ARTICLE

KLOTHO heterozygosity attenuates *APOE4*-related amyloid burden in preclinical AD

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Abstract

Objective

To examine whether the *KLOTHO* gene variant KL-VS attenuates *APOE4*-associated β-amyloid (Aβ) accumulation in a late-middle-aged cohort enriched with Alzheimer disease (AD) risk factors.

Methods

Three hundred nine late-middle-aged adults from the Wisconsin Registry for Alzheimer's Prevention and the Wisconsin Alzheimer's Disease Research Center were genotyped to determine KL-VS and *APOE4* status and underwent CSF sampling (n = 238) and/or ^{11}C-Pittsburgh compound B (PiB)-PET imaging (n = 183). Covariate-adjusted regression analyses were used to investigate whether *APOE4* exerted expected effects on Aβ burden. Follow-up regression analyses stratified by KL-VS genotype (i.e., noncarrier vs heterozygous; there were no homozygous individuals) evaluated whether the influence of *APOE4* on Aβ was different among KL-VS heterozygotes compared to noncarriers.

Results

APOE4 carriers exhibited greater Aβ burden than *APOE4*-negative participants. This effect was stronger in CSF ($t = -5.12$, $p < 0.001$) compared with PiB-PET ($t = 3.93$, $p < 0.001$). In the stratified analyses, this *APOE4* effect on Aβ load was recapitulated among KL-VS noncarriers (CSF: $t = -5.09$, $p < 0.001$; PiB-PET: $t = 3.77$, $p < 0$ .001). In contrast, among KL-VS heterozygotes, *APOE4*-positive individuals did not exhibit higher Aβ burden than *APOE4*-negative individuals (CSF: $t = -1.03$, $p = 0.308$; PiB-PET: $t = 0.92$, $p = 0.363$). These differential *APOE4* effects remained after KL-VS heterozygotes and noncarriers were matched on age and sex.

Conclusion

In a cohort of at-risk late-middle-aged adults, KL-VS heterozygosity was associated with an abatement of *APOE4*-associated Aβ aggregation, suggesting KL-VS heterozygosity confers protections against *APOE4*-linked pathways to disease onset in AD.

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Comment
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Glossary

Aβ = β-amyloid; **AD** = Alzheimer disease; **DVR** = distribution volume ratio; **MCI** = mild cognitive impairment; **PiB** = ^{11}C-Pittsburgh compound B; **ROI** = region of interest.

After aging, the greatest risk factor for developing late-onset Alzheimer disease (AD) is the presence of ≥1 copies of *APOE4*.[1,2] This genetic variant is overrepresented among persons with AD compared with the general population[3] and is associated with a faster rate of cognitive decline[4] and an earlier age at dementia onset[5] among persons with mild cognitive impairment (MCI). Even among cognitively normal adults, *APOE4* carriers exhibit comparatively greater cerebral β-amyloid (Aβ) deposition,[6,7] poorer performance on cognitive tests,[8] accelerated rate of cognitive decline,[9] and higher likelihood of a future diagnosis of MCI or AD dementia.[10] Overall, evidence suggests that a major pathophysiologic phenotype of *APOE4* is an increase in cerebral Aβ deposition.[11]

Klotho is a transmembrane protein and longevity factor[12,13] that increases neural functions and brain resilience.[14–16] It circulates throughout the body and brain to regulate myriad pathways, including insulin,[13] fibroblast growth factor,[17] and NMDA receptor[14–16] signaling. In humans, the *KLOTHO* gene variants F352V and C370S segregate together to form a haplotype (KL-VS) that modulates klotho secretion.[14,18] Carrying 1, but not 2, copies of the KL-VS haplotype increases systemic klotho levels,[14,19] promotes longevity and resilience against age-induced disease,[18,20,21] and is linked to enhanced cognition,[15] greater cortical volumes,[19,22] and increased brain connectivity[23] in aging humans.

KL-VS has been associated with better brain health among those aging normally but has not been extensively examined in those at high risk for developing neurodegenerative disease. Thus, it remains unknown whether positive links with the *KLOTHO* variant potentially extend to neurodegenerative disease. In light of this gap, we probed whether KL-VS exerts an effect on *APOE4*-induced aggregation of cerebral Aβ among late-middle-aged adults at risk for AD. We determined Aβ burden using both CSF sampling and ^{11}C-Pittsburgh compound B (PiB) PET.[24] Because previous studies have demonstrated that KL-VS heterozygosity has a protective effect,[14,18] we predicted that the prototypical *APOE4*-related increase in Aβ load would be attenuated among KL-VS heterozygotes.

Methods

Participants

Three hundred nine late-middle-aged adults from the Wisconsin Registry for Alzheimer's Prevention[25] and the Wisconsin Alzheimer's Disease Research Center participated in this study. They were diagnostically characterized as cognitively normal in standardized, multidisciplinary, consensus conferences on the basis of intact performance on a comprehensive battery of neuropsychological tests, absence of functional impairment, and absence of neurologic/psychiatric conditions that might impair cognition.[26,27] These individuals were selected for the present analyses on the basis of having undergone genotyping for *KLOTHO* and *APOE*, as well as having had either a PiB-PET examination or lumbar puncture assessing CSF $A\beta_{42}$. Specifically, of the 309 participants, 112 had both CSF and PiB-PET data, 126 had CSF but not PiB-PET data, and 71 had PiB-PET but not CSF data. Thus, the CSF analyses presented in this report included 238 participants (i.e., 112 + 126), whereas the PiB-PET analyses included 183 participants (i.e., 112 + 71).

