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FEATURED ARTICLE

APOE ε2 resilience for Alzheimer's disease is mediated by plasma lipid species: Analysis of three independent cohort studies

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Abstract

Introduction: The apolipoprotein E (*APOE*) genotype is the strongest genetic risk factor for late-onset Alzheimer's disease. However, its effect on lipid metabolic pathways, and their mediating effect on disease risk, is poorly understood.

Methods: We performed lipidomic analysis on three independent cohorts (the Australian Imaging, Biomarkers and Lifestyle [AIBL] flagship study, n = 1087; the Alzheimer's Disease Neuroimaging Initiative [ADNI] 1 study, n = 819; and the Busselton Health Study [BHS], n = 4384), and we defined associations between *APOE* ε2 and ε4 and 569 plasma/serum lipid species. Mediation analysis defined the proportion of the treatment effect of the *APOE* genotype mediated by plasma/serum lipid species.

Results: A total of 237 and 104 lipid species were associated with *APOE* ε2 and ε4, respectively. Of these 68 (ε2) and 24 (ε4) were associated with prevalent Alzheimer's disease. Individual lipid species or lipidomic models of *APOE* genotypes mediated up to 30% and 10% of *APOE* ε2 and ε4 treatment effect, respectively.

Discussion: Plasma lipid species mediate the treatment effect of *APOE* genotypes on Alzheimer's disease and as such represent a potential therapeutic target.

KEYWORDS
APOE ε2, *APOE* ε4, Alzheimer's disease, lipidomics, lipid species, mass spectrometry

1 | PART 1–NARRATIVE

The apolipoprotein E (*APOE*) gene is by far the largest genetic risk factor for sporadic Alzheimer's disease (AD).[1,2] Despite its identification and characterization nearly three decades ago, the mechanism by which the gene it influences sporadic AD onset and progression remains to be fully determined. There are two alleles of interest: the ε4 allele dramatically increases risk for sporadic AD, whereas the ε2 allele provides protection or resilience. The encoded protein (apolipoprotein E [apoE]) is involved in lipoprotein transport and metabolism. In peripheral circulation, apoE associates with triglyceride-rich lipoprotein particles (chylomicrons and very low-density lipoprotein). Despite its annotation as a causal genetic variant of sporadic AD, defining the underlying mechanism and the therapeutic potential remain elusive and are topics of considerable interest.

1.1 | Current state of knowledge

Because amyloid beta (Aβ) is central to many hypotheses in both familial and sporadic AD pathogenesis, the relationship between *APOE* and sporadic AD has been largely investigated in the context of Aβ accumulation and clearance. Multiple studies have demonstrated a high proportion of brain Aβ in healthy ε4-positive individuals relative to the other alleles,[3,4] with supporting evidence in human stem-cell–derived neuronal studies highlighting increased Aβ production with the ε4 allele.[5,6] In vitro studies have indicated indirect roles for *APOE* in Aβ clearance via interaction with microglia[7] and other neuronal cells.[8] Despite evidence of the involvement of *APOE* with Aβ, no clear mechanism has been identified. Because therapeutics targeted toward Aβ have largely been unsuccessful, it is likely that several underlying pathways are involved in sporadic AD development.

As a key constituent of lipoproteins and lipid transport, a logical role for *APOE* variants in sporadic AD development would be through perturbations to lipid metabolism. The direct effect of *APOE* variants on human peripheral lipoprotein metabolism has been examined intensively.[9–12] Comprehensive Nuclear Magnetic Resonance (NMR) lipoprotein profiling shows that *APOE ε4* leads to minor increases in nearly all lipoprotein subclasses, whereas *APOE ε2* results in stronger changes to the lipoprotein profile.[13] In the central nervous system, apoE is the most abundant lipoprotein, playing an important role in lipid transport and cholesterol homeostasis.[14] Although lipoproteins are the main carriers of lipids and are studied more extensively in the context of AD, it has been proposed that lipid metabolism— represented by the complex lipid metabolic pathways responsible for the synthesis, interconversion, and catabolism of the small amphiphilic molecules that make up lipoprotein particles in addition to cellular membranes—plays a more critical role in AD pathogenesis.[15,16] The effects of *APOE* variants appear to mildly alter the relationship between peripheral lipid metabolites and the strength of association with AD.[17,18] These findings collectively support a potential relationship between lipid metabolism, *APOE* genotypes, and AD risk.

1.2 | Knowledge gap, the study approach, and other alternatives

Sporadic AD is a complex disease unique to the human population, evident only through our relatively long life-span and higher cognitive function. Thus studies in human populations are important and necessary to understand the complex relationships that exist. Lipidomics is a specialized field examining lipid metabolites in biological systems, and it has typically been limited to small sample sizes. Recent advances have paved the way for population-level approaches that provide the power to examine associations within the variability of human diversity. Owing to the lack of large human studies conducted in the field, the associations between *APOE* genotypes, circulating lipid metabolites, and the relationship with sporadic AD risk have not been examined in detail.

To address this gap in knowledge, we examined the associations between plasma lipid species and *APOE* genotypes in three large cohorts comprising the Busselton Health Study (BHS), the Australian Imaging, Biomarkers and Lifestyle (AIBL) flagship study, and the Alzheimer's Disease Neuroimaging Initiative (ADNI) 1 study. The associations were determined independent of disease using the BHS, a largely healthy population cohort from Australia, to avoid reverse causation. Associations with *APOE* were then contrasted to lipid associations observed with prevalent AD. Finally, mediation analyses using individual lipid species or combined *APOE* lipid summary scores were performed to assess the role of lipid metabolism in mediating risk of *APOE ε2* and *ε4* alleles on AD (Figure 1).

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors reviewed the literature using PubMed and Google to identify recent reports of the risk factors for Alzheimer's disease (AD) and the risk associated with the apolipoprotein E (*APOE*) genotypes. Although the relationship between *APOE* genotypes and lipoprotein levels has been reported previously, no reports on the relationship with the underlying lipid metabolism were found.
2. **Interpretation**: We report detailed associations between *APOE* genotypes and plasma lipid species, indicating an effect on lipid metabolism. The *APOE ε2* allele showed stronger associations with ether lipid species than the *APOE ε4* allele, with these lipid species mediating up to 30% of *APOE ε2* treatment effect, leading to increased resilience.
3. **Future Directions**: Understanding the relationship between ether lipid metabolism, AD risk and resilience presents new therapeutic options to delay or prevent the onset of disease.

1.3 | Findings

The mechanism by which *APOE* genotypes modulate risk has yet to be fully elucidated, and thus identifying this could pave way for additional modulatory therapeutic targets. We summarize the main findings into three major categories. (1) We identified multiple associations with specific lipid classes and species that were independent of clinical lipoprotein measurements, (2) we identified age-specific interactions between the associations of lipid species and *APOE* genotypes, and (3) we demonstrated that lipid species partially mediate the AD risk resulting from inherited *APOE* genotypes.

1.4 | Lipid metabolites are strongly associated with *APOE* genotype

We noted that *APOE* genotypes were associated with circulating lipoprotein levels in all three cohorts only in healthy individuals, in particular, higher high-density lipoprotein cholesterol (HDL-C) and lower total cholesterol were evident in individuals with the *APOE ε2* allele (Table 1). These associations have been observed previously[9–12,19,20] and highlight the importance of considering clinical lipids when examining lipidomic associations. Without adjusting for clinical lipids, associations with lipid species were influenced by the relative levels of lipoproteins in circulation. However, after adjustment for clinical lipids, the resulting associations highlight altered lipid species composition as a

