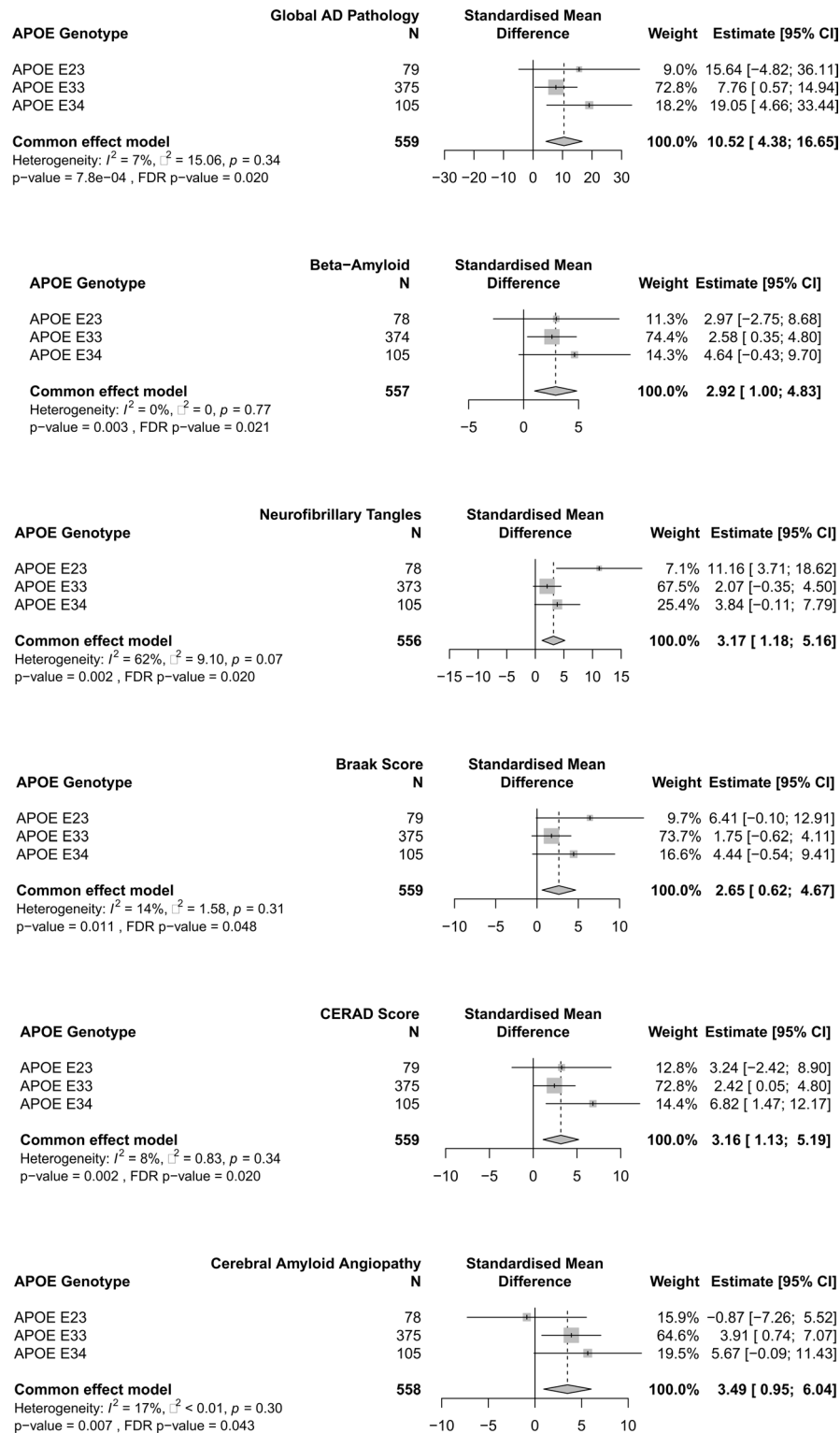
A total of 622 ROS/MAP participants had longitudinal blood lipid measures and neuropathologies. Blood lipids were performed as a give-back to participants. There was no difference in sex, final cognitive diagnosis or *APOE* genotype for people who underwent blood lipid measurement and the rest of the ROS/MAP participants. After restricting to participants with either NCI or MCI at baseline, there were 559 individuals who were included in the main analysis and their demographics are given in table 1.

