KL-VS heterozygosity is associated with lower amyloid-dependent tau accumulation and memory impairment in Alzheimer’s disease

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Klotho-VS heterozygosity (KL-VS\textsuperscript{het}) is associated with reduced risk of Alzheimer’s disease (AD). However, whether KL-VS\textsuperscript{het} is associated with lower levels of pathologic tau, i.e., the key AD pathology driving neurodegeneration and cognitive decline, is unknown. Here, we assessed the interaction between KL-VS\textsuperscript{het} and levels of beta-amyloid, a key driver of tau pathology, on the levels of PET-assessed neurofibrillary tau in 551 controls and patients across the AD continuum. KL-VS\textsuperscript{het} showed lower cross-sectional and longitudinal increase in tau-PET per unit increase in amyloid-PET when compared to that of non-carriers. This association of KL-VS\textsuperscript{het} on tau-PET was stronger in Klotho mRNA-expressing brain regions mapped onto a gene expression atlas. KL-VS\textsuperscript{het} was related to better memory functions in amyloid-positive participants and this association was mediated by lower tau-PET. Amyloid-PET levels did not differ between KL-VS\textsuperscript{het} carriers versus non-carriers. Together, our findings provide evidence to suggest a protective role of KL-VS\textsuperscript{het} against amyloid-related tau pathology and tau-related memory impairments in elderly humans at risk of AD dementia.
Klotho is a transmembrane protein that has been associated with enhanced longevity and better brain health in aging. Klotho is expressed primarily in the kidney and brain, where it has been implicated in a number of vital cellular functions (for review see). Loss-of-function mutations in transgenic mice are associated with reduced Klotho protein expression, accelerated aging phenotypes, and dramatically shortened life span. In humans, two variants in the Klotho gene (KL, 13q13.1), rs9556314 (F352Y) and rs9527025 (C370S), form a functional haplotype. Carrying one copy, but not two copies of the KL-VS haplotype, referred to as KL-VS heterozygosity (KL-VShet), has been previously linked to increased Klotho levels in the blood. KL-VShet occurs in about 20–25% of the population and is associated with higher cognitive performance across the adult life span, larger frontotemporal gray matter volume in cognitively normal individuals, and lower mortality. Together, these results suggest a crucial role of Klotho in the maintenance of cognitive abilities and brain integrity during aging. Beyond the protective role of Klotho in normal aging, recent studies suggest an association between Klotho and reduced risk of Alzheimer’s disease (AD), the most frequent cause of dementia in the elderly. A recent meta-analysis reported that KL-VShet was associated with reduced AD dementia risk and cognitive decline in elderly individuals carrying the ApoE ε4 allele, i.e., the strongest genetic risk factor for AD dementia possibly through elevated levels of primary AD pathology including cortical beta-amyloid (Aβ) aggregation. Importantly, KL-VShet was associated with reduced biomarker levels of Aβ deposition in ApoE ε4 carriers, suggesting that KL-VShet may directly alter levels of primary AD pathology.

Yet, an open question is whether Klotho is associated with altered levels of fibrillary tangles containing pathologic tau, i.e., the key driver of disease progression in AD. In the presence of Aβ deposition, i.e., the earliest primary pathology in AD, neurofibrillary tangles spread from the medial temporal lobe to higher cortical areas. The progressive development of neurofibrillary tangles in the presence of Aβ pathology is closely associated with gray matter atrophy and cognitive worsening and is more predictive of such alterations than Aβ. Due to the high clinical relevance of tau pathology, it is pivotal to understand whether the KL-VShet variant attenuates the accumulation of neurofibrillary tangles at a given level of Aβ deposition, and thus a cognitive decline in AD. Studies in mouse models of Aβ and accelerated aging reported that enhancing KL expression was associated with reduced Aβ burden and phosphorylated tau, although conflicting results were reported as well. However, these mouse models fail to develop neurofibrillary tangles in the presence of Aβ and thus only incompletely recapitulate AD-specific tau pathology in humans. Here, we examined whether KL-VShet attenuates the association between higher Aβ and higher fibrillary tau assessed via positron emission tomography (PET) in a group of 551 elderly asymptomatic and symptomatic individuals recruited within a large North American multicenter study on AD. We found the KL-VShet variant to be associated with an attenuated increase in regional tau-PET at pathological levels of global amyloid-PET, suggesting that KL-VShet was potentially protective against Aβ-related increase in neurofibrillary tangles. This association was pronounced in ApoE ε4 carriers. The strength of the KL-VShet effect on region-specific tau-PET levels was correlated with the regional expression pattern of KL in the brain, supporting the notion that the KL-VShet variant modulates the regional accumulation of tau pathology. Importantly, KL-VShet was associated with higher memory performance and this association was mediated by reduced tau-PET levels in KL-VShet carriers with the elevated amyloid-PET burden. For Aβ, we did not find the previously reported association between KL-VShet and lower Aβ pathology in the current sample, but confirmed this link in a larger sample including all individuals with amyloid-PET but not necessarily tau-PET assessment available indicating that the effect size of KL-VShet on Aβ was smaller than that on tau pathology.