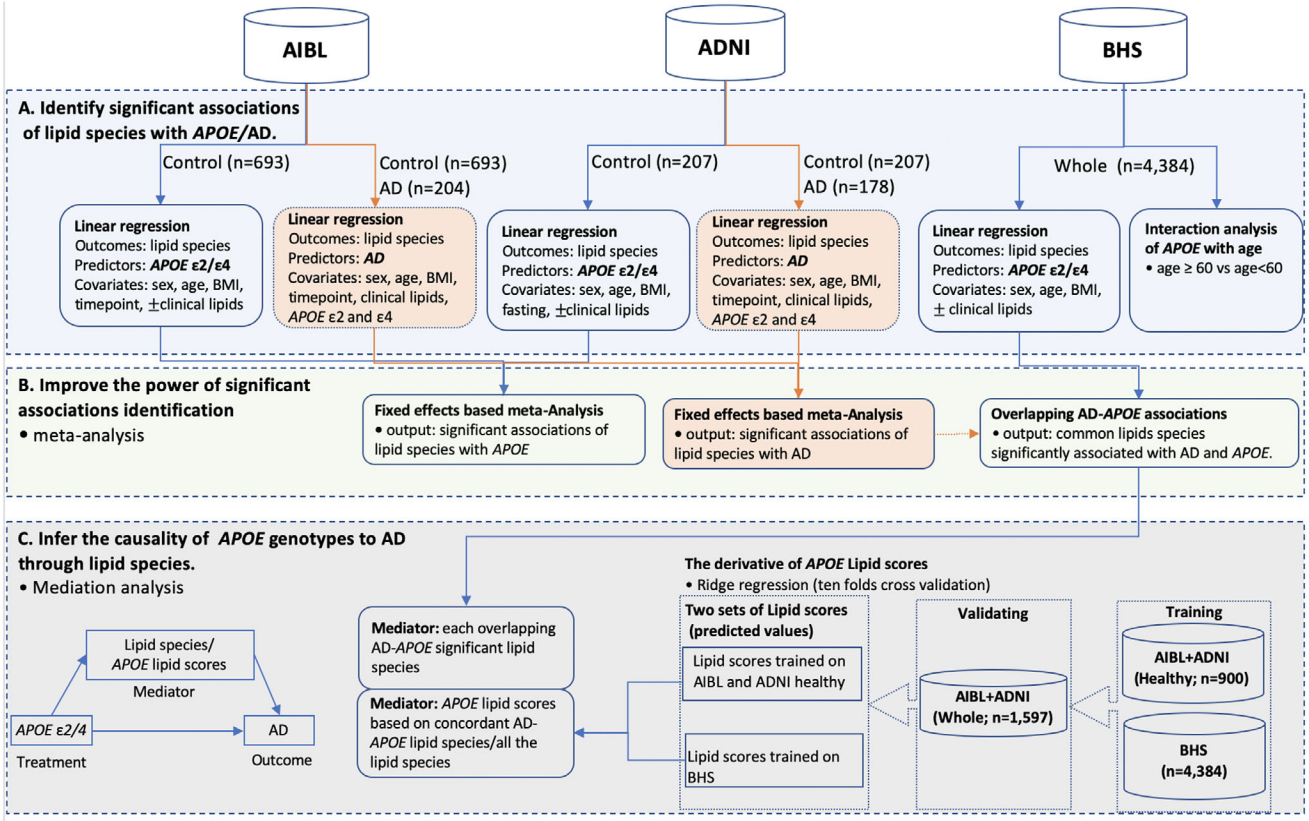


FIGURE 1 Study design. In this study, the analyses include three main sections: the identification of the significant associations of lipid species with the apolipoprotein E(*APOE*) gene and prevalent Alzheimer's disease (AD) (A), the improvement of the power of the associations by meta-analysis combining Australian Imaging, Biomarkers and Lifestyle (AIBL) and Alzheimer's Disease Neuroimaging Initiative (ADNI) (B), and the causality inference of *APOE* genotypes to prevalent AD through lipid species by mediation analysis (C). (A) For each participant, we utilized available samples at their last acquired time point (n = 1087) to maximize the number of participants in the association studies. Lipid association studies with *APOE* were performed using only the control (CN) subset, whereas associations with AD prevalence was examined between the control and AD subsets. Covariates fitted into the models included age, sex, and BMI. Models for ADNI included fasting status, whereas models for AIBL included sample time point. To identify associations that were independent of lipoprotein metabolism, a second set of analyses was performed with further adjustment for clinical lipids (total cholesterol, HDL-C, and triglycerides). Associations were corrected for multiple comparisons using the method of Benjamini Hochberg (BH).[60] In the Busselton Health Study (BHS), interaction of *APOE* genotypes and age was examined using a binary cut-off of 60 years (Table S6). The "±clinical lipids" means the linear regression was performed separately with/without clinical lipids adjustment. (B) Associations between *APOE* genotypes and lipid species and the associations between AD prevalence and lipid species were analyzed using a fixed-effect inverse-variance weighted meta-analysis. Heterogeneity between AIBL and ADNI was assessed using Cochran Q. (C) The mediation analysis was performed on the combined AIBL and ADNI data sets, treating AD as the outcome and *APOE* genotypes as the treatment. There were two types of mediators: (1) individual lipid species (that showed concordant associations with *APOE* and AD from the previous analyze) and (2) *APOE* lipid scores. The lipid scores were created by ridge regression using either the lipid species concordant in association with AD/*APOE* or all the lipid species to predict *APOE* ε2/ε4. The models were trained on either the BHS cohort (n = 4384) or the combined AIBL and ADNI cohorts (Control; n = 900), followed with an external validation on the whole population of combined AIBL and ADNI cohorts (n = 1597). The resulting predicted values on the validate set were the *APOE* lipid scores that were treated as mediators for the mediation analysis

potential effect of *APOE* genotype. Of note, *APOE* ε2 exhibited stronger associations with the plasma lipidome than the *APOE ε4* .

In the BHS study, we observed 29 lipid classes and 347 lipid species significantly associated with *APOE* ε2 after correction for multiple comparisons (Figure 2; Tables S1 and S2). Adjustment for clinical lipids, to identify associations independent of lipoprotein metabolism, resulted in 28 lipids classes and 237 lipid species significantly associated. There were 20 classes and 133 species associated with *APOE* ε2 in the meta-analysis of the fully adjusted AIBL and ADNI models (including healthy individuals only). Comparison of the two analyses identified 120 concordant lipid species, nominally significant in both analyses, including species of 18 lipid classes including ceramide, hexosylceramide, sphingomyelin, plasmalogens, alkyldiacylglycerol, and cholesteryl esters.

Fewer significant associations with *APOE* ε4 were observed in both the BHS and meta-analysis of the AIBL and ADNI cohorts. There were 23 lipid classes and 223 lipid species significantly associated with *APOE* ε4 *in BHS*, after correction for multiple comparisons (Figure S1; Tables S3 and S4). When we further adjusted for clinical lipids, we observed 18 lipid classes and 104 lipid species significantly associated with *APOE* ε4, after correction for multiple comparisons (Figure 2, Tables

TABLE 1 Basic characteristics of participants from the Australian Imaging, Biomarkers and Lifestyle (AIBL) flagship study, Alzheimer's Disease Neuroimaging Initiative (ADNI), and the Busselton Health Study (BHS)

