RESEARCH ARTICLE

AD-associated CSF biomolecular changes are attenuated in KL-VS heterozygotes

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Abstract

Introduction: Dementia as an inevitable aging consequence has been challenged and underscores the need for investigations of the factors that confer resilience. We examine whether the functionally advantageous KL-VS variant of the putative aging suppressor *KLOTHO* gene attenuates age-related cognitive decline and deleterious biomolecular changes.

Methods: Trajectories of change in memory and executive function ($N = 360$; 2–12 visits) and cerebrospinal fluid (CSF) Alzheimer's disease (AD) biomarkers—amyloid beta (Aβ)42, total tau (t-tau), phosphorylated tau (p-tau) ($N = 112$; 2–4 samplings)—were compared between KL-VS non-carriers and heterozygotes in middle-aged and older adults from the Wisconsin Registry for Alzheimer's Prevention and the Wisconsin Alzheimer's Disease Research Center studies.

Results: Memory and executive function declined (p's ≤ 0.001) and CSF t-tau, p-tau, t-tau/Aβ42, and p-tau/Aβ42 levels increased (all p's ≤ 0.004) with age. The rate of p-tau accumulation was attenuated for KL-VS heterozygotes ($p = 0.03$).

Discussion: KL-VS heterozygosity may confer resilience to AD-associated biomolecular changes.

KEYWORDS

Alzheimer's disease, CSF, biomarkers, memory, executive function, tau

1 | BACKGROUND

Alzheimer's disease (AD), a progressive and debilitating neurological disorder of old age, is currently the sixth leading cause of death in the developed world.[1] AD is clinically hallmarked by memory loss and neuropathologically by accumulation of plaques and tangles in the brain.[2] The neuropathological changes are believed to begin years or perhaps even decades before diagnosis.[3,4]

Age is the single biggest risk factor for developing AD.[5] The longstanding belief that dementia is an inevitable consequence of aging has been challenged on multiple fronts in recent years. Not only is it possible to age successfully,[6] but even some individuals at genetic risk for AD[7] or harboring AD neuropathology are able to remain cognitively normal as they age.[8,9] This has refocused research away from risk and onto potentially protective or compensatory factors in hopes of reducing disability and disease incidence.[10]

Klotho, dubbed an anti-aging and longevity factor encoded by the *KLOTHO* gene, is a transmembrane protein and a circulating factor that plays a key role in cellular metabolism and body homeostasis.[11,12] Although the mechanisms of *KLOTHO* action are still not well understood, the available evidence indicates that it enhances synaptic and cognitive functions, protects from neurodegeneration,[12] and may also play a role in central nervous system maturation and aging.[13] Neurons pre-treated with klotho can be rescued in the presence of amyloid beta (Aβ) and glutamate toxicity, suggesting a potential target for therapeutic approaches that might protect against further deterioration and positively affect the outcome for patients with AD.[14–16]

Two *KLOTHO* single-nucleotide polymorphisms (SNPs; rs9536314 and rs9527025) segregate to form a functional haplotype, KL-VS, which modulates klotho secretion in humans.[17–19] Extant meta-analyses of human studies suggest that the functionally advantageous KL-VS heterozygosity (KL-VS^{HET}) is associated with various favorable outcomes, including longevity, better cardiovascular health, and better cognitive function, to name a few.[18–24] The *KLOTHO* literature related to AD and its biomarkers is still nascent. The evidence available thus far suggests that KL-VS^{HET} is associated with lesser Aβ burden[17,25] and lower AD risk in apolipoprotein E (*APOE*) ε4 carriers.[25]

Our group recently reported cross-sectional findings indicating that age-related alterations in cerebrospinal fluid (CSF) biomarkers, and memory and executive function, were attenuated in late-middle-aged, cognitively normal KL-VS^{HET} individuals enrolled in the Wisconsin Registry for Alzheimer's Prevention (WRAP) and the Wisconsin Alzheimer's Disease Research Center (W-ADRC).[26] We now leverage longitudinal data from these same risk-enriched cohorts to examine whether *KLOTHO* attenuates the rate of prospective changes in cognition, specifically decline in memory and executive function, given their sensitivity to incipient AD,[27] and in CSF biomarkers of AD (Aβ42, total tau [t-tau], phosphorylated tau [p-tau], and their respective ratios to Aβ42). We hypothesized that the expected adverse effect of age on the rates of change in both cognitive performance and AD CSF biomarkers will be attenuated in carriers of the functionally advantageous genotype of *KLOTHO* (KL-VS^{HET}).