**Results**

Detailed sample characteristics are presented in Table 1. Among the 551 participants (347 CN, 156 MCI, 48 ADD), there were 144 KL-VShet carriers and 407 non-carriers. Demographics (age, sex, and education) or ApoE ε4 status did not differ between KL-VShet carrier and non-carrier groups (all p > 0.05). Continuous values of global

| Table 1 Sample characteristics. | KL-VShet carriers | KL-VShet non-carriers | p value |
|---------------------------------|------------------|----------------------|--------|
| ADNI, all                       |                  |                      |        |
| N                               | 144              | 407                  |        |
| Age                             | 71.29 (6.61)     | 71.39 (6.72)         | 0.880  |
| Sex, F:M                        | 76.68            | 206.201              | 0.727  |
| Diagnosis, CN:MCI:ADD           | 102.348          | 245.122.40           | 0.059  |
| Education, years                | 16.20 (2.50)     | 16.65 (2.51)         | 0.065  |
| MMSE                            | 28.17 (2.43)     | 28.11 (2.82)         | 0.819  |
| ApoE ε4 status, neg:pos         | 90:54            | 255:152              | 1.000  |
| Global amyloid-PET, CL          | 28.85 (37.94)    | 32.28 (40.30)        | 0.373  |
| Amyloid-PET status, neg:pos     | 89:55            | 232:175              | 0.365  |
| Longitudinal subsample          |                  |                      |        |
| N                               | 52               | 148                  |        |
| Age                             | 70.93 (6.76)     | 71.43 (6.56)         | 0.631  |
| Sex, F:M                        | 69.24            | 69.79                | 0.462  |
| Diagnosis, CN:MCI:ADD           | 36:12:4          | 77:55:16             | 0.097  |
| Education, years                | 15.85 (2.58)     | 16.56 (2.51)         | 0.081  |
| MMSE                            | 28.00 (2.59)     | 27.92 (2.48)         | 0.841  |
| ApoE ε4 status, neg:pos         | 25:27            | 81:67                | 0.506  |
| Global amyloid-PET, CL          | 45.01 (41.44)    | 42.73 (42.59)        | 0.739  |
| Amyloid-PET status, neg:pos     | 22:30            | 61:87                | 1.000  |
| Tau-PET follow-up, years        | 1.54 (0.75)      | 1.66 (0.80)          | 0.330  |

CN cognitively normal, MCI mild cognitive impairment, ADD Alzheimer’s disease dementia, F female, M male, MMSE Mini-Mental State Examination, neg negative, pos positive, CL centroid.
amylod-PET uptake did not differ between KL-VShet carriers versus non-carriers (t(265.06) = 0.92, p = 0.373).

KL-VS heterozygosity is associated with lower amylod-related tau accumulation. In the main analysis, we tested the hypothesis that KL-VShet modifies the association between Aβ and tau pathology (both assessed by continuous measures of PET uptake). In a region-of-interest (ROI)-based analysis, we focused on tau-PET in the inferior temporal cortex (i.e., ROI of early Aβ-related tau pathology[20,26,35–37]) and whole-brain tau-PET levels. The results of a linear regression analysis showed a significant KL-VS het × amylod-PET interaction effect on tau-PET levels in both the inferior temporal ROI (standardized beta = −0.12, p = 0.009, N = 551, effect size measured by Cohen’s f = 0.112) and the global ROI (beta = −0.13, p = 0.008, N = 551, Cohen’s f = 0.114). For both tau-PET ROIs, the increase in tau-PET as a function of rising global amylod-PET was attenuated in KL-VS het carriers versus non-carriers (Fig. 1a, b). The bootstrapped mean t-value of the interaction effect differed significantly from that of the null distribution (inferior temporal ROI: t(1901.9) = −47.43, p < 0.001; global ROI: t(1839.2) = −48.99, p < 0.001; Supplementary Fig. 2). Only the distribution of t-values based on the actual, unshuffled KL-VS het labels was significantly greater than zero (inferior temporal ROI: t(999) = −77.76, p < 0.001; global ROI: t(999) = −83.88, p < 0.001) and the 95% confidence intervals did not include zero (inferior temporal ROI: 95% CI = [−4.847, −0.563]; global ROI: 95% CI = [−4.763, −0.794]). Together, these results confirmed a robust association between KL-VS het and lower Aβ-associated tau accumulation.

In order to determine whether our findings were driven by clinical diagnosis, we repeated the analyses in 156 MCI patients (34 KL-VS het carriers and 122 non-carriers) and found comparable KL-VS het × amyloid-PET interaction effects on tau-PET uptake (inferior temporal ROI: beta = −0.26, p = 0.003, N = 156, Cohen’s f = 0.251; global ROI: beta = −0.25, p = 0.004, N = 156, Cohen’s f = 0.243; Supplementary Fig. 1c, d). Repeating the analysis in 347 CN participants (102 KL-VS het carriers and 245 non-carriers) yielded no KL-VS het × amyloid-PET interaction effects on tau-PET levels in either ROI (both p > 0.05; Supplementary Fig. 1e, f).