AIBL								
	Number of *APOE* ε2 alleles				Number of *APOE* ε4 alleles			
	0	1	2	*P* value	0	1	2	*P*-value
N	564	124	5		520	163	10	
Age (years)	75.2 (6.5)	75.5 (6.9)	75.4 (5.2)	.927	75.7 (6.7)	74.2 (5.8)	71.6 (6.2)	.01
Gender (% female)	327 (58.0)	77 (62.1)	4 (80.0)	.44	303 (58.3)	100 (61.3)	5 (50.0)	.665
BMI (kg/m^2)	26.30(4.4)	26.3 (4.4)	25.6 (5.1)	.927	26.3 (4.2)	26.3 (4.8)	26.6 (4.2)	.966
HDL-C (mmol/L)	1.56 (0.41)	1.67 (0.47)	1.58 (0.54)	.029	1.58 (0.43)	1.56 (0.42)	1.68 (0.46)	.656
Total Cholesterol (mmol/L)	5.28 (1.12)	5.15 (1.05)	5.52 (2.41)	.444	5.23 (1.11)	5.35 (1.16)	5.51 (0.94)	.4
Triglycerides (mmol/L)	1.27 (0.61)	1.19 (0.54)	2.30 (2.42)	.001	1.25 (0.63)	1.32 (0.65)	1.15 (0.46)	.4
1	37 (6.6)	8 (6.5)	0 (0.0)		38 (7.3)	7 (4.3)	0 (0.0)	
2	57 (10.1)	15 (12.1)	1 (20.0)		55 (10.6)	17 (10.4)	1 (10.0)	
3	28 (5.0)	5 (4.0)	0 (0.0)		23 (4.4)	10 (6.1)	0 (0.0)	
4	63 (11.2)	9 (7.3)	0 (0.0)		53 (10.2)	17 (10.4)	2 (20.0)	
5	379 (67.2)	87 (70.2)	4 (80.0)		351 (67.5)	112 (68.7)	7 (70.0)	
ADNI								
N	174	31	2		152	50	5	
Age (years)	75.8 (4.8)	75.2 (5.6)	73.5 (4.7)	.661	75.7 (5.0)	76.0 (5.0)	74.6 (3.8)	.812
Gender (%female)	82 (47.1)	19 (61.3)	0 (0.0)	.133	76 (50.0)	23 (46.0)	2 (40.0)	.819
BMI (kg/m^2)	26.8 (4.4)	26.5 (4.0)	28.5 (1.2)	.782	27.2 (4.5)	25.8 (3.9)	24.8 (2.3)	.087
HDL-C (mmol/L)	1.36 (0.43)	1.43 (0.58)	1.32 (0.44)	.73	1.39 (0.44)	1.35 (0.49)	1.24 (0.44)	.693
Cholesterol (mmol/L)	4.74 (0.93)	4.76 (0.95)	3.37 (1.23)	.119	4.72 (0.94)	4.81 (0.96)	4.37 (0.66)	.566
Triglycerides (mmol/L)	1.42 (0.76)	1.54 (1.06)	0.93 (0.54)	.516	1.37 (0.68)	1.61 (1.11)	1.36 (0.64)	.19
Fasting status (% fasting)	16 (9.2)	3 (9.7)	0 (0.0)	.9	17 (11.2)	0 (0.0)	2 (40.0)	.003
BHS								
N	3625	733	26		3221	1061	102	
Age (years)	50.4 (17.3)	50.7 (17.6)	50.7 (16.7)	.9	50.67 (17.5)	50.2 (17.0)	47.9 (17.0)	.228
Gender (% female)	2032 (56.1)	413 (56.3)	16 (61.5)	.848	1836 (57.0)	572 (53.9)	53 (52.0)	.147
BMI (kg/m^2)	26.0 (4.2)	26.1 (4.2)	25.6 (4.3)	.844	26.0 (4.2)	26.0 (4.2)	25.4 (4.0)	.315
HDL-C (mmol/L)	1.38 (0.39)	1.44 (0.41)	1.45 (0.40)	.001	1.40 (0.39)	1.37 (0.40)	1.32 (0.37)	.024
Cholesterol (mmol/L)	5.65 (1.10)	5.26 (1.10)	4.37 (1.14)	<.001	5.52 (1.12)	5.74 (1.08)	5.65 (1.09)	<.001
Triglycerides(mmol/L)	1.29 (0.91)	1.34 (0.90)	1.38 (1.01)	.43	1.28 (0.89)	1.38 (0.91)	1.33 (1.25)	.009

*In this study, we only used the records at the last time point in AIBL cohort.

S3 and S4). Meta-analysis of the fully adjusted models in the AIBL and ADNI cohorts identified three lipid species associated with *APOE* ε4, after correction, and 91 lipid species that were nominally significant, of which 43 species were also nominally significant in the BHS cohort.

1.5 | *APOE* lipid associations are weaker with increasing age

We observed stronger associations within the BHS cohort than the AIBL and ADNI combined meta-analysis, beyond the expected differences from the larger sample size. Owing to the larger age range of the BHS compared to the AIBL and ADNI cohorts, we hypothesized that age might influence how *APOE* genotype associates with plasma lipids. Interaction analysis with a binary cut-off at age 60 using the BHS cohort (age <60, n = 2884; age ≥ 60, n = 1368; Tables S5) identified a nominal significant interaction of age with the association of *APOE* ε2 with 48 lipid species from 12 classes (Figure 3, Table S6). A greater number of the associations of *APOE* ε4 with lipid species were observed to have interaction effects of age (88 lipid species from 18 classes; Figure 4, Table S7). These included species of phosphatidylethanolamine, alkylphosphatidylethanolamine, alkenylphosphatidylethanolamine, and lysophosphatidylethanolamine

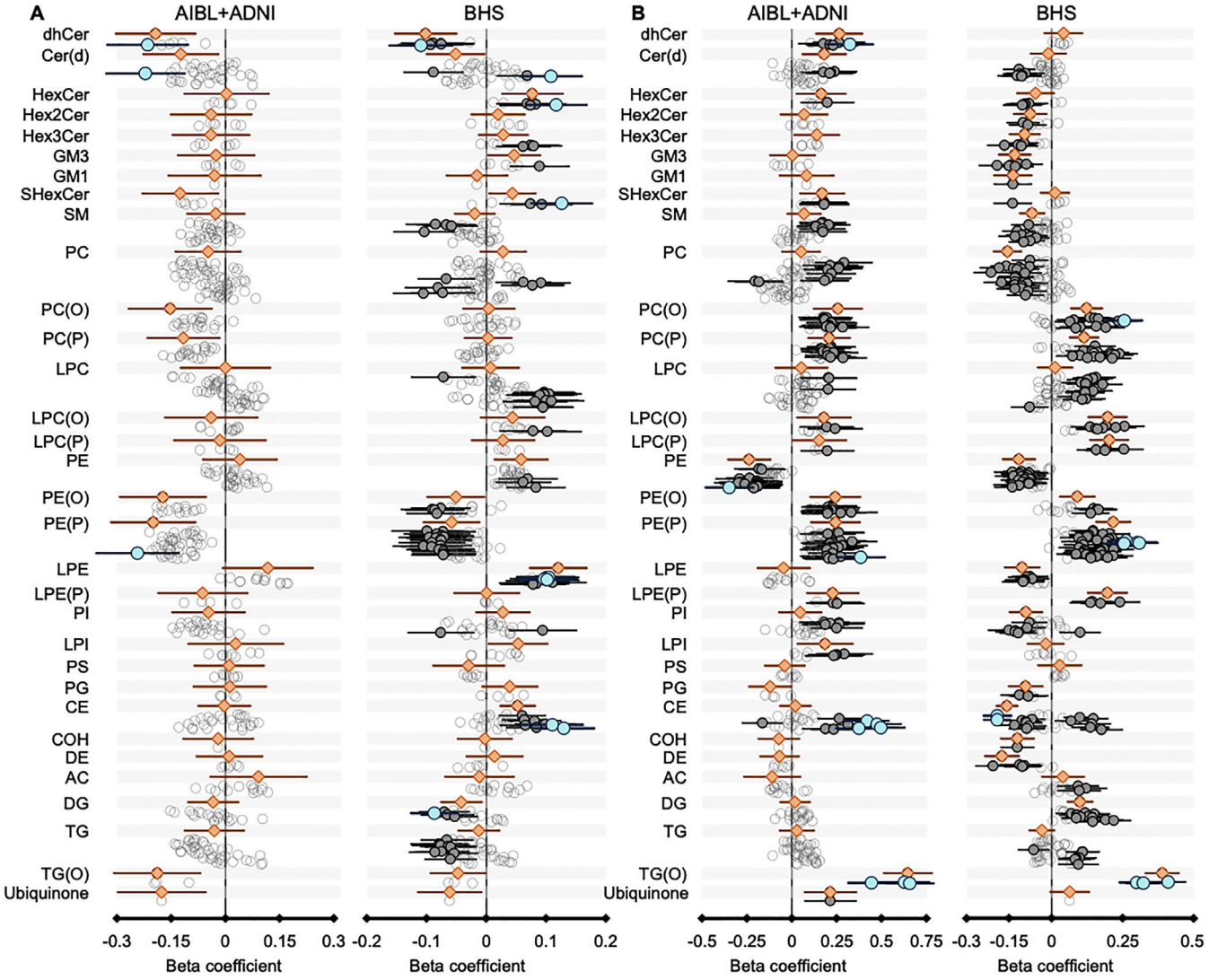


FIGURE 2 Association of *APOE ε4* (A) and *APOE ε2* (B) with lipid species in the Australian imaging, Biomarkers and Lifestyle (AIBL); Alzheimer's Disease Neuroimaging Initiative (ADNI); and Busselton Health Study (BHS) cohorts. ***Linear regression analyses of *APOE ε4*/*ε2* against lipid species were performed adjusting for age, sex, BMI, total cholesterol, HDL-C, triglycerides, timepoint (specific for AIBL), and fasting status (specific for ADNI). Meta-analyses were performed by combining AIBL and ADNI data. Gray open circles, corrected $P > .05$; gray closed circles, corrected $P < .05$; blue circles, top 10 species ranked by *P*-value; orange diamonds, lipid classes

Of interest, these results highlight the amelioration of the genotype effect in the older group (≥60), particularly for the phosphatidylethanolamine classes that show a strong association with AD.[16,17] This coincides with observations in the healthy AIBL and ADNI population where lipid associations with the *APOE* genotype, particularly the *ε4* allele, were weaker. The large meta-analysis conducted by Farrer et al., described a similar age–*APOE* relationship, where the risk of AD from *ε4* allele was considerably reduced at ages > 65 to 70.[1] Although the exact mechanism behind the reduced association with increasing age remains to be determined, one possibility is that individuals who ultimately maintain the lower level of ether lipids progress to develop MCI, AD, or possibly other metabolic diseases including cardiovascular disease, where plasmalogens have shown a negative association,[21,22] leading to a survivorship bias. Because *APOE ε4* associations were determined with healthy controls only, a survivorship bias may result a reduction in the strength of the associations between *APOE ε4* genotype and plasma lipids with increasing age.

