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*KL**VS heterozygosity reduces brain amyloid in asymptomatic at- risk *APOE**4 carriers

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[1]Data used in this manuscript were obtained from the A4 Study publicly available dataset (ida.loni.usc.edu). As such the A4 Study team contributed to the design and data collection of A4 but did not participate in the analyses or writing of this manuscript. A complete listing of the A4 Study Team is available at: a4study.org/a4-study-team.

[2]Data used in this manuscript were obtained from the neuroscience substudy of the 1946 British birth cohort (Insight 46). As such the Insight 46 Study team contributed to the design and data collection of Insight 46 but did not participate in the analyses or writing of this manuscript.

[3]Data used in the preparation of this article were obtained from the Australian Imaging Biomarkers and Lifestyle (AIBL) Study funded by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Unless otherwise listed, AIBL researchers contributed to the design and data collection of AIBL but did not participate in analysis or writing of this report. AIBL researchers are listed at www.aibl.csiro.au.

[4]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). 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/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Disclosure statement

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2021.01.008.

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Abstract

KLOTHO*VS heterozygosity (*KL**VS^{HET+}) was recently shown to be associated with reduced risk of Alzheimer's disease (AD) in *APOE**4 carriers. Additional studies suggest that *KL**VS^{HET+} protects against amyloid burden in cognitively normal older subjects, but sample sizes were too small to draw definitive conclusions. We performed a well-powered meta-analysis across 5 independent studies, comprising 3581 pre-clinical participants ages 60–80, to investigate whether *KL**VS^{HET+} reduces the risk of having an amyloid-positive positron emission tomography scan. Analyses were stratified by *APOE**4 status. *KL**VS^{HET+} reduced the risk of amyloid positivity in *APOE**4 carriers (odds ratio = 0.67 [0.52–0.88]; $p = 3.5 \times 10^{-3}$), but not in *APOE**4 non-carriers (odds ratio = 0.94 [0.73–1.21]; $p = 0.63$). The combination of *APOE**4 and *KL**VS genotypes should help enrich AD clinical trials for pre-symptomatic subjects at increased risk of developing amyloid aggregation and AD. *KL*-related pathways may help elucidate protective mechanisms against amyloid accumulation and merit exploration for novel AD drug targets. Future investigation of the biological mechanisms by which *KL* interacts with *APOE**4 and AD are warranted.

Keywords

Alzheimer's disease; Amyloid; Pre-clinical; PET; APOE4; KLOTHO; Heterozygosity

1. Introduction

With Alzheimer's disease (AD) clinical trials moving toward minimally symptomatic or even pre-symptomatic designs (Cummings et al., 2019; Sperling et al., 2011), which can be lengthy and costly, there is a crucial need to enrich for subjects likely to develop amyloid abnormalities and worsening symptoms. *Apolipoprotein E**4 (*APOE**4) is the strongest genetic risk factor for late-onset AD and a critical mediator of amyloid accumulation in the brain (Belloy et al., 2019). *APOE**4 carriers, compared to non-carriers, are at about 5-fold increased risk of AD (Belloy et al., 2020). Even in pre-symptomatic, cognitively normal subjects during early old age (60–80 years), *APOE**4 carriers are also at about 5-fold increased risk of having an amyloid-positive positron emission tomography (PET) scan (Jansen et al., 2015), increasing the risk for future cerebral tau pathology, cognitive decline,

and ultimately dementia (Jack et al., 2013b). The *APOE**4 genotype is therefore critical in estimating an individual's risk of AD when attempting to enrich AD clinical trials for subjects likely to progress relatively quickly on the AD pathological spectrum (Ballard et al., 2019; Jack et al., 2018; Reiman et al., 2011).