A few participants showed lower tau-PET levels in the ROIs than in the reference region resulting in a tau-PET standard uptake value ratio (SUVR) < 1 (2 participants for the inferior temporal ROI and 51 participants for the global ROI). Yet, the results of the KL-VS het × amyloid interaction analyses on tau-PET levels remained significant after excluding those participants (Supplementary Fig. 1g, f).
KL-VS heterozygosity is related to lower amyloid-dependent tau accumulation over time. In a subsample of 200 participants in whom longitudinal tau-PET data were available, we investigated whether KL-VS\textsuperscript{het} attenuates the association between baseline amyloid-PET levels and the rate of change in tau-PET assessed over a time interval of 1.63 years on average (range: 1–4 years). We found a significant KL-VS\textsuperscript{het} × amyloid-PET interaction effect on tau-PET annual change rates in the inferior temporal ROI (beta = −0.22, p = 0.039, N = 200, Cohen’s f = 0.148), but not in the global ROI (beta = −0.15, p = 0.176, N = 200, Cohen’s f = 0.098). KL-VS\textsuperscript{het} carriers showed lower tau-PET increases in inferior temporal cortices over time as a function of rising global amyloid-PET levels (Fig. 1c, d) suggesting that the KL-VS\textsuperscript{het} variant might be protective against Aβ-associated increase of tau pathology. The main effects of amyloid-PET on tau-PET change rates for KL-VS\textsuperscript{het} carriers and non-carriers are reported in Supplementary Table 1.

Stronger protective effect of KL-VS heterozygosity in ApoE ε4 carriers. Previous studies have reported an ApoE ε4-genotype-dependent effect of KL-VS\textsuperscript{het} on amyloid-PET\textsuperscript{19}. Hence, we additionally explored whether ApoE ε4 carriers showed a stronger association between KL-VS\textsuperscript{het} and lower tau accumulation than ApoE ε4 non-carriers, controlling for age, sex, education, diagnosis, and global amyloid-PET levels in the regression analyses. This analysis yielded a significant KL-VS\textsuperscript{het} × ApoE ε4 interaction effect on tau-PET levels (inferior temporal ROI: beta = −0.11, p = 0.031, N = 551, Cohen’s f = 0.093; global ROI: beta = −0.10, p = 0.041, N = 551, Cohen’s f = 0.088; Supplementary Fig. 3).

Spatial match between KL mRNA expression and the effect of KL-VS heterozygosity on tau-PET. In order to estimate the spatial overlap between the strength of KL gene expression and the test statistic of the KL-VS\textsuperscript{het} × amyloid-PET interaction on tau-PET, we obtained whole-brain mRNA expression levels of KL generated by post-mortem microarray assessments of six healthy brain donors and subsequently mapped to the Allen Brain Atlas\textsuperscript{33,34}. We computed median scores of log2 mRNA expression of KL across the six donors within 34 left-hemispheric regions of the Freesurfer-based Desikan–Killiany brain atlas\textsuperscript{38}. We focused on the left hemisphere since all donors had microarray assessment available for the left hemisphere and only two donors had an assessment for the right hemisphere. Furthermore, we estimated the KL-VS\textsuperscript{het} × amyloid-PET interaction effect on tau-PET levels within the same 34 brain atlas regions using the aforementioned regression model. Surface mapping of both the KL-VS\textsuperscript{het} × amyloid-PET interaction effect (which were all in the same direction) and KL mRNA expression is displayed in Fig. 2a–d. Spatial correlation analysis revealed a significant association (r = 0.46, p = 0.007; Fig. 2e). This result suggests that regions with higher KL mRNA expression levels were more likely to display lower Aβ-related tau-PET levels in KL-VS\textsuperscript{het} carriers versus non-carriers. Visual inspection of the thresholded spatial maps indicated that those areas showing both a significant KL-VS\textsuperscript{het} × amyloid-PET interaction effect (Fig. 2b; see Supplementary Table 2 for detailed statistical results) and high KL mRNA expression (log2 > 75th percentile) (Fig. 2d) were specifically located within the mesiotemporal and inferior and middle temporal brain regions and the posterior cingulum.
Tau mediates the association between KL-VS heterozygosity and less memory impairment. In the main analysis, we assessed whether KL-VS^{het} is beneficial for memory functions via lowering tau pathology. Because the interaction effect of KL-VS^{het} × amyloid-PET on tau-PET levels showed that KL-VS^{het} is associated with lower tau accumulation at higher levels of amyloid-PET, we restricted our analysis to amyloid-positive participants. We used mediation analysis with 10,000 bootstrapping iterations in order to test whether KL-VS^{het} is associated with better memory in individuals with elevated ApoE ε4 carrier status. Boxplots show the 25th percentile, median, 75th percentile (box), and 95% confidence intervals of the median (notch), and 1.5× IQR (whiskers).