1.6 | *APOE ε2* resilience to AD is mediated via the peripheral lipidome

Ether lipids showed concordant risk profiles for *APOE* genotype and AD. Ether lipid associations with AD have been reported previously,[23–25] also within the AIBL and ADNI studies.[16,26] Some studies have suggested that these lipids confer protection and may become depleted in disease progression (see reviews[27–29]). Species

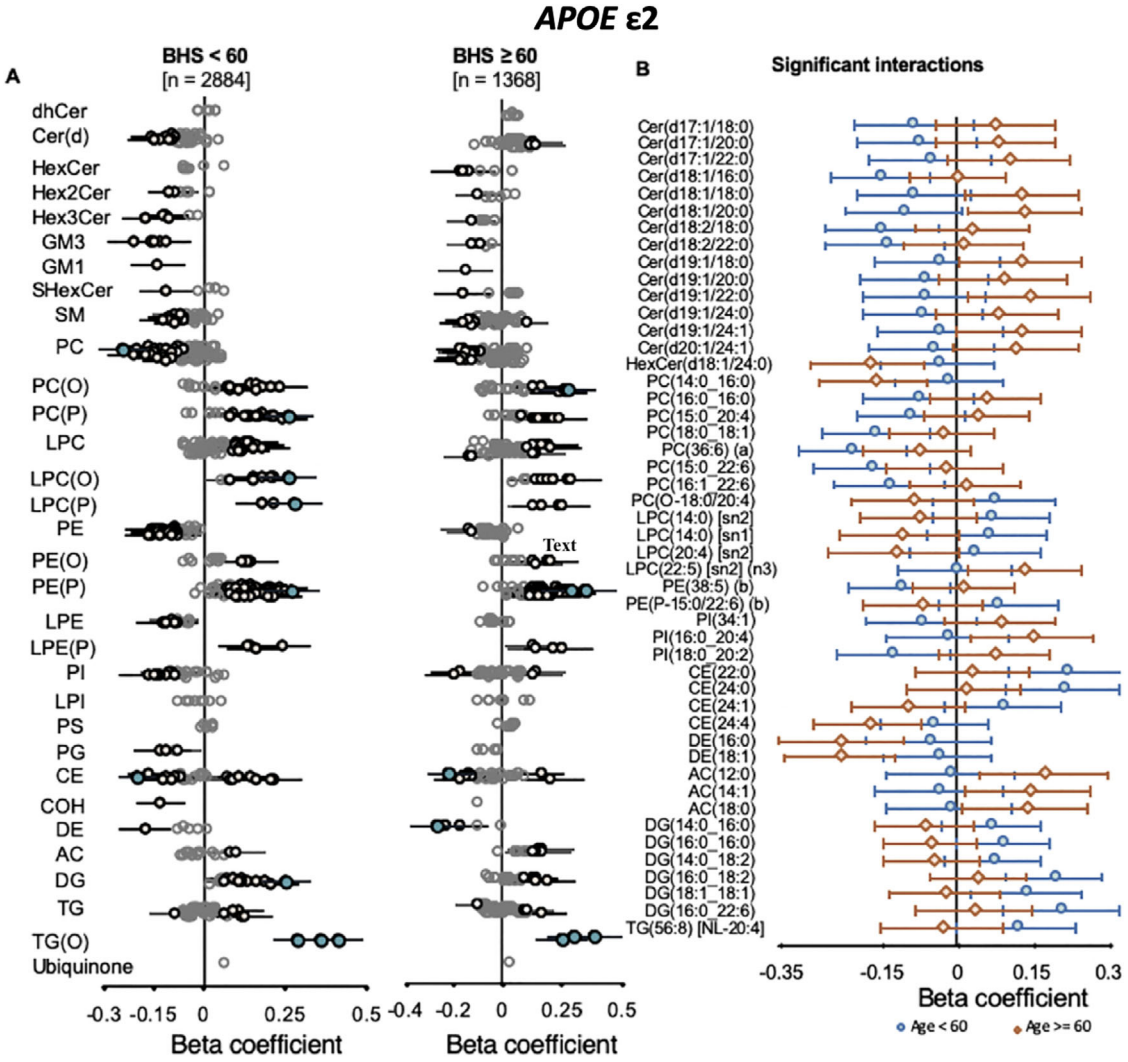


FIGURE 3 Interaction of age on the associations of *APOE* ε2 with peripheral lipid species. Linear regression analyses of *APOE* ε2 against lipid species, adjusted for *APOE* ε4, age, sex, BMI, total cholesterol, HDL-C, and triglycerides with the interaction terms of age (binary cut-off at 60-years-old, Panel A) was performed in the Busselton Health Study (BHS) cohort. Beta coefficients and 95% CI for each group were plotted (left panels). Beta coefficients and 95% CI for lipid species showing significant interaction (*P* value < .05) are plotted together (right panel)

from the ether lipid group are structurally different from other lipids species by having the characteristic fatty alcohol instead of a carboxylic acid in the *sn1* position of the glycerol backbone. Plasmalogens are a subclass of ether lipids, with the characteristic vinyl-ether bond linking the alcohol to the glycerol backbone. These lipids are peroxisome-dependent species that have been highly implicated in AD.

Here we demonstrate that ether lipids, in particular, are lower in individuals with the *APOE* ε4 allele, and higher in individuals with the *APOE* ε2 allele, independent of changes in circulating lipoprotein levels. Furthermore, the same lipid species were associated with AD (Figure 5; Tables S8-S10). In mediation analyses, we observed that up to 36% of the AD risk from *APOE* ε2 was mediated through peripheral lipid species, notably the alkyldiacylglycerol and plasmalogens species, with TG (O-52:2) [NL-16:0] showing the strongest mediation effect (Figure S2, Table S11), whereas lipid scores created against AD mediated up to 24% the AD risk from *APOE* ε2 (Table 2). This mediation of AD risk by peripheral lipid species or lipid scores was much lower for the *APOE* ε4 allele (Table 2; Figure S2; Table S12). These mediation analyses strengthen the evidence for the involvement of ether lipids in AD etiology. Because lipids constitute modifiable risk factors, and dietary supplements are available to increase circulating ether lipids in humans,[27] this raises the possibility of risk reduction through ether lipid modulation.