Standard protocol approvals, registrations, and patient consents

The University of Wisconsin Institutional Review Board approved all study procedures, and each participant provided signed informed consent before participation.

Genotyping

DNA was extracted from whole-blood samples with the PUREGENE DNA Isolation Kit (Gentra Systems, Inc, Minneapolis, MN). DNA concentrations were quantified with ultraviolet spectrophotometry (DU 530 Spectrophotometer, Beckman Coulter, Fullerton, CA). Single nucleotide polymorphisms for *APOE* (rs429358 and rs7412) and *KLOTHO* (rs9536314 for F352V and rs9527025 for C370S) were genotyped by LGC Genomics (Beverly, MA) using competitive allele-specific PCR-based KASP genotyping assays. As expected from HapMap and previous work,[14,19,20] rs9536314 and rs9527025 were in perfect linkage disequilibrium. Quality control (e.g., duplicate sample concordance rates and Hardy-Weinberg equilibrium) has been previously described[7] and was found to be satisfactory.

CSF assessment

Lumbar puncture for collection of CSF samples was performed in the morning after a 12-hour fast with a Sprotte 24- or 25-gauge spinal needle at L3-4 or L4-5 with gentle extraction into polypropylene syringes. Each sample consisted of 22 mL CSF, which was then combined, gently mixed, and centrifuged at 2,000*g* for 10 minutes. Supernatants were frozen in 0.5-mL aliquots in polypropylene tubes and stored at −80°C. The samples were immunoassayed for $A\beta_{42}$ with INNOTEST ELISAs (Fujirebio, Gent, Belgium) by board-certified laboratory technicians who were blinded to clinical data and used protocols accredited by the Swedish Board for Accreditation and Conformity Assessment, as previously described.[28]

PiB-PET protocol

Details on the acquisition and postprocessing of the PiB-PET examinations have been previously described.[26] Briefly, 3-dimensional PiB-PET data were acquired on a Siemens EXACT HR+ scanner (Siemens AG, Erlangen, Germany). Imaging consisted of a 6-minute transmission scan and a 70-minute dynamic scan on bolus injection. Postprocessing was based on an in-house automated pipeline.[29] We derived distribution volume ratio (DVR) maps from the PiB images using the Logan method, with a cerebellar gray matter reference.[30] An anatomic atlas[31] was used to extract mean quantitative DVR data from 8 bilateral regions of interest (ROIs) that are sensitive to Aβ accumulation.[32,33] These ROIs were the angular gyrus, anterior cingulate, posterior cingulate, medial orbitofrontal cortex, precuneus, supramarginal gyrus, middle temporal gyrus, and superior temporal gyrus. The DVR data from the ROIs were also combined to form a composite measure of global Aβ burden.

Statistical analyses

We first ascertained that *APOE4* indeed exerts the expected effect on Aβ load within our relatively young sample by fitting a series of linear regression models that included terms for *APOE4*, age, sex, and parental history of AD. Then, to determine whether the *APOE4* effect was differentially instantiated as a function of KL-VS heterozygosity, we refitted the original model but this time stratified the sample by KL-VS genotype (i.e., fitting separate models for KL-VS noncarriers and KL-VS heterozygotes; there were no KL-VS homozygotes).[34] All analyses were conducted with IBM SPSS, version 21.0 (Armonk, NY). CSF $A\beta_{42}$ and the PiB composite were our primary outcomes and were evaluated at an unadjusted α of 0.05 (2 tailed). The ROI components of the PiB composite were secondary outcomes and were evaluated at a familywise error rate–adjusted α of 0.05 (2 tailed) using the Holm[35]-Bonferroni procedure.

Data availability statement

Anonymized data will be shared by request from any qualified investigator to the corresponding author for purposes of replicating procedures and results.

Results

Background characteristics

Table 1 details background characteristics of the participants for the overall sample and stratified by KL-VS genotype. The average age was 61.39 ± 6.52 years, and 68.9% were women. The average education was 16.10 ± 2.42 years; the mean Mini Mental State Examination score was 29.39 ± 0.86; 41.1% of participants were *APOE4* carriers; and 73.1% had a parental history of dementia. Similar to prior studies,[14,19,20] KL-VS heterozygotes represented 27% of the sample. There were no KL-VS homozygotes in our sample, which is an expected finding considering the allele frequency.[14] KL-VS noncarriers did not differ significantly from KL-VS heterozygotes on any of these background characteristics.

Association between *APOE4* and Aβ burden

As expected, *APOE4* was significantly associated with Aβ load in both CSF and brain platforms, with *APOE4* carriers exhibiting lower CSF $A\beta_{42}$ and higher PiB-PET binding compared with those who were *APOE4* negative (table 2, second column). These findings are depicted graphically in figure 1. This *APOE4* effect was numerically stronger in CSF (R^2 = 0.11, t = −5.12) compared with PiB-PET (largest R^2 [anterior cingulate] = 0.08, t = 4.28).