Is KL-VS heterozygosity associated with lower Aβ accumulation? A recent study found a protective influence of KL-VS^{het} on longitudinal amyloid-PET in cognitively unimpaired ApoE ε4 carriers aged between 60 and 80 years, but not in ApoE ε4 non-carriers or older participants.13 In contrast to this earlier report, we did not find an age-dependent KL-VS^{het} effect on cross-sectional amyloid-PET levels acquired at the time of tau-PET assessment in the current sample (KL-VS^{het} × age interaction: beta = 0.21, p = 0.597, N = 551; Supplementary Fig. 6a) or an ApoE ε4-dependent KL-VS^{het} effect in the subsample of CN participants aged between 60 and 80 years (beta = 0.04, p = 0.586, N = 347, Cohen’s f = 0.030; Supplementary Fig. 5b).
Fig. 6b). However, more subtle effects may have been overlooked in the current more restricted sample of individuals undergoing both amyloid- and tau-PET. Therefore, in a supplementary analysis, we included all participants with amyloid-PET (N = 1067) from ADNI, regardless of whether or not they underwent tau-PET assessment. We found a trend-level significant KL-VShet × age interaction effect one amyloid-PET that demonstrated that KL-VShet carriers in the lower age range (<80 years) displayed lower amyloid-PET levels than non-carriers (β = 0.53, p = 0.046, Cohen’s f = 0.061, N = 1067; Supplementary Fig. 7a). Consistent with the earlier report, we found a significant KL-VShet × ApoE ε4 interaction effect on global amyloid-PET levels in CN participants aged between 60 and 80 years (β = −0.121, p = 0.043, Cohen’s f = 0.095, N = 464; Supplementary Fig. 7b). The same analysis in MCI participants within the same age range showed no significant interaction effect (β = 0.02, p = 0.780, N = 463, Cohen’s f = 0.013), suggesting that the association between KL-VShet and lower amyloid-PET uptake is restricted to a younger age and non-symptomatic cognitive status. See Supplementary Table 3 for detailed sample characteristics of the larger ADNI amyloid-PET sample compared to the current ADNI tau-PET sample. Thus, our results in the larger sample are consistent with those from Belloy et al.’s analysis on the effect of KL-VShet on amyloid-PET stratified by age and ApoE genotype while also showing that the effect size of KL-VShet on tau-PET is stronger than that on amyloid-PET.

Discussion

The heterozygous KL gene variant KL-VShet has been previously associated with higher longevity and cognition performance in adulthood and reduced AD dementia risk. We demonstrate that elderly KL-VShet carriers with elevated Aβ burden, i.e., the earliest primary AD pathology, exhibited lower tau-PET levels and tau-PET annual change rates when compared to those in KL-VShet non-carriers. In amyloid-positive participants, the KL-VShet variant was associated with better memory performance, and this relationship was mediated by lower tau-PET levels, suggesting that lower levels of pathologic tau in the KL-VShet carriers explained the association between KL-VShet and better memory performance. Although our findings do not implicate a causative mechanism of Klotho in AD, we provide evidence for a potential protective role of KL-VShet against Aβ-dependent tau pathology that is the key AD brain alteration linked to cognitive impairment.

To our knowledge, the current study is the first to date that evaluated the interaction between KL-VShet and Aβ on tau accumulation and cognitive decline in humans. There is a growing literature on protective genetic variants in AD but only a few studies have reported genetic variants to be associated with lower tau pathology in AD. For the KL-VShet variant, previous studies reported an association with reduced Aβ accumulation in elderly ApoE ε4 risk-carriers. We extend these previous findings by showing that the relationship between Aβ accumulation and fibrillar tau is modulated by KL-VShet, such that lower local and global tau-PET levels were observed per unit increase of global amyloid-PET burden in KL-VShet carriers when compared to those in non-carriers. This is important because Aβ deposition precedes the development of dementia symptoms by up to 20 years, and as confirmed by a very recent longitudinal amyloid-tau-PET study, high baseline Aβ is associated with subsequent tau accumulation, while Aβ and tau in synergy lead to most pronounced subsequent cognitive decline. The region showing one of the strongest interaction effects between KL-VShet and amyloid-PET on tau-PET was the inferior temporal gyrus (Fig. 2a), a brain area that typically shows an early Aβ-related increase in tau-PET before elevated tau-PET levels extend to other higher cortical brain areas. The protective association between KL-VShet and tau-PET was present selectively in participants with abnormally elevated levels of amyloid-PET and more pronounced in ApoE ε4 carriers. Stratified analyses further revealed a significant KL-VShet effect in the MCI but not in the CN subgroup, which could potentially be due to a stage-dependent beneficial effect of Klotho. However, an alternative explanation is that the levels of both amyloid- and tau-PET are lower in CN compared to those in MCI, and thus any protective effect is likely to be of smaller size and would require a larger sample size to detect. Together, these results support the notion that KL-VShet is associated with an Aβ-related rather than age-related reduction of tau pathology.