For those individuals, the median follow-up duration was 7.0 years prior to death. At baseline, 179 (32%) had MCI and 380 (68%) were cognitively normal. At death, 160 (28.6%) had MCI, 178 (31.8%) had AD and 212 (37.9%) were cognitively normal. All participants were of European ancestry, predominantly women (69.1%), had a high degree of education (average of 15.4 years) and had an average age at first lipid level of 83.7 years. Table 2 gives the characteristics of all individuals included in the main analysis categorised by their final cognitive diagnosis prior to death.

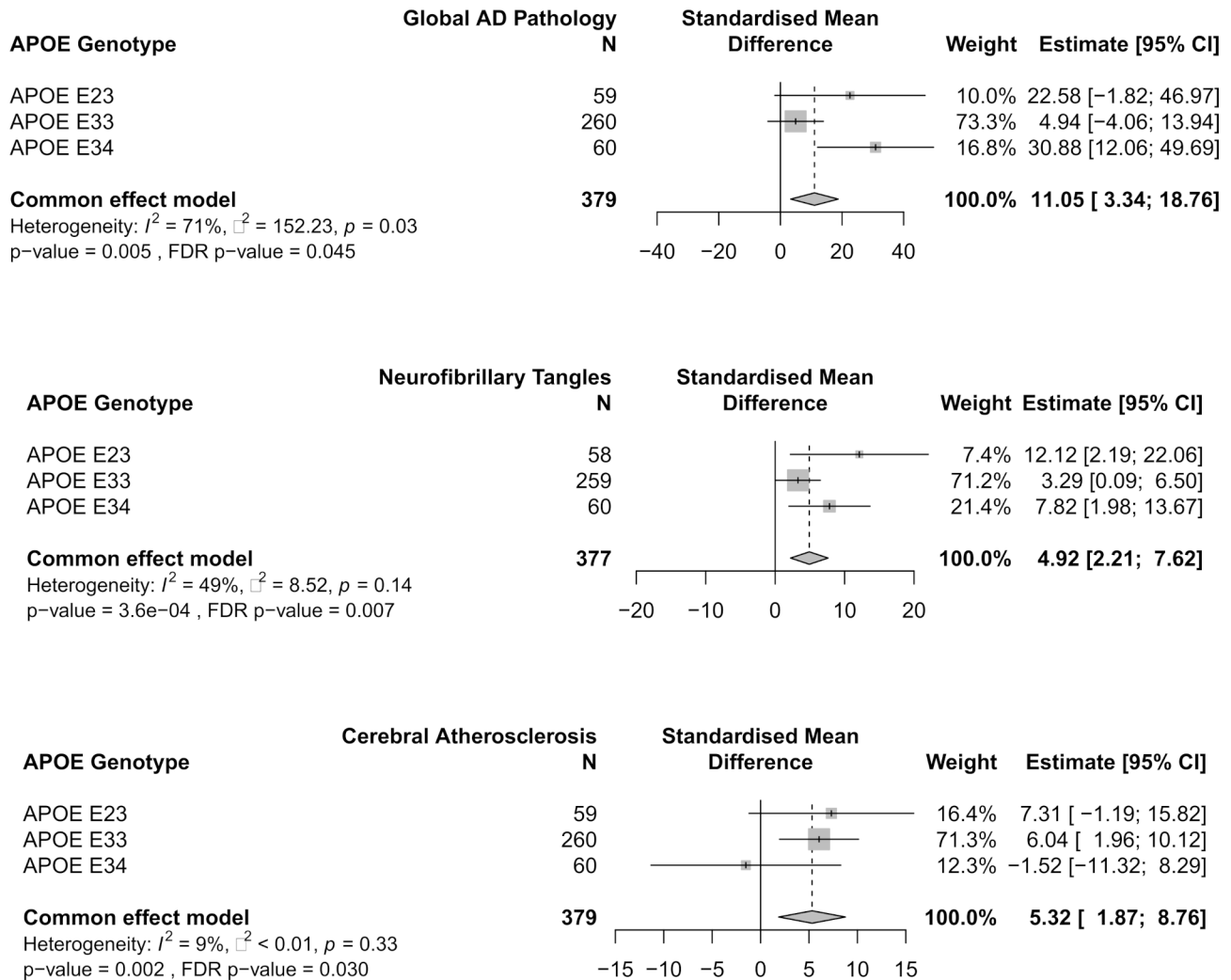
Participants who developed MCI or dementia prior to death had a higher proportion of *APOE E34* genotype (12.3%, 20.0% and 24.2% for NCI, MCI and AD, respectively) and lower proportion of *APOE E23* genotype (20.3%, 8.8% and 11.2% for NCI, MCI and AD, respectively) than cognitively normal participants. As expected, those with either MCI, AD or other dementia had a greater degree of all measures of AD neuropathology (ie, Global AD pathology, beta-amyloid, neurofibrillary tangle, CERAD score and Braak stage). Demographics and clinical characteristics by final cognitive diagnosis are also provided for individuals with NCI at baseline (online supplemental tables 1 and 2) and all available individuals (online supplemental tables 3 and 4) with longitudinal blood lipid measures and neuropathology outcomes.