APOE4-related alteration in aβ burden varies by KL-VS status

Table 2 presents results of the KL-VS–stratified analyses. Among KL-VS noncarriers, *APOE4* carriers consistently exhibited lower CSF $A\beta_{42}$ values and higher PiB-PET retention compared with *APOE4* noncarriers (table 2, third column). In contrast, among KL-VS heterozygotes, *APOE4* carriers did not differ from *APOE4* noncarriers in either CSF $A\beta_{42}$ levels or PiB-PET retention (table 2, fourth column). The percent attenuation in the *APOE4* effect among KL-VS heterozygotes vis-à-vis KL-VS noncarriers ranged from 40% to 63% (table 2, last column). These findings are depicted in figure 1.

Table 1 Background characteristics of study participants

Variable	Total sample (n = 309)	KL-VSnc (n = 227)	KL-VShet (n = 82)	*p* Value
Age, y	61.39 (6.52)	61.51 (6.35)	61.05 (7.06)	0.685
Education, y	16.10 (2.42)	16.00 (2.37)	16.39 (2.53)	0.205
MMSE score	29.39 (0.86)	29.44 (0.84)	29.26 (0.92)	0.112
Women, %	68.9	69.6	67.1	0.671
White, %	96.1	96.5	95.1	0.211
KL-VShet, %	26.5	—	—	—
***APOE4* carrier, %**	41.1	41.9	39.0	0.656
Parental history of dementia, %	73.1	74.0	70.7	0.566

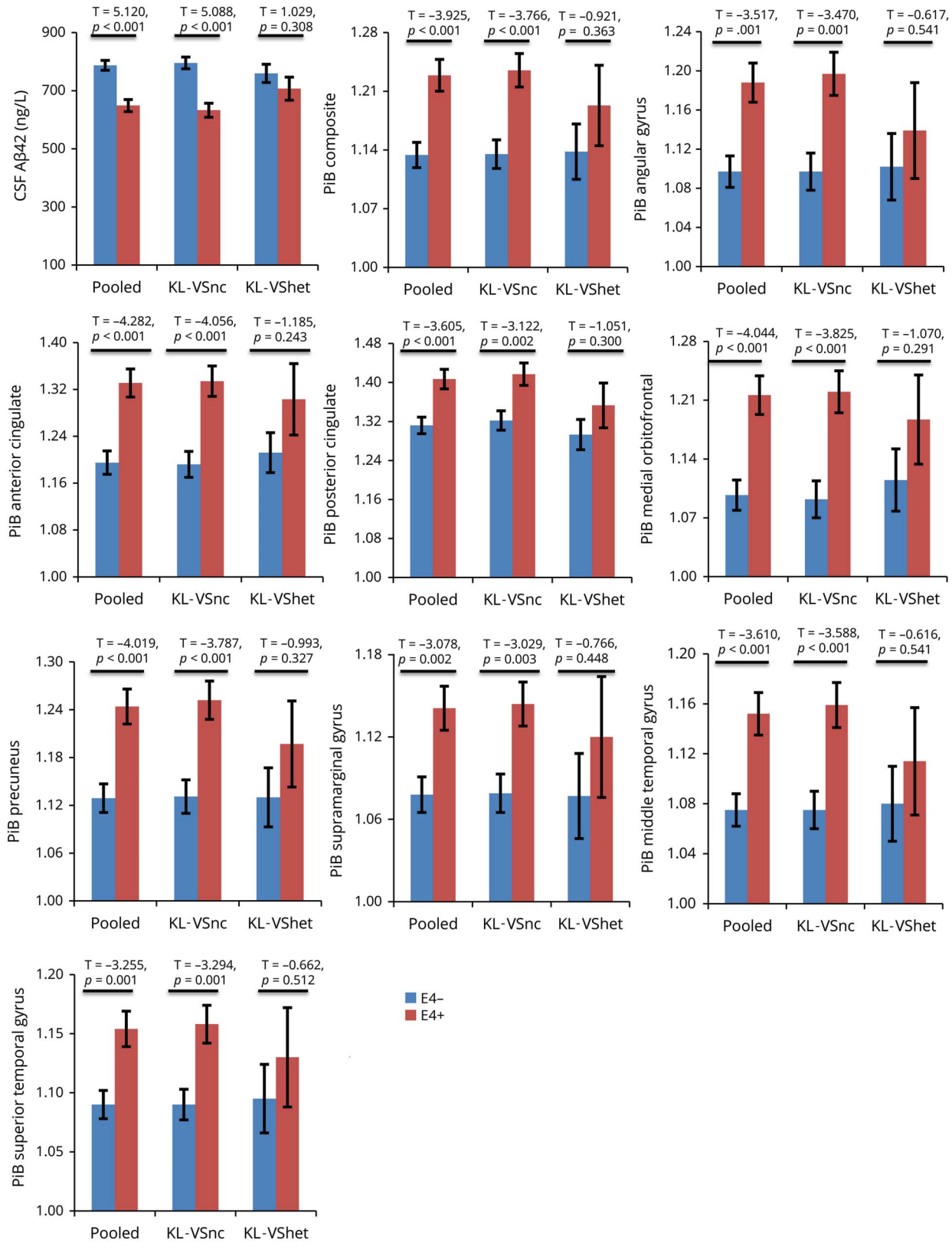
Abbreviations: KL-VShet = KL-VS heterozygote; KL-VSnc = noncarrier of the KL-VS variant; MMSE = Mini-Mental State Examination.