In amyloid-positive individuals, we found KL-VShet to be associated with better memory performance, mediated by the effect of KL-VShet on tau-PET. Our results are broadly consistent with those from studies on health or aging, reporting KL-VShet to be associated with better cognition, and lower risk of conversion from cognitively normal to mild cognitive impairment or AD dementia in ApoE ε4 carriers. Our findings suggest that the association between KL-VShet and lower neurofibrillar tau pathology is of central importance for the association found between KL-VShet and less cognitive impairment. A previously reported absence of an association between KL-VShet and cognitive decline in asymptomatic participants with elevated levels of Aβ did not assess the presence of abnormal neurofibrillary tau, which may have hampered to detect an effect of KL-VShet on cognitive decline in subjects at risk of AD.

Previous studies reported KL-VShet to be associated with lower amyloid-PET in ApoE ε4 carriers (but not in ApoE ε4 non-carriers), which was strongest in the age range between 60 and 80 years. In our primary analysis, we did not confirm age- or ApoE ε4-dependent effects of KL-VShet on tau-PET. By investigating a larger sample of all participants with available amyloid-PET regardless of the availability of tau-PET (N = 1067), we were able to substantiate those earlier findings. Specifically, we showed reduced amyloid-PET burden in younger KL-VShet carriers (<80 years) and, in accordance with previous work, this association was mainly driven by cognitively unimpaired ApoE ε4 carriers rather than non-carriers or MCI patients. Comparing effect sizes, Cohen’s f = 0.061 for the association between KL-VShet and lower amyloid-PET versus f = 0.114 for the association with lower tau-PET, strengthens the important role of changes in tau pathology for understanding the role of Klotho in AD.

The mechanisms linking Klotho to tau pathology remain elusive. Klotho is a pleiotropic protein that has been implicated in multiple biological processes including insulin regulation, growth factor functions, in particular of FGF23, regulation of members of the redox system, and calcium signaling. One possibility of how the Klotho protein might be linked to reduced neurofibrillary tau is its involvement in autophagy, a mechanism that is involved in the clearance of AD pathologies. Lenti-viral overexpression of Klotho protein in an APP-PS1 mouse model of Aβ deposition reduced Aβ plaque load in aged mice and rescued the impaired autophagy possibly by modulating the Akt/mTOR pathway. Since APP-PS1 mice do not develop tau pathology, it remains, however, to be tested whether Klotho-induced autophagy reduces tau pathology. Those mechanistic explanations remain speculative at this point and the current work encourages future studies to investigate the mechanism that could underlie the protection Klotho exerts against the development of Aβ-related tau pathology.

Our findings of the spatial correspondence between the strength of the effect of KL-VShet on regional tau-PET and the spatial distribution of KL mRNA suggest a local effect of Klotho on the development of fibrillar tau, especially in temporal brain areas. Alternative splicing of the human KL mRNA results in both a membrane-bound and a
secreted transcript of Klotho1,4, indicating that Klotho may act both in a cell-autonomous manner and as a humoral factor. Therefore, differences in gene expression in KL in the brain and/or different circulating levels of Klotho linked to KL-VS may influence the development of pathological tau1, but this link remains to be investigated.

Our results have important implications for clinical trials in AD. Since tau pathology correlates more closely with clinical symptoms than Aβ, tau-targeted therapies seem a promising approach to arrest disease progression10. The common KL-VS genotype may inform those clinical trials that target tau pathology. Especially when anti-tau trials aim to include amyloid-positive or ApoE ε4 carrying participants, group differences in the KL-VSε4 variant may be taken into account when estimating the expected change in tau pathology over time, which would be useful in the computation of statistical power to detect a treatment effect. Furthermore, the current findings encourage future studies to test whether enhancing Klotho protein levels could reduce the development of tau pathology in amyloid-positive participants. The Klotho protein is druggable and could thus be made a target in the development of disease-modifying therapeutic approaches.

Several caveats should be considered when interpreting the current results. First, the human KL gene consists of three polymorphic variants. We decided to focus on the KL-VS haplotype given the existing evidence of its beneficial influence on Aβ and cognition in both mice and humans5,8,10,13,22. While the second variant C1818T (rs564481) is located on the fourth exon and likely has no functional consequences itself, the third variant G395A (rs1207568) is located in the promoter region and may be a potential regulatory site of KL. The two latter variants appear more frequent in Asian populations, where they have been linked to cardiovascular risk factors53. Related to the current research question, an investigation across three independent cohorts of oldest-old Danes found different polymorphic variants of KL, besides KL-VS, to be associated with better cognitive functions7. It has yet to be proven whether these other KL variants also support resilience in AD. Another caveat is that we did not measure Klotho protein levels in the serum or CSF. Circulating levels of Klotho decrease during aging54 and are associated with cognitive performance5 and gray matter volume55 in cognitively unimpaired individuals. In patients with AD, CSF levels of Klotho are reduced52, where the experimental reversal of reduced Klotho expression in transgenic mouse models exerted beneficial effects on Aβ and cognition8,11,30. While the KL-VGβet variant has been associated with higher circulating levels of Klotho55, it remains to be investigated whether the association between KL-VSβet and pathological tau are mediated by higher protein levels in the CSF and brain tissue.

