Brief Communication

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

Michael E. Belloya,*, Sarah J. Egera, Yann Le Guena, Valerio Nolanpla, Kacie D. Detersa, Hyun-Sik Yangb, Marzia A. Scelsic, Tenielle Porterd,e, Sarah-Naomi Jamesf, Andrew Wongg, Jonathan M. Schotts,h, Reisa A. Sperlingb, Simon M. Lawsd,e, Elisabeth C. Morminoa, Zihuai Hea,i, Summer S. Hanj, Andre Altmannb, Michael D. Greicuia, for the A4 Study Team1 the Insight 46 Study Team2 the Australian Imaging Biomarkers and Lifestyle (AIBL) Study3 the Alzheimer’s Disease Neuroimaging Initiative4

a Department of Neurology and Neurological Sciences, Stanford University, Stanford, CA, USA
b Department of Neurology, Brigham and Women’s Hospital, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
c Centre for Medical Image Computing (CMIC), University College London, London, UK
d Collaborative Genomics and Translation Group, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia
e School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia
f Medical Research Council Unit for Lifelong Health and Ageing, University College London, London, UK
g Dementia Research Centre, University College London Queen Square Institute of Neurology, University College London, London, UK
h UK Dementia Research Institute, University College London, London, UK
i Department of Medicine, Quantitative Sciences Unit, Stanford University, Stanford, CA, USA
j Department of Neurosurgery, Stanford University, Stanford, CA, USA
k Medical Research Council Unit for Lifelong Health and Ageing, University College London, London, UK
l Department of Medicine, Quantitative Sciences Unit, Stanford University, Stanford, CA, USA
m Collaborative Genomics and Translation Group, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia
n School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia
o Medical Research Council Unit for Lifelong Health and Ageing, University College London, London, UK
p Department of Medicine, Quantitative Sciences Unit, Stanford University, Stanford, CA, USA
q Department of Neurosurgery, Stanford University, Stanford, CA, USA
r Collaborative Genomics and Translation Group, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia
s School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia

derived from the A4 Study publicly available dataset (a4study.org). All 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/a4study-team.

d 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.

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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 × 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.

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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., 2013). 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 (VSHET+) 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(VSHET) 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(VSHET) was associated with reduced amyloid burden in the brains of cognitively normal APOE*4 carriers during early old age. The combination of KL*V 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(VSHET) 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

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Table 1  Demographics of subjects aged 60–80 and cognitively normal at the time of amyloid PET imaging