2 | METHODS

2.1 | Participants

Inclusion of participants in this report was based on the availability of the KL-VS genotype and longitudinal CSF and neuropsychological data, and being characterized as cognitively normal based on standardized, multidisciplinary, consensus conferences.[17,29] This resulted in a sample of 360 individuals (age range 45–65 years at study entry; 68% female) who had two or more visits with available neuropsychological data, each visit occurring biannually. Of these, 112 (69% female) had two or more lumbar puncture procedures to date, which occurred ≈ 2 years apart. Additional information regarding the WRAP and the W-ADRC's IMPACT (**I**nvestigating **M**emory in **P**reclinical **A**D–**C**auses and **T**reatments) cohorts have been published previously. Both cohorts are enriched with risk factors for AD, namely parental history and *APOE ε4* genotype.[17,29]

2.2 | Standard protocol approvals, registrations, and patient consent

Study procedures were approved by the University of Wisconsin Institutional Review Board and all participants signed written consent.

2.3 | Genotyping

DNA was extracted from blood using the PUREGENE DNA Isolation Kit (Gentra Systems, Inc, Minneapolis, MN). DNA concentrations

were quantified using ultraviolet spectrophotometry (DU 530 Spectrophotometer, Beckman Coulter, Fullerton, CA). LGC Genomics (Beverly, MA) performed genotyping for *APOE* (rs429358 and rs7412) and *KLOTHO* (rs9536314 and rs9527025) using competitive allele-specific polymerase chain reaction (PCR)–based KASP genotyping assays. Previously published quality-control procedures are deemed satisfactory.[17,30] Consistent with HapMap and the literature,[17,18,25,26] rs9536314 and rs9527025 were also in perfect linkage disequilibrium in our study population.

2.4 | CSF assessment

Lumbar puncture 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 extraction into polypropylene syringes. Each sample consisted of 22 mL CSF, which was then combined, mixed, and centrifuged at 2000*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 Aβ42, t-tau, and p-tau181 with INNOTEST ELISAs (Fujirebio, Ghent, Belgium) in two batches as described previously,[18,30] with a subset of samples re-assayed. Statistical conversion models were developed based on this subset and applied to adjust for the batch differences and harmonize the values across batches.[31]

2.5 | Neuropsychological testing

Participants completed a comprehensive cognitive test battery at each visit.[28,33,33] The assessment spans five cognitive domains: episodic memory, attention, executive function, language, and visuospatial ability. Here we focus primarily on measures of episodic memory (Rey Auditory Verbal Learning Test [RAVLT])[35] and visual search and processing speed, mental flexibility, and executive function (Trail Making Test [TMT], Parts A & B)[35] given their sensitivity to incipient AD,[27] and also because these tests are common to both WRAP and the W-ADRC batteries. For the RAVLT, we focused on Total Learning (sum of Trials 1–5) and Long Delay Free Recall, whereas for the TMT we analyzed time to test completion.

2.6 | Statistical analyses

All analyses were done in SPSS, v. 26.0 (IBM, Armonk, NY). Participants were split into two groups based on KL-VS status (non-carriers [KL-VS^{NC}] vs heterozygotes [KL-VS^{HET}]) for analytical purposes. KL-VS homozygosity, associated with decreased longevity and worse cognition, is rare; hence, five homozygotes in our sample were omitted from the analysis.