In summary, our findings revealed a protective association of KL-VGβet on tau accumulation that particularly manifested in amyloid-positive individuals, where lower tau pathology was related to better cognitive functions. These findings may be particularly informative for clinical anti-tau trials56 and may encourage future studies on enhancing Klotho protein levels as a therapeutic intervention to slow down the development of tau pathology and dementia in AD.

Methods

Sample characteristics. A total of 551 participants were selected from ADNI phase 3 (ClinicalTrials.gov ID: NCT02854033) based on the availability of KL-VS and ApoE ε4 genotyping, T1-weighted MRI, [18F]-fluorodeoxyglucose (FDG) tau-PET and [18F]-florbetapir (FBP) or [18F]-florbetaben (FBB) amyloid-PET. MR and PET imaging had to be acquired during the same study visit. In addition, a subsample of 200 participants with a follow-up tau-PET assessment was selected for the longitudinal analyses. The two single-nucleotide polymorphisms for KL-VS (rs9536314 for F352V, rs9527025 for C370S) and ApoE (rs29358, rs7412) were genotyped using DNA extracted by Cogenics from a 3 mL aliquot of EDTA blood. Participants were assigned to the heterozygous KL-VS group when they carried 1 but not 2, copies of the KL-VS haplotype. ApoE ε4 carriers were defined as individuals carrying at least one ε4 allele. Clinical classification was performed by the ADNI centers, dividing participants into cognitively normal (CN, Mini-Mental State Examination [MMSE] > 24, CDR = 0, non-demented), mild cognitively impaired (MCI; MMSE > 24, CDR = 0.5, objective memory-loss on the education-adjusted Wechsler Memory) or AD dementia (ADD; CDR ≥ 0.5–1.0, NINCDS/ADRAA criteria for probable AD are fulfilled). All participants provided written informed consent and all work complied with ethical regulations for work with human participants.

MR and PET acquisition and preprocessing. All imaging data were downloaded from the ADNI (https://adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis-method.html). All PET images were coregistered to the corresponding T1-weighted image to make use of Fesersurfer-derived masks in participants’ high-resolution, native space. SUVR scores were obtained by normalizing tau-PET images to the inferior frontal cortex gray matter and amyloid PET images to the whole cerebellum, following the previous recommendations59. In order to make FBP and FBB amyloid-PET measures comparable, we transformed SUVR scores into centiloid (CL) units using the established transformation formula (http://adni.loni.usc.edu/wp-content/themes/freshnews-dev-v2/documents/pet/ADNI Centiloids Final.pdf). For the analysis of longitudinal tau-PET, we additionally calculated annual tau-PET SUVR change rates as the difference between tau-PET SUVR scores measured at the follow-up versus baseline visit divided by the follow-up time in years.

Tau- and amyloid-PET regions of interest. For the analyses of tau-PET, we extracted mean SUVR scores from bilateral inferior temporal gyrri marking Aβ-related increase of tau pathology to neocortical structures26,27,28. In addition, we assessed global tau-PET burden29 as the size-weighted mean SUVR score across all Fesersurfer regions, excluding hippocampus, thalamus, and basal ganglia due to commonly reported tracer off-target binding60.

For the analysis of amyloid-PET images, we computed mean amyloid-PET levels from a global ROI spanning lateral and medial frontal, anterior and posterior cingulate, lateral parietal, and lateral temporal regions. Mean SUVR from these regions was also used for sample stratification into amyloid-positive participants based on established thresholds (SUVR FBB > 1.11 or SUVR FBP > 1.08; see "ADNI UCBERKELEY_AV45_Methods_12.03.15.pdf" and "UCBerkeley_FBB_Methods_D4111.99.pdf" on the ADNI website).

mRNA expression levels of KL. Regional gene expression was obtained from publicly available microarray measurements of regions in the brain based on post-mortem data from the Allen Brain Atlas (http://human.brain-map.org). The Allen Brain atlas is based on more than 60,000 microarray probes collected from 3700 autopsy-based brain tissue samples from a total of six individuals aged 24–57 without a known history of neurological or psychiatric diseases33,34. Here, we specifically extracted median expression of KL mRNA within these Desikan–Killiany ROIs, to test a spatial correlation between KL expression and KL-VSβet effects on local tau-PET uptake. Since microarray assessments and thus KL mRNA expression of all six Allen brain atlas subjects were available only for the left hemisphere (vs. two subjects for the right hemisphere), we restricted the analysis of KL mRNA expression data to the more robust estimates of the left hemisphere in line with previous studies41,62.