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ADNI (n = 229)</th>
<th>A4 (n = 2294)</th>
<th>AIBL (n = 515)</th>
<th>Insight 46 (n = 415)</th>
<th>HABS (n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE*4 status, n (%)</td>
<td>(n = 229)</td>
<td>(n = 2294)</td>
<td>(n = 515)</td>
<td>(n = 415)</td>
<td>(n = 128)</td>
</tr>
<tr>
<td>APOE*4+</td>
<td>72 (31.4%)</td>
<td>876 (38.2%)</td>
<td>144 (28.0%)</td>
<td>117 (28.2%)</td>
<td>43 (33.6%)</td>
</tr>
<tr>
<td>APOE*4-</td>
<td>157 (68.6%)</td>
<td>1418 (61.8%)</td>
<td>371 (72.0%)</td>
<td>298 (71.8%)</td>
<td>85 (66.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APOE genotype, n (%)</th>
<th>(n = 229)</th>
<th>(n = 2294)</th>
<th>(n = 515)</th>
<th>(n = 403)*</th>
<th>(n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/2</td>
<td>0 (0%)</td>
<td>9 (0.4%)</td>
<td>2 (0.4%)</td>
<td>0 (0%)</td>
<td>2 (1.6%)</td>
</tr>
<tr>
<td>2/3</td>
<td>29 (12.7%)</td>
<td>210 (9.2%)</td>
<td>70 (13.6%)</td>
<td>53 (13.1%)</td>
<td>5 (3.9%)</td>
</tr>
<tr>
<td>3/3</td>
<td>64 (27.9%)</td>
<td>732 (31.9%)</td>
<td>116 (22.5%)</td>
<td>100 (24.8%)</td>
<td>36 (28.1%)</td>
</tr>
<tr>
<td>4/4</td>
<td>128 (55.9%)</td>
<td>1199 (52.3%)</td>
<td>299 (58.1%)</td>
<td>233 (57.8%)</td>
<td>77 (60.2%)</td>
</tr>
<tr>
<td>3/4</td>
<td>4 (1.3%)</td>
<td>81 (3.5%)</td>
<td>18 (3.5%)</td>
<td>10 (2.5%)</td>
<td>2 (1.6%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (y), mean (SD)</th>
<th>Mean (n = 229)</th>
<th>Mean (n = 2294)</th>
<th>Mean (n = 515)</th>
<th>Mean (n = 415)</th>
<th>(n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60–80</td>
<td>73.69 (4.40)</td>
<td>70.60 (3.89)</td>
<td>72.10 (5.05)</td>
<td>70.65 (0.67)</td>
<td>73.76 (3.78)</td>
</tr>
<tr>
<td>APOE*4+</td>
<td>72.07 (4.77)</td>
<td>70.24 (3.76)</td>
<td>71.34 (4.98)</td>
<td>70.64 (0.68)</td>
<td>73.14 (3.89)</td>
</tr>
<tr>
<td>APOE*4-</td>
<td>74.44 (4.02)</td>
<td>70.83 (3.95)</td>
<td>72.40 (5.06)</td>
<td>70.66 (0.67)</td>
<td>74.08 (3.71)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex, n (%)</th>
<th>(n = 229)</th>
<th>(n = 2294)</th>
<th>(n = 515)</th>
<th>(n = 415)</th>
<th>(n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>126 (55.0%)</td>
<td>1431 (62.4%)</td>
<td>278 (56.6%)</td>
<td>201 (48.4%)</td>
<td>71 (55.3%)</td>
</tr>
<tr>
<td>APOE*4+</td>
<td>43 (18.8%)</td>
<td>541 (61.8%)</td>
<td>79 (54.9%)</td>
<td>55 (47.0%)</td>
<td>25 (58.1%)</td>
</tr>
<tr>
<td>APOE*4-</td>
<td>83 (35.2%)</td>
<td>890 (38.2%)</td>
<td>215 (40.0%)</td>
<td>146 (49.0%)</td>
<td>46 (54.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education (y)</th>
<th>Mean (n = 229)</th>
<th>Mean (n = 2294)</th>
<th>Mean (n = 515)</th>
<th>Mean (n = 415)</th>
<th>(n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;13</td>
<td>16.54 (2.51)</td>
<td>16.62 (2.68)</td>
<td>16.54 (2.51)</td>
<td>16.62 (2.68)</td>
<td>16.23 (3.00)</td>
</tr>
<tr>
<td>≥13</td>
<td>23 (10.0%)</td>
<td>202 (8.8%)</td>
<td>235 (45.6%)</td>
<td>170 (41.0%)</td>
<td>26 (20.3%)</td>
</tr>
<tr>
<td>MMSE score</td>
<td>Mean (n = 229)</td>
<td>Mean (n = 2294)</td>
<td>Mean (n = 515)</td>
<td>Mean (n = 415)</td>
<td>(n = 128)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>29.12 (1.07)</td>
<td>28.96 (1.13)</td>
<td>28.75 (1.20)</td>
<td>29.28 (0.90)</td>
<td>29.28 (0.87)</td>
</tr>
<tr>
<td>Amyloid PET, n (%)</td>
<td>(n = 229)</td>
<td>(n = 2294)</td>
<td>(n = 515)</td>
<td>(n = 415)</td>
<td>(n = 128)</td>
</tr>
<tr>
<td>Amyloid positive</td>
<td>97 (42.4%)</td>
<td>632 (27.6%)</td>
<td>183 (35.5%)</td>
<td>73 (17.6%)</td>
<td>49 (38.3%)</td>
</tr>
<tr>
<td>APOE*4+</td>
<td>7 (72)</td>
<td>876 (n = 415)</td>
<td>144 (n = 415)</td>
<td>117 (n = 415)</td>
<td>43 (n = 43)</td>
</tr>
<tr>
<td>Amyloid positive</td>
<td>48 (66.7%)</td>
<td>426 (46.8%)</td>
<td>85 (59.0%)</td>
<td>42 (35.9%)</td>
<td>30 (69.8%)</td>
</tr>
<tr>
<td>Amyloid positive</td>
<td>49 (31.2%)</td>
<td>206 (14.5%)</td>
<td>98 (26.4%)</td>
<td>31 (10.4%)</td>
<td>19 (22.4%)</td>
</tr>
</tbody>
</table>

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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; MMSE, mini-mental state examination; PET, positron emission tomography; SD, standard deviation.