We compared the groups on demographic characteristics either using chi-square (χ^2) or independent-samples *t*-tests. With age as the time scale, we used linear mixed-effects regression models to investigate longitudinal changes in our neuropsychological ($N = 360$) and

RESEARCH IN CONTEXT

1. **Systematic review**: The authors performed a traditional literature review related to Alzheimer's disease (AD) biomarkers, cognition, and the *KLOTHO gene*. There is evidence that *KLOTHO* heterozygosity is associated with better cognition and lesser AD biomarker burden. Whether *KLOTHO* heterozygosity also attenuates age-related cognitive decline and the rates of deleterious biomolecular changes is currently unknown.

2. **Interpretation**: Overall, our results suggest that *KLOTHO* heterozygosity attenuates deleterious age-related changes in risk markers for AD within a cohort of non-demented middle-aged and older adults enriched for AD risk. Given the lack of disease-modifying therapies, the identification of new genetic variants that modify AD risk can potentially uncover novel targets for therapeutic interventions.

3. **Future directions**: Additional studies with larger and more diverse samples and CSF biomarker data spanning longer periods would be beneficial to confirm the current findings. In addition, longitudinal prospective studies of older adults with mild cognitive impairment or AD are warranted.

HIGHLIGHTS

- *KLOTHO* is considered a putative aging suppressor gene.
- The functionally advantageous KL-VS variant is linked to favorable health outcomes.
- Memory and executive function decline did not differ based on *KLOTHO* status.
- Phosphorylated tau accumulation is attenuated in KL-VS heterozygotes.
- KL-VS heterozygosity may confer resilience to deleterious age-related changes.

biomarker ($N = 112$) outcomes. Covariates included were *APOE* ε4 status, sex, and parental history of AD. Education was additionally covaried in the neuropsychological models. To ascertain whether the observed trajectories varied as a function of *KLOTHO* genotype, we refitted the models while including *KLOTHO* and age*KLOTHO as additional covariates. The age*KLOTHO term was of primary interest, as it would indicate whether interindividual trajectories in the modeled outcome differed between KL-VS^{HET} and KL-VS^{NC}.

TABLE 1 Background characteristics of study participants

VARIABLE	Sample with repeat Neuropsychological test measures				Sub-sample with repeat CSF measures			
	TOTAL (N = 360)	KL-VSNC (N = 260)	KL-VS^{HET} (N = 100)	p	TOTAL (N = 112)	KL-VS^{NC} (N = 89)	KL-VS^{HET} (N = 23)	p
Age, mean (SD)	66.59 (7.97)	62.31 (6.64)	61.39 (5.98)	0.37	62.12 (6.49)	62.31 (6.64)	61.39 (5.98)	0.54
Education, mean (SD)	16.03 (2.5)	16.00 (2.56)	16.17 (2.35)	0.41	16.42 (2.51)	16.35 (2.56)	16.42 (2.51)	0.59
MMSE	29.23 (1.03)	29.29 (0.87)	29.10 (1.33)	0.22	29.5 (0.72)	29.58 (0.67)	29.21 (0.83)	0.14
Females, N (%)	244 (68)	179 (69)	65 (65)	0.59	78 (69)	65 (73)	13 (54)	0.03
White, N (%)	347 (96)	252 (97)	95 (95)	0.36	106 (95)	85 (95)	21 (92)	0.60
APOE ε4+, N (%)	137 (38)	97 (37)	40 (40)	0.72	45 (40)	35 (40)	10 (42)	0.81
Parental history of AD, N (%)	267 (74)	197 (76)	70 (70)	0.28	89 (80)	71 (80)	18 (86)	0.54
Aβ42 positive, N (%)[a]	46 (12)	37 (14)	9 (9)	0.14	12 (10)	10 (11)	2 (9)	0.21
t-Tau positive, N (%)[a]	53 (14)	38 (14)	15 (15)	0.49	13 (11)	12 (13)	1 (5)	0.73
p-Tau positive, N (%)[a]	53 (14)	36 (13)	17 (17)	0.25	10 (9)	9 (10)	1 (5)	0.64
Number of visits								
1	360	260	100	—	112	89	23	—
2	355	257	98	—	112	89	23	—
3	346	253	93	—	20	15	5	—
4	328	237	91	—	7	4	3	—
5	276	206	70	—	—	—	—	—
6	190	140	50	—	—	—	—	—
7	71	49	22	—	—	—	—	—
8	29	17	12	—	—	—	—	—
9	11	6	5	—	—	—	—	—
10	1	0	1	—	—	—	—	—
Total Visits	1967	1425	542		251	197	54	