Other genetic factors may mitigate *APOE**4-related risk for AD. *KLOTHO* (*KL*) is a compelling candidate, as it has been implicated as a longevity factor promoting cognitive resilience during aging (Arking et al., 2002; Dubal et al., 2014; Kurosu et al., 2005). Specifically, heterozygosity ($^{HET+}$) for the *KL**VS genotype has been associated with increased serum levels of KLOTHO, which in turn was associated with healthy brain aging and synaptic function (Dubal et al., 2014; Yokoyama et al., 2017, 2015). A recent large-scale meta-analysis showed that *KL**VS^{HET+} reduced AD risk in *APOE**4 carriers by as much as 30% (Belloy et al., 2020). Additionally, in line with an earlier study (Erickson et al., 2019), *KL**VS^{HET+} was associated with reduced amyloid burden in the brains of cognitively normal *APOE**4 carriers during early old age. The combination of *KL**VS and *APOE* genotypes may thus be important in refining individual AD risk and in guiding trial recruitment. Prior outcomes on amyloid burden were, however, obtained from cohorts of relatively small sample sizes (Belloy et al., 2020; Erickson et al., 2019). Here, we performed a well-powered meta-analysis across 5 independent studies to evaluate whether *KL**VS^{HET+} reduces the risk of having an amyloid-positive PET scan in cognitively normal *APOE**4 carriers ages 60–80.

2. Materials and methods

2.1. Cohort ascertainment and PET processing

Five AD-related cohorts with genotype and amyloid PET data were included (Table 1). Ascertainment and collection of genotype/phenotype data and PET image processing for each cohort are described in detail elsewhere (Dagley et al., 2017; Ellis et al., 2009; Jagust et al., 2015; Lane et al., 2017; Petersen et al., 2010; Sperling et al., 2020). Briefly, participants were included if they were diagnosed as cognitively normal, based off their respective study's clinical assessments, cognitive battery performance criteria, and scoring above 24 on mini-mental state examinations. Within each cohort, amyloid PET images were normalized to their cerebellar reference region to obtain standardized uptake value ratios (SUVR) or distribution volume ratios (DVR) in a composite of cortical brain areas. PET scans were then dichotomized as positive (abnormal) or negative (normal) using SUVR or DVR cutoffs defined independently in each of the 5 studies (Dagley et al., 2017; Ellis et al., 2009; Jagust et al., 2015; Lane et al., 2017; Petersen et al., 2010; Sperling et al., 2020).

Participants provided written informed consents in the original studies. The Stanford Institutional Review Board granted the current study protocol an exemption because the analyses were carried out on "de-identified, off-the-shelf" data.

2.2. Genetic data processing

Genetic data underwent standard quality control, processing, and ancestry determination as previously described (Belloy et al., 2020; Yang et al., 2019). Only non-Hispanic subjects

from Northwestern European ancestry were included to obtain the largest, most homogenous sample. For the AIBL cohort, genetic data for ancestry determination were not directly available, so included subjects were non-Hispanic Whites of European ancestry. For the HABS cohort, processing was slightly augmented with regard to prior work: 2 genotyping batches were first processed separately (retaining subjects/variants with genotyping rate >0.98, genotype missing rate >0.98, Hardy Weinberg equilibrium $p < 10^{-6}$, and identity-by-descent pi-hat <0.125) and then merged (Yang et al., 2019).

2.3. Study design and statistical analyses

We evaluated the association of *KL**VS^{HET+} with dichotomized amyloid PET outcome by *APOE**4 status. All analyses were restricted to PET scans acquired when subjects were diagnosed as cognitively normal and between the ages of 60–80 years, consistent with prior work (Belloy et al., 2020). In longitudinal studies (ADNI, AIBL, and HABS), only a single time point and related age-at-scan was retained per subject: (1) for subjects that only had amyloid negative outcomes, the latest time point was retained, and (2) for subjects that had an amyloid positive outcome at any time, the first amyloid positive time point was retained. Analyses were stratified to *APOE**4 carriers (*APOE**2/4, 3/4, 4/4) and non-carriers (*APOE**2/2, 2/3, 3/3), or to the full sample to test the formal interaction between *APOE**4 status and *KL**VS^{HET+}. Outcomes were evaluated per cohort using logistic regression analyses and combined using fixed-effects inverse-variance weighted meta-analysis (testing heterogeneity with Cochran's *Q* test). In all stratified models, the outcome was adjusted for age, sex, and the first 3 genetic principal components (where available) to account for population substructure. To evaluate the interaction between *APOE**4 status and *KL**VS^{HET+} in the full model, we additionally added terms for *APOE**4 status and the *APOE**4-by-*KL**VS^{HET+} cross-product. Significance was determined as $p < 0.05$ and effects are shown as odds ratios (OR) with 95% confidence intervals [CI].