* 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.
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 presymptomatic amyloid positive subjects are likely to convert to AD (Burnham et al., 2016; Jack et al., 2013). 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.
Table 2
Association of KL*V5\text{HET}+ with amyloid PET positivity status, stratified by APOE\(4\) status, in cognitively normal subjects aged 60–80

<table>
<thead>
<tr>
<th>Study stratum</th>
<th>Association between KL*V5\text{HET}+ and Amy+ by APOE(4) status</th>
<th>Interaction between KL*V5\text{HET}+ and Amy+ by APOE(4) status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amy− with KL*V5\text{HET}+ (N/total)</td>
<td>Amy+ with KL*V5\text{HET}+ (N/total)</td>
</tr>
<tr>
<td>ADNI</td>
<td>14/24 (58.3%)</td>
<td>10/48 (20.8%)</td>
</tr>
<tr>
<td>A4</td>
<td>129/450 (28.7%)</td>
<td>98/426 (23.0%)</td>
</tr>
<tr>
<td>APOE(4)+</td>
<td>317/1212 (26.2%)</td>
<td>21/206 (10.3%)</td>
</tr>
<tr>
<td>APOE(4+)</td>
<td>21/59 (35.6%)</td>
<td>19/85 (22.4%)</td>
</tr>
<tr>
<td>HABs</td>
<td>1/13 (7.7%)</td>
<td>8/627 (12.5%)</td>
</tr>
<tr>
<td>Meta-analysisa</td>
<td>182/621 (29.3%)</td>
<td>145/631 (23.0%)</td>
</tr>
<tr>
<td>without ADNib</td>
<td>168/597 (28.1%)</td>
<td>135/583 (23.2%)</td>
</tr>
</tbody>
</table>

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). Bold indicates significance at p < 0.05. Italics represents trending towards significance at p < 0.10.

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).
size of KL*VShET+ in APOE4 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 APOE4 carriers from the large A4 cohort, KL*VShET+ 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*VShET+ and APOE4 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 APOE4-by-KL*VShET+ 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 APOE4-stratified analyses, it is important to consider that APOE-related risk for AD and amyloid pathology varies widely across APOE2 and APOE4 dosage, even within the considered APOE4 positive and negative strata. In models that were adjusted for APOE2 and APOE4 dosage, we observed no clear differences with the main analyses, suggesting that the protective effect of KL*VShET+ may be observed regardless of APOE2/4, 3/4, or 4/4 status. Future larger-scale studies will be required to specifically investigate the role of KL*VShET+ 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 APOE4 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*VShET+ in different ancestral groups.

A functional link between KL*VShET+ and AD may be reflected in the association of KL*VShET+ with increased KLOTHO protein levels, but it currently remains unclear how KL*VShET+ interacts with APOE4 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 DISINTTEGRIN 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 APOE4 with regard to AD is to increase amyloid burden, this may explain why the protective effect of KL*VShET+ on amyloid burden appears stratified to APOE4 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*VShET+ in AD and its potential value for clinical trial enrichment, it will also be relevant to evaluate whether KL*VShET+ affects tau pathology, the key driver of disease progression in AD (Bejanin et al., 2017). Finally, the rarer KL*VShET+ homozgyous 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 homozgyosity is associated with increased amyloid burden.

5. Conclusion

Overall, our findings suggest that KL*VShET+ reduces the risk of having an amyloid positive PET scan in cognitively normal APOE4 carriers between the ages of 60 and 80, thereby validating prior findings that KL*VShET+ is associated with reduced amyloid burden and AD risk in APOE4 carriers. This suggests that KL*VS genotype may prove useful for clinical trial enrichment. Specifically, restricting APOE4 carriers to those without KL*VShET+ 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.

Disclosure statement

The authors report no conflicts of interest.

CRediT authorship contribution statement

Michael E. Belloy: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft. Sarah J. Eger: Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - original draft. Yann Le Guen: Data curation, Writing - review & editing. Valerio Napolioli: Data curation, Supervision, Writing - review & editing. Kacie D. Deters: Data curation, Writing - review & editing. Hyun-Sik Yang: Data curation, Formal analysis, Validation, Writing - review & editing. Marzia A. Scelsi: Data curation, Writing - review & editing. Tenielle Porter: Data curation, Validation, Writing - review & editing. Sarah-Naomi James: Data curation, Writing - review & editing. Andrew Wong: Data curation, Writing - review & editing. Jonathan M. Schott: Resources, Writing - review & editing. Reisa A. Sperling: Resources, Writing - review & editing. Simon M. Laws: Resources, Writing - review & editing. Elisabeth C. Mormino: Data curation, Methodology, Writing - review & editing, Supervision. Zhihua He: Funding acquisition, Methodology, Supervision, Writing - review & editing. Summer S. Han: Methodology, Supervision, Writing - review & editing. Andre Altmann: Data curation, Formal analysis, Funding acquisition, Validation, Writing - review & editing. Michael D. Greicius: Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft.

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Appendix A. Supplementary data

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References