Alkyl diacylglycerols is notable owing to its ether-linkage in the *sn1* position (with fatty acyl linkages in the *sn2* and *sn3* positions), placing them in the same family as ether lipids and plasmalogens, a lipid class reported to be negatively associated with AD.[16,23,30,31] Although the biosynthetic origin of the alkyldiacylglycerols in the periphery remains largely uncharacterized, it is clear that they are derived from the peroxisomal pathway that synthesizes 1-O-alkyl-dihydroxyacetone phosphate, which is then converted into 1-O-alkyl-2-acylglycerol in the endoplasmic reticulum, at which point it can be converted into either

TABLE 2 Mediation analysis of lipid species–based lipid scores for prevalent Alzheimer's disease (AD) cases and *APOE* ε4 (A)/ε2 (B)

	Concordant lipid species–based lipid scores						All lipid species–based lipid scores					
	Lipid score trained on combined AIBL and ADNI (Healthy)			Lipid score trained on BHS			Lipid score trained on combined AIBL and ADNI (Healthy)			Lipid score trained on BHS		
	Est.	95% CI	*P*-value	Est.	95% CI	*P*-value	Est.	95% CI	*P*-value	Est.	95% CI	*P*-value
A. *APOE* ε2												
Total effect	−0.13	(−0.18 - −0.07)	<2e-16	−0.13	(−0.18 - −0.07)	<2e-16	−0.12	(−0.18 - −0.07)	<2e-16	−0.13	(−0.18 - −0.07)	<2e-16
Prop. mediated (Cont)	0.28	(0.12 - 0.55)	8.0e-04	0.24	(0.13 - 0.43)	<2e-16	−0.09	(−0.4 - 0.15)	4.3e-01	0.27	(0.15 - 0.51)	<2e-16
Prop. mediated (AD)	0.19	(0.06 - 0.48)	8.0e-04	0.16	(0.07 - 0.37)	<2e-16	−0.06	(−0.25 - 0.1)	4.3e-01	0.19	(0.08 - 0.45)	<2e-16
ACME (Avg)	−0.03	(−0.05 - −0.01)	8.0e-04	−0.03	(−0.04 - −0.01)	<2e-16	0.01	(−0.02 - 0.03)	4.3e-01	−0.03	(−0.04 - −0.02)	<2e-16
ADE (Avg)	−0.10	(−0.15 - −0.04)	1.6e-03	−0.10	(−0.16 - −0.05)	1.2e-03	−0.13	(−0.19 - −0.07)	<2e-16	−0.10	(−0.15 - −0.04)	2.8e-03
Prop. mediated (Avg)	0.24	(0.09 - 0.52)	8.0e-04	0.20	(0.1 - 0.4)	<2e-16	−0.08	(−0.32 - 0.13)	4.3e-01	0.23	(0.12 - 0.48)	<2e-16
B. *APOE* ε4												
Total effect	0.19	(0.16 - 0.22)	<2e-16	0.19	(0.16 - 0.22)	<2e-16	0.18	(0.16 - 0.22)	<2e-16	0.19	(0.16 - 0.22)	<2e-16
Prop. mediated (Cont)	0.05	(0.02 - 0.08)	1.4e-03	0.07	(0.04 - 0.11)	<2e-16	−0.01	(−0.05 - 0.03)	8.1e-01	0.05	(0.03 - 0.09)	<2e-16
Prop. mediated (AD)	0.08	(0.03 - 0.13)	1.4e-03	0.11	(0.07 - 0.17)	<2e-16	−0.01	(−0.09 - 0.07)	8.1e-01	0.09	(0.04 - 0.13)	<2e-16
ACME (Avg)	0.01	(0 - 0.02)	1.4e-03	0.02	(0.01 - 0.03)	<2e-16	0.00	(−0.01 - 0.01)	8.1e-01	0.01	(0.01 - 0.02)	<2e-16
ADE (Avg)	0.18	(0.15 - 0.21)	<2e-16	0.17	(0.14 - 0.2)	<2e-16	0.19	(0.16 - 0.23)	<2e-16	0.18	(0.14 - 0.21)	<2e-16
Prop. mediated (Avg)	0.06	(0.02 - 0.11)	1.4e-03	0.09	(0.06 - 0.14)	<2e-16	−0.01	(−0.07, 0.06)	8.1e-01	0.07	(0.03 - 0.11)	<2e-16

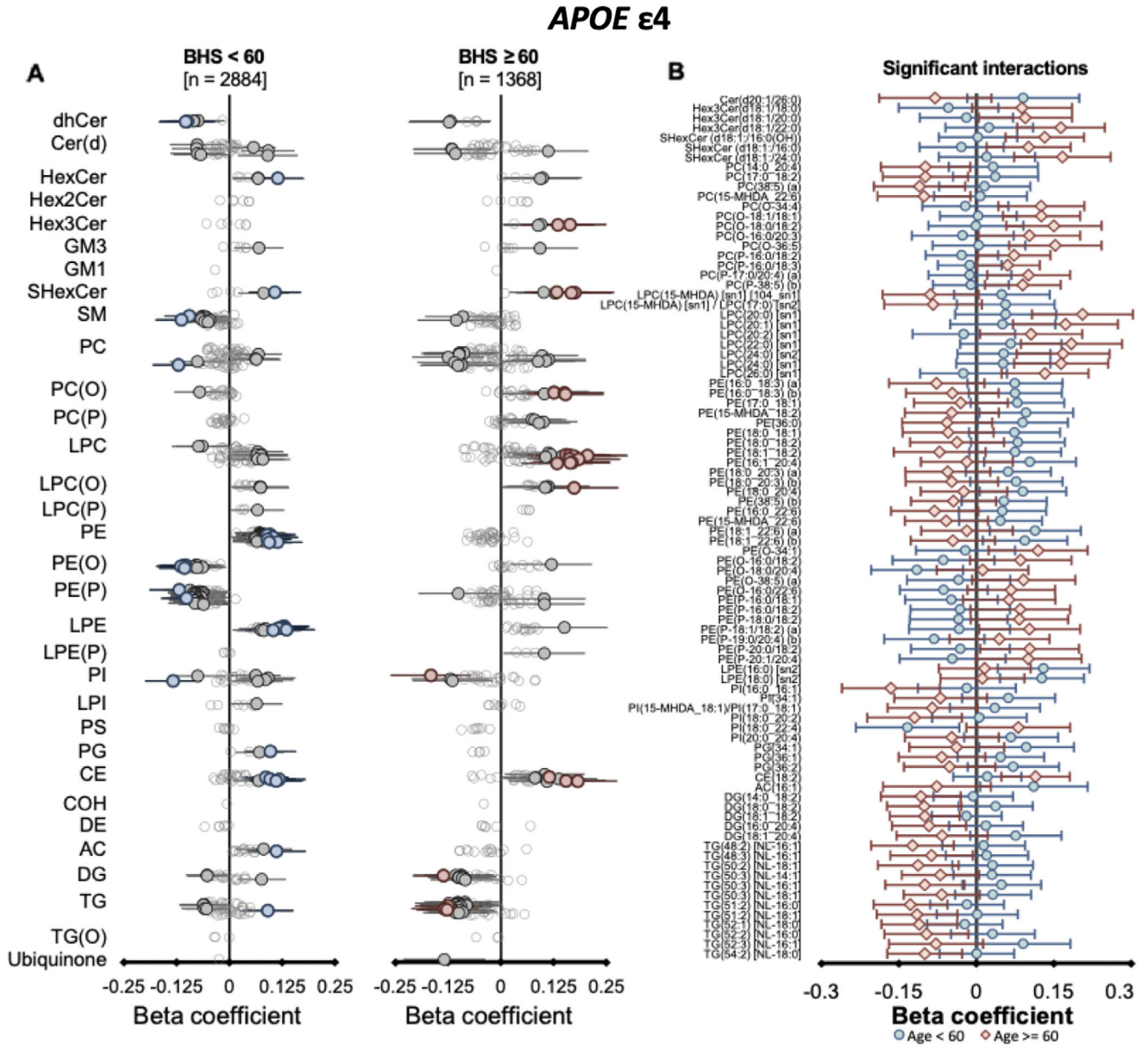
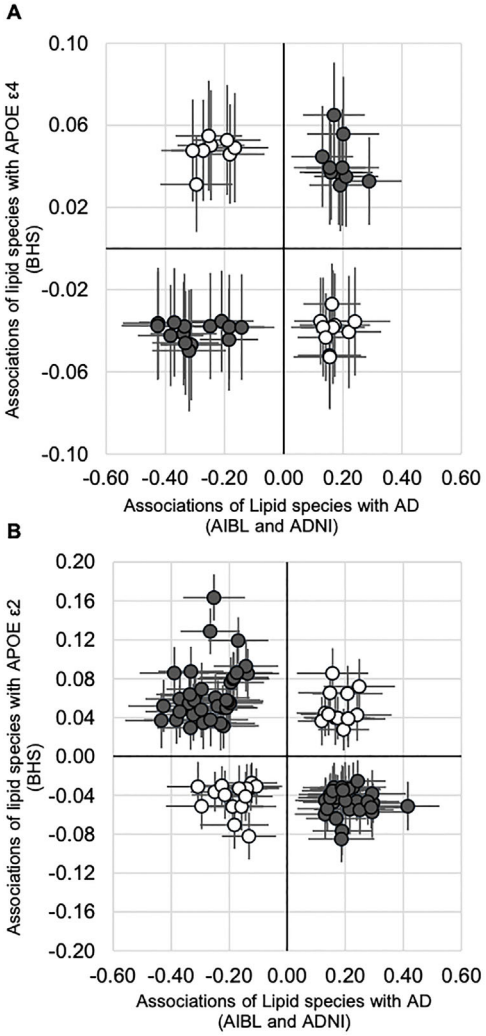