Due to the wide range of sample sizes across cohorts, we conducted power analysis for each cohort for a range of a priori defined parameters and effect sizes at a significance level of $p < 0.05$. Specifically, power was calculated for OR values ranging from 0.6 to 0.8, which is consistent with expectations from previously reported effect sizes of *KL**VS^{HET+} on AD case-control status in *APOE**4 carriers (OR = 0.69) and for the *APOE**4-by-*KL**VS^{HET+} interaction effect (OR = 0.73) (Belloy et al., 2020). This choice is motivated by the large correlation between amyloid status in cognitively normal subjects and prospective case-control status (Jansen et al., 2015). Estimates for prevalence and *APOE**4-related risk of amyloid positivity in cognitively normal subjects were obtained from a prior large-scale amyloid PET meta-analysis (Jansen et al., 2015). Estimates of *APOE**4 and *KL**VS^{HET+} frequencies in cognitively normal subjects were derived from prior large-scale AD case-control meta-analyses (Belloy et al., 2020; Farrer et al., 1997).

All analyses were performed in R v3.6.0 (metafor and simple-boot packages).

3. Results

We evaluated the association of *KL**VS^{HET+} with amyloid PET positivity in cognitively normal subjects across 5 independent cohorts, comprising 1252 *APOE**4 carriers and 2329

*APOE**4 non-carriers (Table 1). For each cohort and their respective meta-analyses, outcomes and power estimates for *APOE**4-stratified and *APOE**4-by-*KL**VS^{HET+} interaction tests are listed in Table 2. *KL**VS^{HET+} was significantly associated with decreased risk for amyloid positivity in *APOE**4 carriers (OR = 0.67 [0.52–0.88]; $p = 3.5 \times 10^{-3}$), but not in *APOE**4 non-carriers (OR = 0.94 [0.73–1.21]; $p = 0.63$). The *APOE**4-by-*KL**VS^{HET+} interaction was such that *KL**VS^{HET+} displayed a stronger protective effect against amyloid positivity in *APOE**4 carriers than in non-carriers, but this effect only reached trend-level significance (OR = 0.70 [0.48–1.02]; $p = 0.062$).

As a sensitivity test, meta-analyses were repeated after selecting PET time points closest to age 70.6 (study mean age) in amyloid negative subjects, rather than selecting their last time point. Meta-analysis in *APOE**4 carriers indicated the same effect as observed in the main analysis (OR = 0.68 [0.52–0.88]; $p = 3.8 \times 10^{-3}$). Furthermore, to ensure an independent validation effort of prior studies, meta-analyses were repeated after excluding the ADNI cohort, in which the association of *KL**VS^{HET+} with amyloid PET burden was investigated previously (Belloy et al., 2020). Meta-analysis in *APOE**4 carriers indicated significantly reduced risk for amyloid positivity (OR = 0.72 [0.55–0.95]; $p = 0.020$) in this fully independent set of studies. In our final sensitivity analysis, we added *APOE**2 and *APOE**4 dosage, in addition to the other covariates, to the model. Findings were highly consistent with those of the main analyses (Table S1). For all presented meta-analyses, heterogeneity tests were non-significant.

4. Discussion

Our results show that *KL**VS^{HET+} reduces the risk of an amyloid-positive PET scan in cognitively normal *APOE**4 carriers between the ages of 60 and 80. This finding replicates and strengthens prior observations that *KL**VS^{HET+} reduces amyloid burden in cognitively normal *APOE**4 carriers during early old age.

The effect size for the association of *KL**VS^{HET+} with amyloid positivity in *APOE**4 carriers (OR = 0.67) was highly consistent with the previously reported effect size for the association of *KL**VS^{HET+} with case-control status in *APOE**4 carriers (OR = 0.69) (Belloy et al., 2020). Notably, both *APOE**4-stratified analyses had a power greater than 0.8 to detect the meta-analyzed effect size of *KL**VS^{HET+} in *APOE**4 carriers, indicating that the lack of effect in *APOE**4 non-carriers was not due to power limitations. These findings thus validate the protective effect of *KL**VS^{HET+} on AD risk specifically in *APOE**4 carriers and align with observations that pre-symptomatic amyloid positive subjects are likely to convert to AD (Burnham et al., 2016; Jack et al., 2013a). Notably, in *APOE**4 carriers, *KL**VS^{HET+} only displayed a small protective effect in the Insight 46 cohort (OR = 0.90) and a risk increasing effect in HABS (OR = 6.09). However, both samples had low power to detect the expected effect size of *KL**VS^{HET+} in *APOE**4 carriers and displayed large variance on their outcome estimates. Particularly HABS had a small sample size compared to other cohorts, which could have led to spurious non-concordant associations. In contrast, in *APOE**4 carriers from the large A4 cohort, *KL**VS^{HET+} was associated with significantly decreased risk for amyloid positivity with a power close to 0.8.