### LDL-C is associated with a higher burden of AD neuropathology

The primary goal of this study was to examine the relationship between blood lipids before clinical dementia onset and eventual AD neuropathologies. Nevertheless, we tested the relationship between each measured blood lipid (ie, LDL-C, HDL-C and TG) and each of the 12 measured neuropathologies. To rigorously account for the effect of *APOE* on blood lipid and AD neuropathologies, individuals with the same *APOE* genotype were analysed together, and overall association between blood lipids and neuropathology was estimated by meta-analysis. To avoid any potential effect dementia diagnosis may have on blood lipid levels and to appropriately account for the longitudinal nature of the lipid assessments, a linear mixed model was used for each individual analysis including only lipid levels measured before participants had a diagnosis of dementia. All analyses were adjusted for sex, age, years of formal educational, smoking, use of cholesterol lowering medication, BMI and study (ROS vs MAP). Significant associations at FDR <0.05 were observed between LDL-C and global AD pathology (N=559, standardised mean difference (SMD) of 10.5, 95% CI 4.4 to 16.6), neurofibrillary tangles (N=556, SMD of 3.2, 95% CI 1.2 to 5.2), beta-amyloid (N=557, SMD 2.9 with a 95% CI 1.0 to 4.8), Braak score (N=559, SMD of 2.6, 95% CI 0.6 to 4.7), CERAD score (N=559, SMD of 3.2, 95% CI 1.1 to 5.2) and CAA (N=558, SMD of 3.5; 95% CI 0.9 to 6.0; figure 1). LDL-C was also nominally associated with cerebral atherosclerosis (p=0.034), but no significant relationship was identified between LDL-C and either gross cerebral infarctions, hippocampal sclerosis, Lewy bodies, microinfarcts or TDP-43 pathologies (online supplemental figure 1). For HDL-C, there was a significant association with hippocampal sclerosis (N=559, SMD of 6.6, 95% CI 1.7 to 11.5) and Lewy body pathology (N=559, SMD of -4.97, 95% CI -8.0