Abbreviations: KL-VS^{NC} = KL-VS non-carriers; KL-VS^{HET} = KL-VS heterozygotes; MMSE = Mini-Mental State Examination; APOE ε4+ = APOE ε4 carrier; Aβ42 = amyloid beta 42; t-Tau = total tau; p-Tau = phosphorylated tau; mean (SD) = mean (standard deviation).

[a]Positive based on our center's derived cut point for CSF AD biomarkers.[27]

3 | RESULTS

3.1 | Sample characteristics

Characteristics of both the larger sample with multiple neuropsychological visits and the smaller sub-sample with two or more lumbar punctures are detailed in Table 1 . Overall, participants were predominantly White (96%) and female (68%), with a mean age ± SD of 67 ± 8 and 16 years of education. The sample was enriched for AD risk; 38% are APOE ε4 carriers and 74% have a parental history of dementia. There were no significant differences between the larger sample with multiple neuropsychological visits and the sub-sample with two or more lumbar punctures, or between KL-VS^{HET} and KL-VS^{NC}, within either the CSF or the cognitive sample, in any of the aforementioned characteristics (all p's ≥ 0.05), except for sex in the CSF biomarker sub-sample whereby 73% of KL-VS^{NC} but only 54% of KL-VS^{HET} were female (p = 0.03).

We also assessed how many of the participants in each sample would be considered positive (i.e., abnormal) based on our center's derived cut points for CSF AD biomarkers (Aβ42, ≤471; p-tau, ≥59.5; and t-tau, ≥461.[26,36] Although most of the sample was negative for AD biomarkers (>88%), the number of biomarker-positive participants at baseline (<14%) did not differ significantly between KL-VS^{HET} and KL-VS^{NC} (see Table 1): 14% of KL-VS^{NC} versus 9% of KL-VS^{HET} were considered positive (p = 0.14) for Aβ42; 14% of KL-VS^{NC} versus 15% of KL-VS^{HET} were considered positive (p = 0.49) for t-tau; and 13% of KL-VS^{NC} versus 17% of KL-VS^{HET} were considered positive (p = 0.25) for p-tau.

3.2 | Trajectories of neuropsychological performance tapping memory and executive function in KL-VS non-carriers and heterozygotes

Performance on neuropsychological tests tapping memory (RAVLT trials 1–5 and long delay) and executive function (TMT B) declined significantly with age across both KLOTHO genotypes taken together (all

TABLE 2 Trajectories of change in cognitive outcomes and CSF biomarkers of AD as a function of the KLOTHO KL-VS polymorphism

VARIABLE		ESTIMATE	SE	t	p
COGNITIVE OUTCOMES[a]					
RAVLT Trials 1–5	Age	−0.12	0.03	−3.45	0.001
	KLOTHO	−0.32	0.82	−0.39	0.69
	Age x KLOTHO	−0.07	0.07	1.09	0.27
RAVLT Long Delay	Age	−0.04	0.01	−3.20	0.001
	KLOTHO	−0.34	0.28	−1.23	0.22
	Age x KLOTHO	−0.0006	0.02	−0.03	0.97
TMT A	Age	−0.002	0.04	−0.07	0.95
	KLOTHO	−0.03	0.90	−0.03	0.97
	Age x KLOTHO	−0.03	0.07	−0.39	0.70
TMT B	Age	0.64	0.09	6.42	<0.001
	KLOTHO	1.22	2.56	0.48	0.63
	Age x KLOTHO	−0.16	0.19	−0.85	0.39
CSF BIOMARKERS[b]					
$A\beta42$	Age	−4.16	2.22	−1.79	0.07
	KLOTHO	−26.52	35.99	−0.74	0.46
	Age x KLOTHO	−5.46	5.57	−0.99	0.33
t-Tau	Age	5.35	1.31	4.08	<0.001
	KLOTHO	−33.35	24.17	−1.38	0.17
	Age x KLOTHO	−4.09	3.06	−1.34	0.18
p-Tau	Age	0.49	0.18	2.93	0.004
	KLOTHO	−1.78	2.56	−0.69	0.49
	Age x KLOTHO	−1.78	0.40	−2.26	0.02
t-Tau/$A\beta42$	Age	0.01	0.002	5.33	<0.001
	KLOTHO	−0.06	0.05	−1.23	0.22
	Age x KLOTHO	−0.006	0.006	−1.03	0.31
p-Tau/$A\beta42$	Age	0.001	0.0003	4.23	<0.001
	KLOTHO	−0.004	0.005	−0.76	0.45
	Age x KLOTHO	−0.001	0.001	−1.42	0.16