Table 2 Association between *APOE4* carriage and amyloid burden within entire sample: Pooled and KL-VS–stratified analyses

	Pooled analyses			KL-VSnc analyses			KL-VShet analyses			
Amyloid measure	B **(SE)**	***t* Statistic**	***p* Value**	B **(SE)**	***t* Statistic**	***p* Value**	B **(SE)**	***t* Statistic**	***p* Value**	**Reduction in *APOE4* effect, %**
CSF $A\beta_{42}$[a]	−138.43 (27.04)	−5.12	<0.001	−162.67 (31.97)	−5.09	<0.001	−52.44 (50.95)	−1.03	0.308	62.12
PiB composite[b]	0.10 (0.02)	3.93	<0.001	0.10 (0.03)	3.77	<0.001	0.06 (0.06)	0.92	0.363	40.00
PiB angular gyrus	0.09 (0.03)	3.52	0.001	0.10 (0.03)	3.47	0.001	0.04 (0.06)	0.62	0.541	55.56
PiB anterior cingulate	0.14 (0.03)	4.28	<0.001	0.14 (0.04)	4.06	<0.001	0.09 (0.08)	1.19	0.243	35.71
PiB posterior cingulate	0.10 (0.03)	3.61	<0.001	0.10 (0.03)	3.12	0.002	0.06 (0.06)	1.05	0.300	40.00
PiB medial orbitofrontal cortex	0.12 (0.03)	4.04	<0.001	0.13 (0.03)	3.83	<0.001	0.07 (0.07)	1.07	0.291	46.15
PiB precuneus	0.12 (0.03)	4.02	<0.001	0.12 (0.03)	3.79	<0.001	0.07 (0.07)	0.99	0.327	41.67
PiB supramarginal gyrus	0.06 (0.02)	3.08	0.002	0.07 (0.02)	3.03	0.003	0.04 (0.06)	0.77	0.448	42.86
PiB middle temporal gyrus	0.08 (0.02)	3.61	<0.001	0.08 (0.02)	3.59	<0.001	0.03 (0.05)	0.62	0.541	62.50
PiB superior temporal gyrus	0.06 (0.02)	3.26	0.001	0.07 (0.02)	3.29	0.001	0.04 (0.05)	0.66	0.512	42.86

Abbreviations: $A\beta_{42}$ = β-amyloid$_{42}$; KL-VShet = KL-VS heterozygote; KL-VSnc = noncarrier of the KL-VS variant; PiB = Pittsburgh compound B.
[a] For the CSF models, total sample was 238 individuals (142 *APOE4*−, 96 *APOE4*+), of whom 176 were KL-VSnc (104 *APOE4*−, 72 *APOE4*+) and 62 were KL-VShet (38 *APOE4*−, 24 *APOE4*+).
[b] For the PiB models, total sample was 183 individuals (110 *APOE4*−, 73 *APOE4*+), of whom 138 were KL-VSnc (80 *APOE4*−, 58 *APOE4*+) and 45 were KL-VShet (30 *APOE4*−, 15 *APOE4*+).

Figure 1 *APOE4* differentially associates with amyloid burden as a function of KL-VS status (whole-sample analyses)



Bar graphs depicting group differences in amyloid between *APOE4*− (blue bars) and *APOE4*+ individuals (red bars). Analyses were performed using all participants with available data. Specifically, for the CSF graph, total (i.e., pooled) sample was 238 individuals (142 *APOE4*−, 96 *APOE4*+), of whom 176 were noncarriers of the KL-VS variant (KL-VSnc) (104 *APOE4*−, 72 *APOE4*+) and 62 were KL-VS heterozygotes (KL-VShet) (38 *APOE4*−, 24 *APOE4*+). For the Pittsburgh compound B (PiB) graphs, total sample was 183 individuals (110 *APOE4*−, 73 *APOE4*+), of whom 138 were KL-VSnc (80 *APOE4*−, 58 *APOE4*+) and 45 were KL-VShet (30 *APOE4*−, 15 *APOE4*+). $A\beta_{42}$ = β-amyloid$_{42}$.

Because there were considerably more KL-VS noncarriers than KL-VS heterozygotes (176 vs 62 for CSF analyses, 138 vs 45 for PiB-PET analyses; see table 2 footnote), there is the possibility that the differential effect of *APOE4* within KL-VS noncarriers vs KL-VS heterozygotes might simply be due to this differing sample sizes. To exclude this possibility, the CSF analyses reported in table 2 were repeated after the 62 KL-VS heterozygotes with CSF data were age and sex matched to 62 KL-VS noncarriers (mean ± SD age 61.22 ± 7.71 vs 61.30 ± 8.27 years, respectively, $p = 0.956$; female 66.1% vs 64.5%, $p = 0.850$). Similarly, the PiB-PET analyses shown in table 2 were repeated after the 45 KL-VS heterozygotes with PiB-PET data were age and sex matched to 45 KL-VS noncarriers (mean ± SD age 61.05 ± 7.06 vs 61.09 ± 7.12, respectively, $p = 0.979$; female 62.2% vs 62.2%, $p = 1.00$).