**Figure 1** Longitudinally measured LDL-C in NCI or MCI associated with measured brain pathologies. Results for association testing between longitudinally measured LDL-C in individuals with NCI or MCI at baseline with censoring of LDL-C for a diagnosis of dementia. Each neuropathology was considered in a separate linear mixed model stratified by *APOE* genotype and adjusted for relevant covariates. Final significance was determined by meta-analysis shown by in the forest plot, and the nominal p value and FDR p values are given. Results of each *APOE* genotype are shown with their sample size, standardised mean difference estimate (small vertical black line), the 95% CI of the standardised mean difference estimate (horizontal black line), and their relative contribution to the meta-analysis (grey shaded box around the standardised mean difference estimate). The result of the fixed-effect meta-analysis is shown as a vertical dotted line and the 95% CI as a diamond. Measures of the heterogeneity between groups are given,  $I^2$ , and the residual heterogeneity,  $\tau^2$ , and estimated p value are given. The standardised mean difference may be considered as the difference in the neuropathology score per unit of blood lipid measured. Full results of LDL-C, HDL-C and TG and all 12 neuropathologies are given in online supplemental table 5 and additional plots are given in online supplemental figures 1–4. AD, Alzheimer’s disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MCI, mild cognitive impairment; NCI, no cognitive impairment; FDR, false discovery rate.



**Figure 2** Longitudinally measured LDL-C in people with NCI associated with measured brain pathologies. Results for association testing between longitudinally measured LDL-C in individuals with NCI at baseline with censoring of LDL-C for a diagnosis of either MCI or dementia. Each neuropathology was considered in a separate linear mixed model stratified by APOE genotype and adjusted for relevant covariates. Final significance was determined by meta-analysis and is shown by forest plot following the same approach as described in figure 1. Full results of LDL-C, HDL-C, and TG and all 12 neuropathologies are given in online supplemental table 6 and additional plots are given in online supplemental figures 5–8. AD, Alzheimer’s disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MCI, mild cognitive impairment; NCI, no cognitive impairment; TG, triglycerides.

to -2.0; online supplemental figure 2) and the remainder of the results were non-significant (online supplemental figure 3). TGs were only nominally associated with cerebral atherosclerosis (p=0.029) and remainder of the results were non-significant (online supplemental figure 4). Full meta-analysis results for each measured blood lipid (ie, LDL-C, HDL-C and TG) and all 12 measured neuropathologies are given in online supplemental table 5.

To remove the effect that any cognitive impairment or likely presence of significant brain pathology may have on the relationship between blood lipids and brain neuropathology, we performed a sensitivity analysis restricted to the 379 individuals with NCI at baseline and censored lipid levels if the diagnosis changed to either MCI or dementia. This analysis is not as well powered compared with the main analysis due to the reduced sample size. Nevertheless, significant associations at FDR<0.05 were observed between LDL-C and global AD pathology (N=379, SMD of 11.05, 95% CI 3.34 to 18.76), neurofibrillary tangles (N=377, SMD of 4.92, 95% CI 2.21 to 7.62) and

cerebral atherosclerosis (N=379, SMD of 5.32; 95% CI 1.87 to 8.76; figure 2). Nominal association was found between LDL-C and beta-amyloid (p=0.011 and FDR p=0.071; N=377, SMD 3.05 with a 95% CI 0.69 to 5.41), Braak Score (p=0.012 and FDR p=0.071; N=379, SMD of 3.23; 95% CI of 0.71 to 5.75) and CAA (p=0.018 and FDR p=0.092; N=378, SMD of 3.87; 95% CI 0.67 to 7.08; online supplemental figure 5). HDL-C remained significantly associated with Lewy Body pathology (N=366, SMD of -6.75, 95% CI of -10.31 to -3.18; online supplemental figure 6) after FDR adjustment. The remainder of the associations tested were non-significant (online supplemental figures 7 and 8). Full meta-analysis results are given in online supplemental table 6.