We did not observe a significant interaction between *KL**VS^{HET+} and *APOE**4 to lower risk for amyloid positivity, contrary to what was previously reported for case-control association testing (Belloy et al., 2020). However, the current effect size for the interaction (OR = 0.70) was highly consistent with the previously reported one (OR = 0.73) (Belloy et al., 2020) and the *p*-value was less than 0.1. In this study, the full meta-analysis on 3581 individuals with amyloid PET scans only showed a moderate power of 0.65 to detect the *APOE**4-by-*KL**VS^{HET+} interaction. Increasing the sample size of subjects with amyloid PET scans may therefore increase power sufficiently to observe a significant interaction effect in future studies. Furthermore, while we focused on *APOE**4-stratified analyses, it is important to consider that *APOE*-related risk for AD and amyloid pathology varies strongly across *APOE**2 and *APOE**4 dosages, even within the considered *APOE**4 positive and negative strata. In models that were adjusted for *APOE**2 and *APOE**4 dosage, we observed no clear differences with the main analyses, suggesting that the protective effect of *KL**VS^{HET+} may be observed regardless of *APOE**2/4, 3/4, or 4/4 status. Future larger-scale studies will be required to specifically investigate the role of *KL**VS^{HET+} per *APOE* genotype, as the current study did not provide sufficient power in these substrata.

One limitation is that across the included cohorts, the use of different acquisition methods, PET tracers, and study-specific SUVR/DVR thresholds, precluded a single harmonized analysis. Because raw SUVR/DVR values were not available for all cohorts, it was also not possible to implement a standardization procedure for amyloid positivity inference (Mormino et al., 2014). However, these limitations were largely addressed by performing cross-cohort meta-analyses that showed no significant heterogeneity. Only in *APOE**4 carriers heterogeneity tests reached trend-level significance, but this was due to large sways in effect sizes in ADNI and HABS, which was likely a consequence of these cohorts' small sample sizes. Indeed, prior work indicated that amyloid PET positivity outcomes compare well across different amyloid PET tracers (Landau et al., 2014), supporting the current study design. Finally, due to the lack of information on Northwestern European ancestry and genetic principal components in AIBL, the reported outcomes in AIBL may have higher intrinsic variance. The current study focused on subjects of Northwestern European ancestry to obtain the largest genetically homogenous sample (majority of the subjects), which precludes generalization of our findings. When larger, ethnically diverse samples with amyloid PET or cerebrospinal fluid measurements become available, future studies should explore the effect of *KL**VS^{HET+} in different ancestral groups.

A functional link between *KL**VS^{HET+} and AD may be reflected in the association of *KL**VS^{HET+} with increased KLOTHO protein levels, but it currently remains unclear how *KL**VS interacts with *APOE**4 to modulate amyloid pathology. Some evidence suggests that *AMYLOID BETA PRECURSOR PROTEIN* (*APP*) regulates *KL* expression (Li et al., 2010), which in turn may increase levels of DISINTEGRIN AND METALLOPROTEINASE DOMAIN-CONTAINING PROTEIN 10 (ADAM10) to reduce amyloid beta burden through autophagy-mediated clearance (Kuang et al., 2017; Zeng et al., 2019). Because the most prominent effect of *APOE**4 with regard to AD is to increase amyloid burden, this may explain why the protective effect of *KL**VS on amyloid burden appears stratified to *APOE**4 carriers. These hypotheses require empirical interrogation. Furthermore, since amyloid pathology only reflects the initial aspect of AD pathology, to

fully understand the role of *KL**VS in AD and its potential value for clinical trial enrichment, it will also be relevant to evaluate whether *KL**VS^{HET+} affects tau pathology, the key driver of disease progression in AD (Bejanin et al., 2017). Finally, the rarer *KL**VS homozygous genotype, in contrast to *KL**VS heterozygosity, has been associated with negative effects on lifespan (Arking et al., 2002), brain-aging resilience (Yokoyama et al., 2017), cognition (Yokoyama et al., 2015), and KLOTHO serum levels (Yokoyama et al., 2017). It will therefore be relevant for larger subsequent studies to evaluate whether *KL**VS homozygosity is associated with increased amyloid burden.