FIGURE 4 Interaction of age on the associations of *APOE* ε4 with peripheral lipid species. Linear regression analyses of *APOE* ε4 against lipid species, adjusted for *APOE* ε2, age, sex, BMI, total cholesterol, HDL-C, and triglycerides with the interaction terms of age (binary cut-off at 60-years-old, Panel A) was performed in the Busselton Health Study (BHS) cohort. Beta coefficients and 95% CI for each group were plotted (left panels). Beta coefficients and 95% CI for lipid species showing significant interaction (*P* value < .05) are plotted together (right panel)

plasmalogens or alkyl diacylglycerol. *APOE* ε2 dramatically elevates the levels of both of these lipid classes, above those of typical phospholipid or triglyceride species. The *APOE* ε2 polymorphism may have selective preference for alkyldiacylglycerols and other ether lipids in incorporation into lipoproteins. Alternatively, the turnover rate of lipoproteins with *APOE* variants has been highlighted previously, where changes to the metabolic flux rate of lipoproteins due to their interactions with the low-density lipoprotein (LDL) receptor may influence its composition.[32] Although ether lipids are a diverse group of lipids, it is well known that plasmalogens represent the more biologically active lipid class, having been linked to anti-oxidative and anti-inflammatory properties.[27,33,34] Potentially the alkyldiacylglycerol species rather represent sensitive markers of ether lipid synthesis or turnover perturbation. In contrast to *APOE* ε2, the *APOE* ε4 allele showed a weaker association with lipid species, and those same species and the lipid scores mediated only a small proportion of the *APOE* ε4 risk on AD (7% and 9%, respectively), although here also the plasmalogen species were the strongest mediators (Table 2; Figure S2; Table S12). This raises the possibility that the two common polymorphisms of *APOE* mediate risk through alternate mechanisms, with the resilient effect of *APOE* ε2 being more strongly influenced by its effect on ether lipid metabolism in the periphery.



C

Positive with ε2 Negative with AD	Negative with ε2 Positive with AD	Positive with ε4 Positive with AD
PC(O-32:2)	Cer(d18:1/16:0)	HexCer(d18:1/24:1)
PC(O-34:4)	HexCer(d18:1/24:1)	Hex3Cer(d18:1/22:0)
PC(O-16:0/22:6)	Hex3Cer(d18:1/16:0)	Hex3Cer(d18:1/24:1)
PC(O-18:0/22:6)	Hex3Cer(d18:1/22:0)	GM3(d18:1/24:0)
PC(O-40:7) (a)	Hex3Cer(d18:1/24:1)	LPC(O-24:1)
PC(P-16:0/22:6)	GM3(d18:1/16:0)	PE(16:1_20:4)
PC(P-18:0/22:6)	GM3(d18:1/20:0)	LPE(20:4) [sn2]
PC(P-18:1/22:6)	GM3(d18:1/22:0)	LPE(20:4) [sn1]
LPC(20:5) [sn2]	GM3(d18:1/24:0)	CE(22:4)
LPC(20:5) [sn1]	GM3(d18:1/24:1)	CE(24:1)
PE(O-36:5)	GM1(d18:1/16:0)	
PE(O-18:1/22:6)	SM(d18:2/18:1)	**Negative with ε4 Negative with AD**
PE(P-15:0/22:6) (a)	SM(43:2) (b)	dhCer(d18:0/22:0)
PE(P-16:0/20:4)	PC(33:2)	dhCer(d18:0/24:0)
PE(P-16:0/20:5)	PC(16:0_18:1)	Cer(d18:1/24:0)
PE(P-16:0/22:5) (n3)	PC(16:1_18:2)	PE(O-18:1/22:6)
PE(P-16:0/22:6)	PC(16:0_18:3) (a)	PE(P-15:0/22:6) (a)
PE(P-17:0/20:4) (b)	PC(15-MHDA_18:2)	PE(P-15:0/22:6) (b)
PE(P-17:0/22:6) (a)	PC(15-MHDA_20:4)	PE(P-16:0/20:5)
PE(P-17:0/22:6) (b)	PE(16:0_18:3) (b)	PE(P-16:0/22:5) (n3)
PE(P-18:0/20:5)	PE(15-MHDA_18:1)	PE(P-16:0/22:6)
PE(P-18:0/22:5) (n3)	PE(17:0_18:1)	PE(P-17:0/22:6) (a)
PE(P-18:0/22:6)	PE(15-MHDA_18:2)	PE(P-17:0/22:6) (b)
PE(P-18:1/20:5) (a)	PE(17:0_18:2)	PE(P-18:0/22:5) (n3)
PE(P-18:1/20:5) (b)	PE(16:0_20:4)	PE(P-18:0/22:6)
PE(P-18:1/22:6) (a)	PE(15-MHDA_20:4)	PE(P-18:1/20:5) (a)
PE(P-19:0/20:4) (b)	PE(17:0_20:4)	
PE(P-20:0/22:6)	PE(38:5) (a)	
LPE(P-16:0)	CE(22:4)	
AC(13:0)	CE(22:5) (n6)	
AC(15:0) (a)	CE(24:4)	
DG(16:0_22:6)	TG(50:3) [NL-16:1]	
TG(58:8) [NL-22:6]		
TG(O-50:1) [NL-16:0]		
TG(O-52:0) [NL-16:0]		
TG(O-52:2) [NL-16:0]		

FIGURE 5 Lipid species profiles significantly associated with apolipoprotein E (*APOE*) gene and prevalent Alzheimer's disease (AD). Meta-analysis of the Australian Imaging, Biomarkers and Lifestyle (AIBL) and Alzheimer's disease Neuroimaging Initiative (ADNI) cohorts was performed to identify lipid species associated with prevalent AD (linear regression of AD against lipid species, adjusted for *APOE* ε2, *APOE* ε4, age, sex, BMI, total cholesterol, HDL-C, and triglycerides). Linear regression of *APOE* ε4 (A) or *APOE* ε2 (B) against lipid species, adjusted for age, sex, BMI, total cholesterol, HDL-C, and triglycerides was performed in the in Busselton Health Study (BHS) cohort. The beta-coefficients for lipid species significant in both analyses were plotted against each other. Dark closed circles highlight species that are in a concordant direction with ε4 risk increase (A) or ε2 risk reduction (B)

1.7 | Next steps

Our analyses demonstrate that up to 36% of the AD resilience associated with *APOE* ε2 is mediated by lipid species (primarily ether lipids, alkyl diacylglycerol, and plasmalogen) and, to a lesser extent, the increased AD risk associated with *APOE* ε4 is also mediated by some of the same lipid species. These lipid species then represent a potential therapeutic target to reduce the risk of AD. However, it will be important to understand the mechanism(s) by which such ether lipids may attenuate disease risk.

Alkyl diacylglycerol is a naturally occurring class of lipids, particularly enriched in the livers of several species of sharks[35] and relatively abundant in human breast milk,[36] which, upon ingestion, can be metabolized into plasmalogens and other ether lipid species, to increase the levels of these lipids in circulation and within immune cells.[37] The use of alkyldiacylglycerol as a nutraceutical has been examined in the context of immune modulation[38 and a] potential treatment for specific cancers,[39] and the immune-modulating properties of ether lipids has been reviewed.[27,40]

There is growing evidence of an immune component in AD pathogenesis[41] and genetic evidence linking both immunity and lipid metabolism involvement with AD risk.[42] The involvement of *APOE* in the immune response[43,44] suggests that its potential role in risk reduction may be via modulation of immune cell function and behavior. More recently, ether lipids have been linked directly to ferroptosis,[45,46] a novel cell death mechanism that links together immunity,[47] iron metabolism, and AD.[48,49] Plasmalogens, an end product of the ether lipid biosynthetic pathway resulting from the formation of the vinyl

ether bond by the desaturase PEDS1/TMEM189, appear to play complex and possibly contradicting roles in ferroptosis.[45] Although our data do not directly link ferroptosis, *APOE* polymorphisms and AD together, the critical role of ether lipids in mediating these biological processes necessitates further examination. In vitro and in vivo studies exploring the role of ether lipids, ferroptosis, and immune cell function in the context of AD will likely provide some of the answers to the mechanisms by which ether lipids may be mediating the risk reduction afforded by *APOE ε2*.