To understand the effect that clinical dementia could have on the relationship between the blood lipids and neuropathology, we performed a sensitivity analysis that included all available measures of blood lipids at baseline regardless of diagnosis (ie, NCI, MCI or AD) and did not censor blood lipid levels based on cognitive status using the same covariates, APOE genotype



stratification and meta-analysis as was used in the primary analysis. The blood lipids that showed associations with neuropathologies in the primary analysis remained significant in the sensitivity analysis. Specifically, after multiple testing correction, LDL-C remained associated with AD-related neuropathologies at  $FDR < 0.05$  (online supplemental figure 9) except for Braak Stage that only remained nominally associated ( $p = 0.015$  and  $FDR p = 0.067$ ; online supplemental figure 10). For HDL-C, lower level remained associated with the presence of Lewy body pathology (online supplemental figure 11) but not with hippocampal sclerosis ( $p = 0.52$  and  $FDR p = 0.82$ ; online supplemental figure 12). The only new finding was that TG was significantly associated with cerebral atherosclerosis after adjusting for multiple testing ( $N = 622$ ,  $SMD: 7.3$ ,  $95\% CI 1.9$  to  $12.7$ ,  $FDR p = 0.043$ ; online supplemental figure 13) while the other associations tested remained non-significant (online supplemental figure 14). Full meta-analyses results are given in online supplemental table 7.

## DISCUSSION

In this study, we examined the association between premorbid longitudinal blood lipids and AD neuropathology, which has not been previously described to the best of our knowledge. We observed that higher LDL-C was associated with a higher burden of all AD neuropathologies independent of the effect of *APOE* (ie, neuritic plaques and neurofibrillary tangles using either an antibody-based quantitative measure or the traditional silver staining semiquantitative scoring of those pathologies). These findings suggest that blood LDL-C plays a role in AD pathogenesis. They also suggest that some of the effect of *APOE* on AD may be through blood lipids, which may explain why *APOE* appears to have a non-additive effect on AD risk.<sup>30</sup>

These results should be interpreted in light of the study's limitation in that ROS/MAP recruited participants of normal cognitive functioning and over 65 years of age with relatively few medical comorbidities, which may result in a healthy survivor bias. Additionally, all ROS/MAP participants in this study were of European descent. These factors could limit the generalisability of our findings.

Strengths of this study include premorbid longitudinal measures of LDL-C and systematic detailed cognitive, medical and neuropathological assessments. Specifically, blood lipids were measured annually over a median of 4 years (range 1–13). Annual cognitive assessments allowed us to censor blood lipid measurements if the cognitive diagnosis changes. In the first sensitivity analysis, only individuals with NCI at baseline were included and lipid measures were censored if the diagnosis changed to either MCI or dementia. Despite the loss of power due to the loss of about 180 individuals compared with the main analysis, results of this control-only sensitivity analysis show higher LDL-C is associated with higher burden of AD neuropathology, which is consistent findings from the main analysis. To explore the potential for reverse causality of dementia on blood lipids, our second sensitivity analysis included all blood lipids, regardless of diagnosis and these results show a mild attenuation of the strength of significant associations identified in the primary analysis, which suggests that dementia may alter blood lipids. The detailed medication records enabled us to account for lipid lowering medications, most commonly statin drugs, in our analyses. The comprehensive and systematic neuropathological assessment provided 12 measures of age-related brain pathologies for each individual and enabled us to comprehensively survey brain neuropathologies and blood lipids. The

comprehensive nature of these neuropathologies shows the specificity of the association between LDL-C and measures of AD neuropathology. Additionally, the orthogonal assessments of AD pathology (with silver staining or immunohistochemistry) strengthen the findings given all measures of AD neuropathology were associated with premorbid LDL-C. Finally, ROS/MAP studies are community-based with a low lost to follow-up (8.7%) and high rate of autopsy completion (86.7%), which aids generalisability of these findings.

Our findings suggest lowering premorbid LDL-C would reduce AD neuropathology and, consequently, this would be expected to mitigate prevalence of AD, dementia and cognitive decline in the general population. Future work should investigate basic mechanisms of the association between LDL-C and AD neuropathology, and public health recommendations should strongly consider adding LDL-C to the list of potentially modifiable dementia risk factors.

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