5. Conclusion

Overall, our findings suggest that *KL**VS^{HET+} reduces the risk of having an amyloid positive PET scan in cognitively normal *APOE**4 carriers between the ages of 60 and 80, thereby validating prior findings that *KL**VS^{HET+} is associated with reduced amyloid burden and AD risk in *APOE**4 carriers. This suggests that *KL**VS genotype may prove useful for clinical trial enrichment. Specifically, restricting *APOE**4 carriers to those without *KL**VS^{HET+} should enrich pre-symptomatic recruitment studies for subjects at increased risk of developing amyloid aggregation and AD. Future investigations of the biological mechanisms by which *KL* interacts with AD are warranted and will support exploration of *KL*-related pathways for novel AD drug targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The A4 Study is a secondary prevention trial in preclinical Alzheimer's disease, aiming to slow cognitive decline associated with brain amyloid accumulation in clinically normal older individuals. The A4 Study is funded by a public-private-philanthropic partnership, including funding from the National Institutes of Health-National Institute on Aging, Eli Lilly and Company, Alzheimer's Association, Accelerating Medicines Partnership, GHR Foundation, an anonymous foundation and additional private donors, with in-kind support from Avid and Cogstate. The companion observational Longitudinal Evaluation of Amyloid Risk and Neurodegeneration (LEARN) Study is

funded by the Alzheimer's Association and GHR Foundation. The A4 and LEARN Studies are led by Dr. Reisa Sperling at Brigham and Women's Hospital, Harvard Medical School and Dr. Paul Aisen at the Alzheimer's Therapeutic Research Institute (ATRI), University of Southern California. The A4 and LEARN Studies are coordinated by ATRI at the University of Southern California, and the data are made available through the Laboratory for Neuro Imaging at the University of Southern California. The participants screening for the A4 Study provided permission to share their de-identified data in order to advance the quest to find a successful treatment for Alzheimer's disease. We would like to acknowledge the dedication of all the participants, the site personnel, and all of the partnership team members who continue to make the A4 and LEARN Studies possible. The complete A4 Study Team list is available on: a4study.org/a4-study-team .

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Table 1

Demographics of subjects aged 60–80 and cognitively normal at the time of amyloid PET imaging

Characteristic	ADNI (n = 229)	A4 (n = 2294)	AIBL(n = 515)	Insight 46 (n = 415)	HABS (n = 128)
*APOE**4 status, n (%)	(n = 229)	(n = 2294)	(n = 515)	(n = 415)	(n = 128)
*APOE**4+	72 (31.4%)	876 (38.2%)	144 (28.0%)	117 (28.2%)	43 (33.6%)
*APOE**4−	157 (68.6%)	1418 (61.8%)	371 (72.0%)	298 (71.8%)	85 (66.4%)
APOE genotype, n (%)	(n = 229)	(n = 2294)	(n = 515)	(n = 403)[a]	(n = 128)
2/2	0 (0%)	9 (0.4%)	2 (0.4%)	0 (0%)	2 (1.6%)
2/3	29 (12.7%)	210 (9.2%)	70 (13.6%)	53 (13.1%)	5 (3.9%)
2/4	5 (2.2%)	63 (2.7%)	10 (1.9%)	7 (1.7%)	5 (3.9%)
3/3	128 (55.9%)	1199 (52.3%)	299 (58.1%)	233 (57.8%)	77 (60.2%)
3/4	64 (27.9%)	732 (31.9%)	116 (22.5%)	100 (24.8%)	36 (28.1%)
4/4	3 (1.3%)	81 (3.5%)	18 (3.5%)	10 (2.5%)	2 (1.6%)
Age (y), mean (SD)	(n = 229)	(n = 2294)	(n = 515)	(n = 415)	(n = 128)
60–80	73.69 (4.40)	70.60 (3.89)	72.10 (5.05)	70.65 (0.67)	73.76 (3.78)
*APOE**4+	72.07 (4.77)	70.24 (3.76)	71.34 (4.98)	70.64 (0.68)	73.14 (3.89)
*APOE**4−	74.44 (4.02)	70.83 (3.95)	72.40 (5.06)	70.66 (0.67)	74.08 (3.71)
Sex, n (%)	(n = 229)	(n = 2294)	(n = 515)	(n = 415)	(n = 128)
Female	126 (55.0%)	1431 (62.4%)	278 (56.6%)	201 (48.4%)	71 (55.5%)
*APOE**4+	(n = 72)	(n = 876)	(n = 144)	in = 117)	(n = 43)
Female	43 (59.7%)	541 (61.8%)	79 (54.9%)	55 (47.0%)	25 (58.1%)
*APOE**4−	(n = 157)	(n = 1418)	(n = 371)	(n = 298)	(n = 85)
Female	83 (52.9%)	890 (62.8%)	215 (60.0%)	146 (49.0%)	46 (54.1%)
Education (y)	(n = 229)	(n = 2292)	(n = 515)	(n = 415)	(n = 128)
Mean (SD)	16.54 (2.51)	16.62 (2.68)	–	–	16.23 (3.00)
<13, n (%)	23 (10.0%)	202 (8.8%)	235 (45.6%)	170 (41.0%)	26 (20.3%)
≥13, n (%)	206 (90.0%)	2090 (91.1%)	280 (54.4%)	245 (59.0%)	102 (79.7%)
MMSE score	(n = 229)	(n = 2294)	(n = 515)	(n = 415)	(n = 128)
Mean (SD)	29.12 (1.07)	28.96 (1.13)	28.75 (1.20)	29.28 (0.90)	29.28 (0.87)
Amyloid PET, n (%)	(n = 229)	(n = 2294)	(n = 515)	(n = 415)	(n = 128)
Amyloid positive	97 (42.4%)	632 (27.6%)	183 (35.5%)	73 (17.6%)	49 (38.3%)