As in the full-sample analyses, the matched-sample analyses revealed that, for KL-VS noncarriers, *APOE4* carriage was consistently associated with lower CSF $A\beta_{42}$ values and higher PiB-PET retention (table 3, third column), which was not the case for KL-VS heterozygotes (table 3, fourth column). In this matched sample, the percent attenuation in the *APOE4* effect among KL-VS heterozygotes vis-à-vis KL-VS noncarriers ranged from 53% to 75% (table 3, last column). These findings are also shown in figure 2.

To delineate the spatial reach of the PiB-PET findings on a whole-brain level, we implemented exploratory voxel-wise regression analyses in SPM8 (fil.ion.ucl.ac.uk/spm) within the matched sample (n = 90) while imposing a $p_{voxel} < 0.005$ uncorrected threshold and a cluster size minimum of 100 contiguous voxels for statistical significance. The analysis adjusted for the same covariates as in the ROI models. We found that, within the entire matched sample, *APOE4* carriage was significantly associated with greater Aβ burden in several brain regions, including right posterior cingulate, right middle temporal gyrus, right superior temporal gyrus, right superior frontal gyrus, left inferior temporal gyrus, bilateral inferior frontal gyrus, bilateral anterior cingulate, bilateral inferior parietal lobule, bilateral precuneus, and bilateral middle frontal gyrus (figure 3A). With stratification by KL-VS genotype, these findings were reproduced among KL-VS noncarriers with remarkable similarity (figure 3B). On the other hand, we did not detect any *APOE4* effects among KL-VS heterozygotes at the set threshold (figure 3C).

Direct effects of KL-VS on Aβ deposition

Although not a distinct aim of the study, we exploratorily examined whether KL-VS status is directly associated with Aβ burden. We ran these analyses in both the full sample (n = 183 for PiB, n = 238 for CSF $A\beta_{42}$) and the age- and sex-matched sample (n = 90 for PiB, n = 124 for CSF $A\beta_{42}$). For completeness sake, we additionally stratified by *APOE4* status. The only effect detected was of KL-VS on CSF $A\beta_{42}$ among *APOE4* carriers within the age- and sex-matched sample. Specifically, KL-VS heterozygotes in this subsample had higher $A\beta_{42}$ (714.81 ± 44.22) compared with KL-VS noncarriers (582.44 ± 44.22; $p = 0.049$).

Discussion

This study showed that, in a late-middle-aged cohort at risk for AD, KL-VS heterozygosity attenuates *APOE4* effects on Aβ burden, assessed with both PET imaging and CSF analyses. The relationship between *KLOTHO* and *APOE4* status has not been extensively investigated in humans. These data suggest that beneficial effects of the longevity factor klotho in the brain may mitigate deleterious mechanisms linked to *APOE4*. Furthermore, in line with previous findings, this study observed the well-described relationship between *APOE4* status and increased Aβ burden, even though our participants were relatively young (mean age 61 years).

APOE4 carriage has long been established as the most potent genetic risk factor for late-onset AD. Initial studies found that it exerted a strong gene dose effect on prevalence of AD.[5,36,37] Specifically, in a large case-control study, the distribution of cases diagnosed with AD at age 75 years was 24% among *APOE4* noncarriers, 61% among *APOE4* heterozygotes, and 86% among *APOE4* homozygotes.[5] The same study found that, relative to *APOE4* noncarriers, *APOE4* heterozygotes were at 2.8-fold increased hazard for developing AD, whereas *APOE4* homozygotes were at an 8.1-fold higher hazard for the disease.[5] Building on prior in vitro findings of a role for the ApoE protein in Aβ metabolism,[38] later in vivo studies demonstrated that, of the pathologic features of AD, *APOE4* was selectively linked to an increase in cerebral Aβ deposition.[3,39,40] In the current study, we similarly found that middle-aged adults who were *APOE4* carriers had increased cerebral Aβ and reduced CSF $A\beta_{42}$ compared with *APOE4* noncarriers.[41] We also found that this *APOE4* differential was more pronounced in CSF relative to PET imaging, suggesting a comparatively higher sensitivity of CSF $A\beta_{42}$ vis-à-vis PiB-PET to cerebral amyloidosis in this preclinical stage.[7,42–44] This finding corroborates prior studies suggesting that CSF-derived measures of β-amyloidosis are more sensitive to underlying AD pathology than neuroimaging-based measures.[42–44]