Neuropsychological assessment. The ADNI neuropsychological test battery contains multiple indicators for memory functions, on which basis a composite score (ADNI-MEM) has been established39. ADNI-MEM summarizes test performance on the Rey Auditory Verbal Learning Test, elements from the AD Assessment Scale–Cerebral Subscale, word recall from the MMSE, and the Wechsler Logical Memory Subscale. The Allen Brain atlas is based on more than 60,000 microarray probes collected from 3700 autopsy-based brain tissue samples from a total of six individuals aged 24–57 without a known history of neurological or psychiatric diseases33,34. Here, we specifically extracted median expression of KL mRNA within these Desikan–Killiany ROIs, to test a spatial correlation between KL expression and KL-VSβet effects on local tau-PET uptake. Since microarray assessments and thus KL mRNA expression of all six Allen brain atlas subjects were available only for the left hemisphere (vs. two subjects for the right hemisphere), we restricted the analysis of KL mRNA expression data to the more robust estimates of the left hemisphere in line with previous studies41,62.

Statistical analysis. All statistical analyses were conducted with R statistical software (version 3.6.1). P values were considered significant when meeting a two-tailed alpha threshold of 0.05. Baseline tau-PET SUVR values were entered as log-
is KL-VS heterozygosity associated with lower ApoE accumulation? Lastly, we performed an exploratory analysis with the aim to confirm previously observed age- and ApoE-dependent associations between KL-VS het and lower amyloid-PET burden. For this purpose, we tested for a KL-VS het × age effect on global amyloid-PET levels in the current sample (N = 551) and in a larger ADNI sample (N = 1067) including all participants with amyloid-PET assessment and KL-VS status (regardless of whether or not they underwent tau-PET assessment). Sex, education, and diagnosis were considered covariates. To this end, we investigated age-dependent effects of KL-VS het on amyloid-PET levels in a subgroup including only CN participants aged between 60 and 80 years. Age, sex, and education were considered as covariates.

Data availability

The data that support the findings of this study were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) and are available from the ADNI database (adni.loni.usc.edu) upon registration and compliance with the data use agreement. A list including the anonymized participant identifiers of the currently used sample and the source file can be downloaded from the ADNI database (tau-PET data release in May 2020; UCBERKELEYV1451_05_12_20.csv). The Allen Brain Atlas (http://human.brain-map.org) and FreeSurfer-mapped transcriptomic data from the Allen Brain Atlas (https://figshare.com/articles/A_Freesurfer_view_of_the_cortical_transcriptome_generated_from_the_Allen_Human_Brain_Atlas/1439749) are freely available online. Source data underlying Fig. 2 are provided with this paper.

Code availability

The R code pertaining to the figures in this manuscript is provided at https://github.com/njulanietz/NatCommun2021_KL-VS. Costume R code can be obtained from the first author upon request.

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Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

References

1. Kuro-o, M. et al. Mutation of the mouse klotho gene leads to a syndrome resembling aging. Nature 390, 45–51 (1997).
2. Zhu, Z. et al. Klotho gene polymorphisms are associated with healthy aging and longevity: Evidence from a meta-analysis. Mech. Ageing Dev. 178, 33–40 (2019).
3. Kuro-o, M. The Klotho proteins in health and disease. Nat. Rev. Nephrol. 15, 27–44 (2019).
4. Kuraku, H. Suppression of aging in mice by the hormone Klotho. Science 309, 1829–1833 (2005).
5. Dubal, D. B. et al. Life extension factor klotho enhances cognition. Cell Rep. 7, 1065–1076 (2014).
6. Arking, D. E., Atzmon, G., Arking, A., Barzilai, N. & Dietz, H. C. Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. Circ. Res. 96, 412–418 (2005).
7. Mengel-From, J. et al. Genetic variants in KLOTHO associate with cognitive function in the oldest old group. J. Gerontol. A Biol. Sci. Med. Sci. 71, 1151–1159 (2016).
8. Yokoyama, J. S. et al. Variation in longevity gene KLOTHO is associated with greater cortical volumes. Ann. Clin. Transl. Neuro. 2, 215–230 (2015).
9. Deary, I. J. et al. KLOTHO genotype and cognitive ability in childhood and old age in the same individuals. Neurosci. Lett. 378, 22–27 (2005).
10. de Vries, C. F. et al. Klotho, APOEε4, cognitive ability, brain size, atrophy, and survival: a study in the Aberdeen Birth Cohort of 1936. Neurobiol. Aging 55, 91–98 (2017).
11. Zeng, C.-Y. et al. Lifetival vector-mediated overexpression of Klotho in the brain improves Alzheimer’s disease–like pathology and cognitive deficits in mice. Neurobiol. Aging 78, 18–28 (2019).
12. Barker, W. W. et al. Relative frequencies of Alzheimer disease, Lewy body, and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank. Alzheimer Dis. Assoc. Disord. 16, 203–212 (2002).
13. Belloy, M. E. et al. Association of Klotho-VS heterozygosity with risk of Alzheimer disease in individuals who carry APOE4. JAMA Neurol. 7, 849–862 (2020).
14. Jansen, W. J. et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA 313, 1924–1938 (2015).
15. Corder, E. H. et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science 261, 921–923 (1993).

16. Eckman, C. M. et al. Klotho heterozygosity attenuates APOE-related amyloid burden in preclinical AD. Neurology 92, e1878–e1889 (2019).

17. Bejanin, A. et al. Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer’s disease. Brain 140, 3286–3300 (2017).

18. Bateman, R. J. et al. Clinical and biomarker changes in dominantly inherited Alzheimer’s disease. N. Engl. J. Med. 367, 795–804 (2012).