Characteristic	ADNI (n = 229)	A4 (n = 2294)	AIBL(n = 515)	Insight 46 (n = 415)	HABS (n = 128)
*APOE**4+	(n = 72)	(n = 876)	(n = 144)	(n = 117)	(n = 43)
Amyloid positive	48 (66.7%)	426 (48.6%)	85 (59.0%)	42 (35.9%)	30 (69.8%)
*APOE**4−	(n = 157)	(n = 1418)	(n = 371)	(n = 298)	(n = 85)
Amyloid positive	49 (31.2%)	206 (14.5%)	98 (26.4%)	31 (10.4%)	19 (22.4%)

Data were available from the Alzheimer's Disease Neuroimaging Initiative (ADNI), the Anti-Amyloid Treatment in Asymptomatic Alzheimer disease Study (A4), the Australian Imaging Biomarkers and Lifestyle Study of Aging (AIBL), Insight 46 (a neuroscience sub-study of the MRC National Survey of Health and Development), and the Harvard Aging Brain Study (HABS).

Key: APOE, *Apolipoprotein E**4; MMSE, mini-mental state examination; PET, positron emission tomography; SD, standard deviation.

[a]In the Insight 46 cohort, the rs7412 variant (which provides information on *APOE**2 status) was not directly genotyped in all subjects and could not be imputed with high reliability.

Table 2

Association of *KL**VS^{HET+} with amyloid PET positivity status, stratified by *APOE**4 status, in cognitively normal subjects aged 60–80