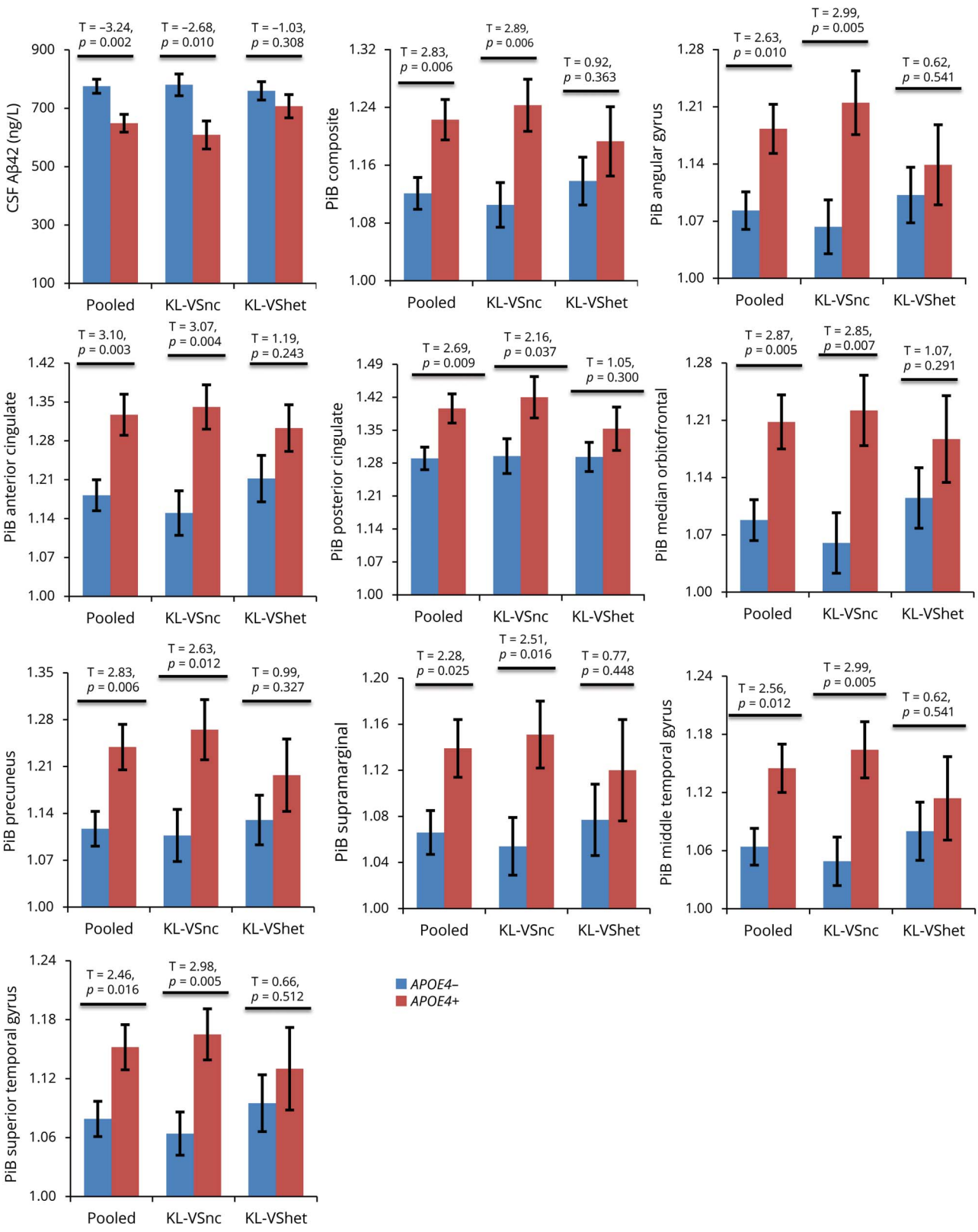
The KL-VS allele of the longevity gene *KLOTHO* has been associated with positive brain and cognitive health during normal human aging in a recent series of studies.[14,18,20,21] One such study[14] found that, in 3 separate cohorts of cognitively healthy adults, KL-VS heterozygotes had enhanced global cognition compared to KL-VS noncarriers. The average effect size of this enhancement across cohorts was a Cohen *d* of 0.34,[14] which exceeded the effect size of *APOE4* of 0.27 in similar healthy aging cohorts.[45] In 2 independent studies,[19,22] KL-VS heterozygosity was associated with greater volume in the right dorsolateral prefrontal cortex and better executive function,[19] as well as slower cognitive decline.[22] There is also evidence that KL-VS heterozygosity resulted in higher serum

Table 3 Association between *APOE4* carriage and amyloid burden within age- and sex-matched subsample: Pooled and KL-VS–stratified analyses

	Pooled analyses			KL-VSnc analyses			KL-VShet analyses			
Amyloid measure	**B (SE)**	***t* Statistic**	***p* Value**	**B (SE)**	***t* Statistic**	***p* Value**	**B (SE)**	***t* Statistic**	***p* Value**	**Reduction in *APOE4* effect, %**
CSF $A\beta_{42}$[a]	−126.77 (39.19)	−3.24	0.002	−171.80 (64.21)	−2.68	0.010	−52.44 (50.95)	−1.03	0.308	69.48
PiB composite[b]	0.10 (0.04)	2.83	0.006	0.14 (0.05)	2.89	0.006	0.06 (0.06)	0.92	0.363	57.14
PiB angular gyrus	0.10 (0.04)	2.63	0.010	0.15 (0.05)	2.99	0.005	0.04 (0.06)	0.62	0.541	73.33
PiB anterior cingulate	0.15 (0.05)	3.10	0.003	0.19 (0.06)	3.07	0.004	0.09 (0.08)	1.19	0.243	52.63
PiB posterior cingulate	0.11 (0.04)	2.69	0.009	0.13 (0.06)	2.16	0.037	0.06 (0.06)	1.05	0.300	53.85
PiB medial orbitofrontal cortex	0.12 (0.04)	2.87	0.005	0.16 (0.06)	2.85	0.007	0.07 (0.07)	1.07	0.291	56.25
PiB precuneus	0.12 (0.04)	2.83	0.006	0.16 (0.06)	2.63	0.012	0.07 (0.07)	0.99	0.327	56.25
PiB supramarginal gyrus	0.07 (0.03)	2.28	0.025	0.10 (0.04)	2.51	0.016	0.04 (0.06)	0.77	0.448	60.00
PiB middle temporal gyrus	0.08 (0.03)	2.56	0.012	0.12 (0.04)	2.99	0.005	0.03 (0.05)	0.62	0.541	75.00
PiB superior temporal gyrus	0.07 (0.03)	2.46	0.016	0.10 (0.03)	2.98	0.005	0.04 (0.05)	0.66	0.512	60.00