19. Mattsson-Carlsgren, N. et al. Abeta deposition is associated with increases in soluble and phosphorylated tau that precede a positive Tau PET in Alzheimer’s disease. Sci. Adv. 6, eaaz2387 (2020).

20. Schöll, M. et al. PET imaging of tau deposition in the aging human brain. Neurofibril 89, 971–982 (2016).

21. Guo, T. et al. Longitudinal cognitive and biomarker measurements support a unidirectional pathway in Alzheimer’s Disease pathophysiology. Biol. Psychiatry 89, 786–794 (2021).

22. Jack, C. R. Jr et al. Predicting future rates of tau accumulation on PET. Brain 143, 3136–3150 (2020).

23. Harrison, T. M. et al. Longitudinal tau accumulation and atrophy in aging and Alzheimer disease. Ann. Neurol. 85, 229–240 (2019).

24. LaPoint, M. R. et al. The association between tau PET and retrospective cortical thinning in clinically normal elderly. Neuroimage 157, 612–622 (2017).

25. Hanseeuw, B. J. et al. Fluoroexoxyglucose metabolism associated with tau-amyloid interaction predicts memory decline. Ann. Neurol. 81, 583–596 (2017).

26. Johnson, K. A. et al. Tau positron emission tomographic imaging in aging and early Alzheimer disease. Ann. Neurol. 79, 110–119 (2016).

27. Ossenkoppele, R. et al. Associations between tau, Aβ, and cortical thickness with cognition in Alzheimer disease. Neurology 92, e601–e612 (2019).

28. Alzheimers, A. J. et al. Klotho allele status is not associated with APOE and APOE epsilon4-related cognitive decline in preclinical Alzheimer’s disease. Neurobiol. Aging 76, 770–774 (2020).

29. Uddin, M. S. et al. Autophagy and Alzheimer disease pathology. Acta Neuropathol. 139, 1052–1044 (2020).

30. Porter, T. et al. Klotho allele status is not associated with Abeta and APOE epsilon4-related cognitive decline in preclinical Alzheimer’s disease. Neurobiol. Aging 76, 162–165 (2019).

31. Uddin, M. et al. Autophagy and Alzheimer’s disease: from molecular mechanisms to therapeutic implications. Front Aging Neurosci. 10, 4 (2018).

32. Corder, E. H. et al. The neuroprotective effect of Klotho is mediated via regulation of members of the redox system. J. Biol. Chem. 289, 24700–24715 (2014).

33. Chang, Q. et al. The b-glucuronidase Klotho hydrolyzes and activates the TRPV5 channel. Science 310, 490–493 (2005).

34. Fernandez, A. F. et al. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. Nature 558, 136–140 (2018).

35. Pontecorvo, M. J. et al. Relationships between beta-amyloid and hippocampal disconnection in Alzheimer’s disease. Nature 558, 37–40 (2014).

36. Rhee, E. et al. Relationship between polymorphisms G395A in promoter and C1818T in exon 4 of the KLOTHO gene with glucose metabolism and cardiovascular risk factors in Korean women. J. Endocrinol. Invest. 29, 613–618 (2006).

37. Yokoyama, J. S. et al. Systemic klotho is associated with KLOTHO variation and predicts intrinsic connectivity in healthy human aging. Brain Imaging Behav. 11, 391–400 (2017).

38. Dun, M. A. & Seabrook, G. R. On the horizon—the value and promise of the global pipeline of Alzheimer’s disease therapeutics. Alzheimers Dement 6, e12009 (2020).

39. Desikan, R. S. et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31, 986–998 (2006).

40. Lampert, H. G. & Kley, B. Promyelocytic leukaemia protein (PML) and Klotho: a new role for an old partner? Biochem. Biophys. Res. Commun. 398, 513–518 (2010).

41. Franzmeier, N. et al. The BDNF Val66Met SNP modulates the association between beta-amyloid and hippocampal volume in healthy controls. Neuroimage 89, 165–177 (2017).

42. Chang, Q. et al. The neuroprotective effect of Klotho is mediated via regulation of members of the redox system. J. Biol. Chem. 289, 24700–24715 (2014).

43. Porter, T. et al. Klotho allele status is not associated with APOE and APOE epsilon4-related cognitive decline in preclinical Alzheimer’s disease. Neurobiol. Aging 76, 162–165 (2019).

44. Urakawa, I. et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 444, 770–774 (2006).
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J.N.: study concept and design, data processing, statistical analysis, interpretation of the results, and writing the manuscript. N.F.: critical revision of the manuscript. A.R.: data processing and critical revision of the manuscript. M.D.: critical revision of the manuscript. M.B.: critical revision of the manuscript. M.E.: study concept and design, interpretation of the results, and writing the manuscript. ADNI provided all data used for this study.

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Competing interests

M.B. received speaker honoraria from GE healthcare and LMI and is an advisor of LMI. All other authors declare no competing interests.

Additional information

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