With this insight, a next logical step would be to modulate ether lipid species with a view toward preventing or attenuating AD onset and progression, or to influence surrogate AD risk markers (A*β*, phosphorylated tau, or cognition) in the early stages of disease. Modulation of ether lipid species in humans has been demonstrated in several studies,[31,37] where the biologically active precursor, alkylglycerols, that can be synthesized[50] or derived from natural sources in various marine animals[27,51] has been used to bypass the rate-limiting peroxisomal step to upregulate plasmalogen synthesis. Because the vinyl-ether bond of plasmalogen species is highly susceptible to acid hydrolysis, ingestion of these species may not be the optimal approach to raise plasmalogen levels. Thus the non-plasmalogen precursors may serve as better and more-stable dietary interventions for raising plasmalogens.

The expected development of sporadic AD likely spans decades prior to the onset of symptoms[52]; therefore, early intervention will be required. Nutraceuticals comprising alkyldiacylglycerols are potentially low-cost, low-risk dietary supplements that may provide tangible risk reductions and so represent prime targets as a proactive preventative measure for AD. To demonstrate efficacy for such an intervention will require substantial investment in clinical trials of sufficient size and duration to reach statistical significance. However, an additional application of our findings is the development of these ether lipids as biomarkers to not only identify those at increased risk (for inclusion in clinical studies) but also those who will most benefit from ether lipid-modulation therapy.

1.8 | Limitations and remaining questions

Our study examines the relationship between *APOE* polymorphisms and plasma lipid species in the context of AD using three large independent cohorts. The classification of AD and non-AD dementia is clinically difficult and is confirmed only through post-mortem examination. Such misclassification could lead to confounding and underestimation of effect sizes in our analyses. Further to this, in neurological diseases, the importance of peripheral biomarkers remains contentious, as they may not accurately reflect the neurological pathophysiology. However, there are many biological processes that can ultimately influence the pathogenesis of neurological diseases, including the innate and adaptive immune systems. Additional research into lipid metabolic changes within both the immune system and the brain, in relation to AD and *APOE* variants, will shed light on the mechanisms by which dysregulated lipid metabolism may influence AD risk.

Finally, although we were able to validate many of our analyses across cohorts, the interaction of age with the association between plasma lipid species and *APOE* variants requires external validation on an independent cohort. The sample size and age range required meant that this effect could be explored only in the BHS cohort in this study.

1.9 | Conclusion

Here, we combine the power of two large clinical studies of AD with an Australian population study to elucidate the relationship between *APOE* variants and lipid metabolism. We demonstrate a strong relationship of *APOE ε2* and *ε4* alleles with ether lipid species. We further demonstrate that these same lipid species strongly mediate the resilient effects of *APOE ε2* on AD risk, thereby presenting a therapeutic opportunity.

2 | PART 2–CONSOLIDATED RESULTS AND STUDY DESIGN

2.1 | Study design

This study includes three main sections of analyze (Figure 1). First, lipid association studies with *APOE* were performed using only the cognitively healthy individuals to avoid any associations driven by reverse causation, whereas associations with AD prevalence were examined between the CN and AD subsets. In BHS, interaction of *APOE* alleles and age was examined using a binary cut-off at 60 years (Table S5).

Next, associations between *APOE* genotypes and lipid species and the associations between AD prevalence and lipid species were analyzed using a fixed-effect inverse-variance weighted meta-analysis. Heterogeneity between AIBL and ADNI was assessed using Cochran Q.

Then, the mediation analysis was performed on the aligned AIBL and ADNI data sets to assess whether lipid species mediate the effects of *APOE* on AD. We investigated two types of mediators: (1) individual lipid species (which showed concordant associations with *APOE* and AD from the previous analyses) and (2) *APOE* lipid scores. The lipid scores were created by ridge regression using either the lipid species concordant in association with AD/*APOE* or all the lipid species to predict *APOE ε2/ε4*. Causal mediation analysis was then performed to estimate the proportion of risk in the outcome model explained by a direct effect of *APOE* genotype on prevalent AD and the proportion that was mediated by individual lipid species or lipid scores.

2.2 | Results

2.2.1 | The association of *APOE* genotypes with plasma lipid species

In the BHS study, we observed 29 lipid classes and 347 lipid species significantly associated with *APOE ε2* after correction for multiple

comparisons (Figure 2; Tables S1 and S2). Adjustment for clinical lipids, to identify associations that are independent of lipoprotein metabolism, resulted in 28 lipids classes and 237 lipid species significantly associated. There were 20 classes and 133 species associated with *APOE* ε2 in the meta-analysis of the fully adjusted AIBL and ADNI models. A total of 120 concordant lipid species from 18 lipid classes including ceramide, hexosylceramide, sphingomyelin, plasmalogen, alkyl diacylglycerol, and cholesteryl ester, were identified to be nominally significant in both analyses.

A lower number of significant associations with *APOE* ε4 were observed in both the BHS and meta-analysis of the AIBL and ADNI cohorts. There were 23 lipid classes and 223 lipid species significantly associated with *APOE* ε4 *in BHS*, after correction for multiple comparisons (Figure S1; Tables S3 and S4). When we further adjusted for clinical lipids, we observed 18 lipid classes and 104 lipid species significantly associated with *APOE* ε4, after correction for multiple comparisons (Figure 2, Tables S3 and S4). Meta-analysis of the fully adjusted models in the AIBL and ADNI cohorts identified only three lipid species associated with *APOE* ε4, after correction. Ninety-one lipid species were nominally significant, of which 43 species were also nominally significant in the BHS cohort.

2.2.2 | Interaction of age with the associations between *APOE* and lipid species in the BHS cohort

We observed considerably stronger associations within the BHS cohort than the AIBL and ADNI meta-analysis, beyond that expected from the larger sample size. Owing to the larger age range of the BHS compared to the AIBL and ADNI cohorts, we hypothesized that age might influence how *APOE* genotype associates with plasma lipids. Interaction analysis with a binary cut-off at age 60 using the BHS cohort (age < 60, n = 2884; age ≥ 60, n = 1368) identified a nominal significant interaction of age with the association of *APOE* ε2 with 48 lipid species from 12 classes (Figure 3, Table S6). A greater number of the associations of *APOE* ε4 with lipid species was observed to have interaction effects of age (88 lipid species from 18 classes; Figure 4, Table S7). These included species of phosphatidylethanolamine, alkylphosphatidylethanolamine, alkenylphosphatidylethanolamine, and lysophosphatidylethanolamine. Of interest, these results highlight weaker associations between lipid species and *APOE* ε4 genotype with increasing age.

2.2.3 | Concordance of the associations of *APOE* alleles and prevalent AD with lipid species

We rationalized that lipids increased by the ε2 allele, but negatively associated with AD may be involved in mediating risk reduction. Similarly, lipids that associate in the same direction with AD and *APOE* ε4 are likely important in disease pathology. After aligning the associations of lipids species with AD (Table S8) and *APOE* ε2, we observed 68 lipid species displaying an inverse association with AD diagnosis and *APOE* ε2 (putative protective lipid species, Figure 4; Table S9). These lipid species included 37 species of ether phospholipids and 29 lipid species of sphingolipids, phosphatidylethanolamine, and dehydrocholesterol species.

We observed fewer lipid species (24) showing concordant associations with AD and *APOE* ε4, which fall predominantly within the ceramide, trihexosylceramide, alkenylphosphatidylethanolamine, and lysophosphatidylethanolamine classes (Figure 4; Table S10).

2.2.4 | Mediating role of the plasma lipidome on the effects of *APOE* on AD risk

We identified 11 lipid species mediating the effect of *APOE* ε2 on AD, after correcting for multiple comparisons (Figure S2; Table S11). These lipid species differed slightly from those mediating the effects of *APOE* ε4, largely comprising the alkyl phosphatidylcholine and phosphatidylethanolamine and alkenyl phosphatidylethanolamine classes. Of particular interest were three species of alkyldiacylglycerol TG (O-50:1) [NL -16:0], TG (O-52:0) [NL -16:0], and TG (O-52:2) [NL -16:0], which showed strong mediating effects, accounting for up to 30% of the total effects of *APOE* ε2 on AD. There were 14 lipid species that showed a significant mediation effect between *APOE* ε4 and AD, after correcting for multiple comparisons (Figure S2; Table S12). These lipid species were of the dihydroceramide, alkylphosphatidylethanolamine, and alkenylphosphatidylethanolamine classes.