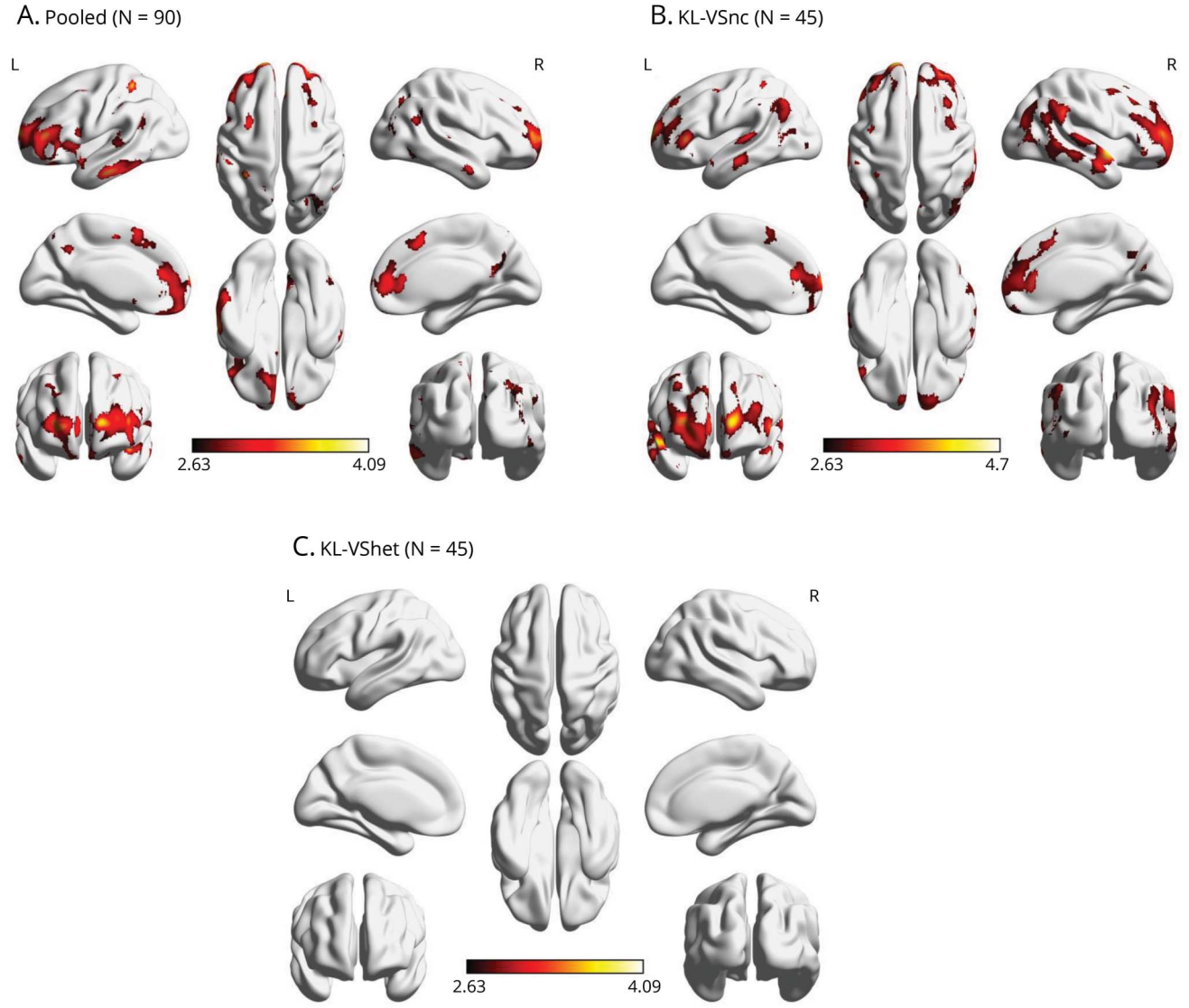
Abbreviations: $A\beta_{42}$ = β-amyloid$_{42}$; KL-VShet = KL-VS heterozygote; KL-VSnc = noncarrier of the KL-VS variant; PiB = Pittsburgh compound B.
[a] The CSF models included the 62 KL-VShet individuals (38 *APOE4*−, 24 *APOE4*+) who provided data for the analyses shown in table 2. They were age and sex matched to 62 KL-VSnc individuals (38 *APOE4*−, 24 *APOE4*+) for a total of 124 individuals (76 *APOE4*−, 48 *APOE4*+) in the pooled analyses.
[b] The PiB models included the 45 KL-VShet individuals (30 *APOE4*−, 15 *APOE4*+) who provided data for the analyses shown in table 2. They were age and sex matched to 45 KL-VSnc individuals (26 *APOE4*−, 19 *APOE4*+) for a total of 90 individuals (56 *APOE4*−, 34 *APOE4*+) in the pooled analyses.