Study stratum	Association between *KL**VS^{HET+} and Amy+ by *APOE**4 status					Interaction between *KL**VS^{HET+} and Amy+ by *APOE**4 status				
	Amy− with *KL**VS^{HET+} (N/total)	Amy+ with *KL**VS^{HET+} (N/total)	Odds ratio [95% CI]	*p*-value	Power	Amy− with *KL**VS^{HET+} (N/total)	Amy+ with *KL**VS^{HET+} (N/total)	Odds ratio [95% CI]	*p*-value	Power
ADNI										
*APOE**4+	14/24 (58.3%)	10/48 (20.8%)	0.18 [0.06–0.59]	0.0044	0.16 (0.11–0.21)	47/132 (35.6%)	25/97 (25.8%)	0.18 [0.05–0.70]	0.013	0.08 [0.05–0.14]
*APOE**4−	33/108 (30.6%)	15/49 (30.6%)	0.99 [0.66–1.32]	0.98	0.14 [0.08–0.18]					
A4										
*APOE**4+	129/450 (28.7%)	98/426 (23.0%)	**0.72 [0.53–0.98]**	**0.038**	**0.77 [0.45–0.96]**	446/1662 (26.8%)	149/632 (23.6%)	0.77 [0.49–1.23]	0.28	0.50 [0.25–0.72]
*APOE**4−	317/1212 (26.2%)	51/206 (24.8%)	0.93 [0.66–1.32]	0.69	0.62 [0.32–0.88]					
AIBL										
*APOE**4+	21/59 (35.6%)	19/85 (22.4%)	0.53 [0.25–1.11]	0.092	0.27 [0.15–0.38]	90/332 (27.1%)	41/183 (22.4%)	0.61 [0.24–1.55]	0.30	0.19 [0.15–0.32]
*APOE**4−	69/273 (25.3%)	22/98 (22.4%)	0.87 [0.50–1.51]	0.62	0.31 [0.19–0.54]					
Insight 46										
*APOE**4+	17/75 (22.7%)	9/42 (21.5%)	0.90 [0.35–2.29]	0.82	0.18 [0.10–0.26]	85/342 (24.9%)	18/73 (24.7%)	0.80 [0.23–2.77]	0.73	0.08 [0.05–0.10]
*APOE**4−	68/267 (25.5%)	9/31 (26.7%)	1.33 [0.57–3.07]	0.51	0.02 [0.02–0.02]					
HABS										
*APOE**4+	1/13 (7.7%)	9/30 (30.0%)	6.09 [0.56–66.5]	0.14	0.04 [0.04–0.06]	18/79 (22.8%)	13/49 (26.5%)	7.47 [0.54–102.6]	0.13	0.03 [0.02–0.03]
*AP0E*4–*	17/66 (25.8%)	4/19 (21.1%)	0.56 [0.13–2.37]	0.43	0.021 [0.02–0.02]					
Meta-analysis[a]										
*APOE**4+	182/621 (29.3%)	145/631 (23.0%)	**0.67 [0.52–0.88]**	**0.0035**	**0.90 [0.57–0.99]**	686/2547 (26.9%)	246/1034 (23.8%)	*0.70 [0.48–1.021]*	*0.061*	*0.65 [10.33–0.88]*
*APOE**4−	504/1926 (26.2%)	101/403 (25.1%)	0.94 [0.73–1.21]	0.63	0.86 [0.52–0.99]					

Study stratum	Association between *KL**VS^{HET+} and Amy+ by *APOE**4 status					Interaction between *KL**VS^{HET+} and Amy+ by *APOE**4 status				
	Amy− with *KLVS^{HET+} (N/ total)**	**Amy+ with *KL**VS^{HET+} (N/ total)**	**Odds ratio [95% CI]**	***p*-value**	**Power**	**Amy− with *KL**VS^{HET+} (N/ total)**	**Amy+ with *KL**VS^{HET+} (N/ total)**	**Odds ratio [95% CI]**	***p*-value**	**Power**
Meta-analysis without ADNI[b]										
*APOE**4+	168/597 (28.1%)	135/583 (23.2%)	**0.72 [0.55–0.95]**	**0.020**	**0.85 [0.54–0.99]**	639/2415 (26.5%)	221/937 (23.6%)	0.78 [0.53–1.16]	0.22	0.60 [0.34–0.86]
*APOE**4−	471/1818 (25.9%)	86/354 (24.3%)	0.93 [0.71–1.23]	0.62	0.82 [0.49–0.98]					

Power is directly reported in the table for an OR of 0.7 and additionally for OR values ranging from 0.6 to 0.8 [denoted by square brackets], corresponding to a priori expected effect sizes (cf. methods).

Key: Amy+, amyloid positive; Amy−, amyloid negative; HET+, heterozygous carriers; OR, odds ratio; CI, confidence interval.

[a]Cochran's Q tests for heterogeneity were non-significant for the displayed meta-analyses across all cohorts in the *APOE**4+ ($Q = 9.01$, $p = 0.06$), *APOE**4− ($Q = 1.24$, $p = 0.87$), and full sample ($Q = 7.24$, $p = 0.12$).

[b]Meta-analyses were repeated after excluding ADNI to ensure a fully independent validation effort of prior work (Belloy et al., 2020). Cochran's Q tests for heterogeneity were non-significant for the displayed meta-analyses across cohorts, when excluding ADNI, in the *APOE**4+ ($Q = 3.95$, $p = 0.27$), *APOE**4− ($Q = 1.22$, $p = 0.75$), and full sample ($Q = 3.12$, $p = 0.37$).