The *APOE* ε2 lipid scores derived from BHS and healthy AIBL/ADNI both showed strong mediating effects on AD risk of 20% and 24%, respectively (Table 2). In contrast, the mediating effects of the *APOE* ε4 lipid scores were smaller, but still significant, at 9% and 6% for the BHS and healthy AIBL/ADNI derived scores, respectively (Table 2). Mediation analysis with lipid scores derived from all lipid species showed similar performance when built using the BHS cohort, with a 23% and 7% mediation effect for *APOE* ε2 and *APOE* ε4, respectively.

3 | PART 3–DETAILED METHODS AND RESULTS

More details of methodology including the data and statistical methods are described.

3.1 | Study cohorts

3.1.1 | The Busselton Health study (BHS)

BHS is a community-based population study for which participants were recruited in Western Australia in the 1960s. The BHS holds extensive phenotype data (eg, cardiovascular disease traits), high-density single-nucleotide polymorphism (SNP) panels, and plasma lipidomic profiling data.[53–55] In this study, we used data from 4492 participants who provided plasma samples at the 1994 to 1995 recall.

3.1.2 | The Australian Imaging, Biomarkers and Lifestyle (AIBL) flagship study

AIBL is a longitudinal study that initially recruited 1112 participants who are older than 60 years of age. This included 768 cognitive normal individuals (CN), 133 with mild cognitive impairment (MCI), and 211 with AD at the last time point. The participants were recalled at 18-month intervals for up to 72 months. *APOE* genotype and other biochemical data were collected. Lipidomics analysis was performed on all available plasma samples, with 4106 fasted plasma samples examined from baseline up to the fifth time point.[56]

3.1.3 | Alzheimer's Disease Neuroimaging Initiative (ADNI)

ADNI is a multi-site longitudinal study using a non-randomized, natural history, non-treatment design. The first phase (ADNI-1), launched in 2004, aimed to track disease progression using biomarkers and to identify features of MCI that may predict cognitive decline. Clinical follow-up is available for up to 2 to 3 years post screening in the ADNI-1 study, with participants carried forward into subsequent ADNI2-GO studies. The ADNI-1 cohort includes 819 participants (229 CN, 398 MCI, and 192 AD). Lipidomics was performed on all participants with available samples at baseline.

3.1.4 | Ethics approval and consent to participate

For all the above cohorts, written informed consent was obtained from all participants before protocol-specific procedures were performed.

3.2 | Lipid extraction and mass spectrometry analysis

Extensive details on the lipidomic profiling of the BHS, ADNI, and AIBL cohorts have been published previously.[16,26,55] Lipid extractions were performed on plasma (AIBL) and serum (ADNI, BHS) samples as described previously.[57] Lipidomic profiling (569 lipid species from 32 classes) was carried out using scheduled multiple reaction monitoring on an Agilent 6490 QqQ mass spectrometer.[57]

3.3 | Definition of AD state

In AIBL, clinical criteria used to determine disease status included a Mini Mental State Examination score of <28, failure on the Logical Memory test, other evidence of possible significant cognitive difficulty on neuropsychological testing, a Clinical Dementia Rating score of ≥0.5, a medical history suggestive of the presence of illnesses likely to impair cognitive function, and an informant or personal history suggestive of impaired cognitive function.[56] The definition of possible AD in ADNI followed the the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA) criteria,[58] whereas the classification of MCI is defined according to the criteria proposed by Petersen et al.[59]

3.4 | Statistical analysis

3.4.1 | Cohort stratification

We sought to examine the association of *APOE* genotypes with lipid species independent of disease to avoid any associations driven by reverse causation. Thus we utilized only the cognitively healthy individuals in the AIBL and ADNI cohorts. After removing samples with missing records, we had a total of 5284 participants: AIBL (n = 693), ADNI (n = 207), and BHS (n = 4384). The characteristics of each cohort are presented in Table 1. All the lipid species were log10 transformed, followed by normalization to zero mean and one-unit standard deviation.

3.4.2 | Association of *APOE* genotypes with lipid species

The associations between *APOE* genotype (ε2 and ε4) and lipid species were determined by linear regression in healthy individuals in each cohort separately. BHS is a population cohort with voluntary enrolment and a median age of 48; we treated the BHS population as non-AD. Adjustment for covariates and meta-analyses are described in Figure 1.

3.4.3 | Identifying concordant associations with AD

Linear regression was used to determine the associations between lipid species and AD, relative to healthy control. To identify associations independent of *APOE* genotype, these analyses were adjusted for both *APOE* ε2 and ε4. Other covariates included age, sex, BMI, and clinical lipids. We then selected the lipid species that were associated with both AD and *APOE* genotype (ε2 or ε4).

To identify lipids of potential biological relevance, we highlighted lipid species that were concordant in their association with *APOE* and AD. Concordant species between *APOE* ε2 and AD were negatively associated with *APOE* ε2 but positively associated with AD, and vice versa. Conversely, concordant species between *APOE* ε4 and AD were positively or negatively associated with both *APOE* ε4 and AD.

3.4.4 | Mediation of the *APOE* genotype effect on AD by lipid species

To assess whether lipid species mediate the effect of *APOE* on AD, we performed mediation analysis (using the R package "mediation") on the combined AIBL and ADNI data sets (n = 1597). The analysis was con-

ducted using either individual lipid species that showed concordant associations with *APOE* and AD, or lipid scores for *APOE* genotypes. Two lipid scores (Figure 1, lower panel) were created by ridge regression (R package "glmnet") using either: (1) the lipid species concordant in association with AD/*APOE*; or (2) all lipid species. Penalty parameters were optimized using internal 10-fold cross-validation. As illustrated in Figure 1 (lower panel), models were created using the BHS cohort (n = 4384) or the combined AIBL and ADNI cohorts (healthy individuals; n = 900), adjusting for age, sex, BMI, fasting status, HDL-C, total cholesterol, and triglycerides. The resulting predicted values on the whole population of combined AIBL and ADNI cohorts (n = 1597) were the *APOE* lipid scores that were treated as mediators in the mediation analysis.

Causal mediation analysis (Figure 1, lower panel) was performed by first estimating the total effect of *APOE* genotypes on prevalent AD using logistic regression, adjusted for age, sex, BMI, HDL-C, total cholesterol, and triglycerides. The mediator model is constructed, looking at the association of *APOE* genotype with lipid species and lipid scores, adjusting for the same covariates. Causal mediation analysis was then used to estimate the proportion of risk in the outcome model explained by a direct effect of *APOE* genotype on prevalent AD—the average direct effect (ADE)—and the proportion that was mediated by lipid species or lipid scores—the average causal mediation effect (ACME). To test for moderation effects, an interaction term was introduced between lipid species and *APOE* genotype. Confidence intervals were estimated using resampling (10,000 empirical bootstraps).

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The authors who made direct contribution to this study have been listed as authors in this article. As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wpcontent/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf. Part of the data used in preparation of this article were generated by the Alzheimer's Disease Metabolomics Consortium (ADMC). The authors who made direct contribution to this study have been listed as authors in this article. Investigators within the ADMC provided data but did not participate in analysis or writing of this report can be found at https://sites.duke.edu/adnimetab/team/. Metabolomics data and results from the ADNI study have been made accessible

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest with the contents of this manuscript.

AUTHOR CONTRIBUTIONS

Meikle and Kaddurah-Daouk led the study design team. Wang, Huynh, and Giles led the statistical analyses presented in this study. Mellett, Duong, Nguyen, Lim, Smith, Olshansky, Huynh, and Giles supported the acquisition and processing of the lipidomic data for the three cohorts. Cadby, Hung, Hui, Beilby, Watts, and Moses were key members of the Busselton Health Study team. Chatterjee, I Martins, Laws, Bush, Rowe, Villemagne, Ames, Masters, Taddei, Doré, Fripp, and Martins were key members of the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing team. Arnold, Kastenmüller, Nho, Saykin, Baillie, Han, and Kaddurah-Daouk were key members of the Alzheimer's Disease Neuroimaging Initiative team and represent the Alzheimer's Disease Metabolomics consortium (ADMC): A complete listing of ADMC investigators can be found at https://sites.duke.edu/adnimetab/who-we-are/.

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SUPPORTING INFORMATION

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