Figure 2 *APOE4* differentially associates with amyloid burden as a function of KL-VS status (matched-sample analyses)



Bar graphs depicting group differences in amyloid between *APOE4*− (blue bars) and *APOE4*+ (red bars) individuals. Analyses were performed among age- and sex-matched participants. Specifically, the CSF graph included the 62 KL-VS heterozygote (KL-VShet) individuals (38 *APOE4*−, 24 *APOE4*+) who provided data for the results shown in figure 1. They were age and sex matched to 62 noncarriers of the KL-VS variant (KL-VSnc) (38 *APOE4*−, 24 *APOE4*+) for a total (i.e., pooled) sample of 124 individuals (76 *APOE4*−, 48 *APOE4*+). The Pittsburgh compound B (PiB) graphs included the 45 KL-VShet individuals (30 *APOE4*−, 15 *APOE4*+) who provided data for the results shown in figure 1. They were age and sex matched to 45 KL-VSnc individuals (26 *APOE4*−, 19 *APOE4*+) for a total sample of 90 individuals (56 *APOE4*−, 34 *APOE4*+). $A\beta_{42}$ = β-amyloid$_{42}$.

Figure 3 Spatial representation of *APOE4*–amyloid burden relationship as a function of KL-VS status (tested within age- and sex-matched subsample)



A 3-dimensional rendering of the effect of *APOE4* carriership on cerebral amyloid among (A) a pooled sample of 90 participants comprising 45 KL-VS heterozygote (KL-VShet) individuals who were age and sex matched to 45 noncarriers of the KL-VS variant (KL-VSnc), (B) the 45 KL-VSnc individuals, and (C) the 45 KL-VShet individuals. Results were thresholded at $p_{voxel} < 0.005$ and a minimum of 100 contiguous voxels on the basis of Monte Carlo simulations (3dClustSim, AFNI, afni.nimh.nih.gov).

klotho, which, in turn, predicted greater intrinsic connectivity in key cortical hubs such as the default mode network.[23] Even so, it should be noted that beneficial effects of KL-VS were not observed in other populations at particularly advanced ages.[46,47] The culmination of these studies is that KL-VS heterozygosity largely wields a salutary influence on brain aging. In this study, we extend prior reports by showing that KL-VS heterozygosity abates the potent effect of *APOE4* carriage on Aβ burden in those at risk for AD.

This study demonstrates that the *KLOTHO* variant KL-VS is associated with attenuation of a key pathogenic protein and biomarker of neurodegenerative disease. It is interesting to speculate that, since the klotho protein is a longevity factor, its protective effects could relate to mechanisms that delay aging itself.[14] Alternatively, klotho effects could be related directly or indirectly to the ApoE protein and its known effects on Aβ aggregation and clearance.[48–50] Whether klotho and ApoE interact directly or indirectly and whether klotho blocks ApoE-induced mechanisms should be determined. In addition to the prognostic implications of carrying the KL-VS gene variant of *KLOTHO*, these data suggest that *KLOTHO* pathways may counter deleterious effects of *APOE4* in aging and disease.

A limitation to our study is the demographic composition. Participants were mostly highly educated and white, thus potentially limiting the generalizability of our study findings.

Another limitation is the relatively high prevalence of *APOE4* carriership within the cohort compared to the general population. In addition, given the cross-sectional nature of this study, we do not know whether KL-VS heterozygosity modifies *APOE4*-related trajectories in prospective cognitive course or in the attainment of clinically relevant endpoints of MCI/dementia. Because we are following up the Wisconsin Registry for Alzheimer's Prevention/Wisconsin Alzheimer's Disease Research Center cohorts prospectively, we will be well positioned to tackle this important question in the future.

Our key finding is that KL-VS heterozygosity attenuates the effect of *APOE4* on Aβ deposition, suggesting that KL-VS heterozygosity potentially alters *APOE4*-associated differences in disease pathology, thus conferring resilience to *APOE4*-linked pathways to disease onset in AD.

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Disclosure

C. Erickson, S. Schultz, J. Oh, B. Darst, Y. Ma, D. Norton, T. Betthauser, C. Gallagher, C. Carlsson, B. Bendlin, S. Asthana, B. Hermann, and M. Sager report no disclosures relevant to the manuscript. K. Blennow has served as a consultant or on advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Pfizer, and Roche Diagnostics and is a cofounder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures–based platform company at the University of Gothenburg. H. Zetterberg has served at advisory board for Eli Lilly, Roche Diagnostics, and Wave; has received travel support from Teva; and is cofounder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures–based platform company at the University of Gothenburg. C. Engelman and B. Christian report no disclosures relevant to the manuscript. S. Johnson has served on an advisory board for Roche Diagnostics. D. Dubal has served as a consultant for Unity Biotechnology. Klotho is the subject of a pending international patent application held by the Regents of the University of California. O. Okonkwo reports no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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Continued

Appendix (continued)

Name	Location	Role	Contribution
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