KLOTHO denotes the estimated mean difference at age 0 (mean age for the whole sample) in the outcome of interest between KL-VS[NC] and KL-VS[HET]. Age indicates the annual rate of change in the outcome of interest for KL-VS[NC]. Age x KLOTHO denotes the difference in the annual rate of change between KL-VS[NC] and KL-VS[HET] (this estimate must be added to the estimate for age to determine annual rate of change for KL-VS[HET]).

Abbreviations: KL-VS[NC] = KL-VS non-carriers; KL-VS[HET] = KL-VS heterozygotes; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test, $A\beta42$ = amyloid beta 42; t-Tau = total tau; p-Tau = phosphorylated tau; SD = standard deviation.

[a]**Covariates**: APOE ε4, sex, parental history of AD, education.

[b]**Covariates**: APOE ε4, sex, parental history of AD.

p's < 0.001), with no significant genotype differences in longitudinal rates of performance decline (Table 2).

3.3 | Trajectories of CSF biomarkers in KL-VS non-carriers and heterozygotes

Except for a non-significant trend for $A\beta42$ (p = 0.07), all biomarkers investigated showed significant longitudinal change with age in both groups taken together (all p's ≤ 0.004). Genotype differences in rates of longitudinal accumulation (Table 2) were observed for p-tau only,

with that rate being slower for KL-VS[HET] ($t_{131.9}$ = −2.26, p = 0.03; Figure 1).

4 | DISCUSSION

We report that in a late-middle-aged cohort enriched for AD risk, unfavorable accumulation of p-tau with age was attenuated in carriers of a functionally favorable KL-VS genotype. Specifically, KL-VS[NC] exhibited a steeper rate of expected age-related alterations in CSF p-tau compared to KL-VS[HET].
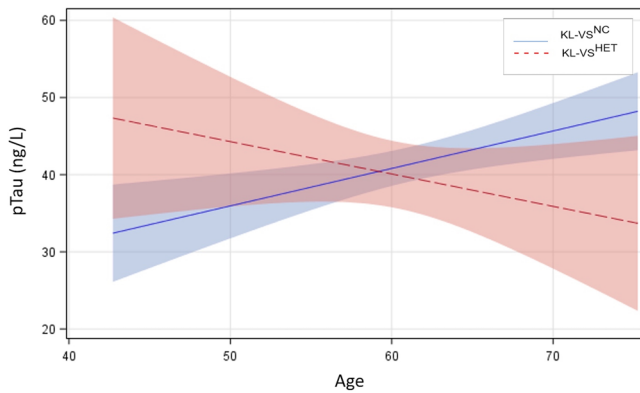
FIGURE 1 Estimated trajectories of change in phosphorylated tau p-tau as a function of *KL-VS* status. Rate of cerebrospinal fluid (CSF) p-tau accumulation decreases in KL-VS[HET] but increases in KL-VS[NC] with age ($p = 0.03$)

In a prior cross-sectional study from this same cohort, we reported that KL-VS[NC] exhibited an expected age-related pattern of associations with CSF biomarkers, memory, and executive function, which were all attenuated in KL-VS[HET].[26] Our current study expands on those previous findings and further adds to the literature by demonstrating for the first time that the favorable effects of KL-VS[HET] extend to prospective trajectories of age-related changes in a core AD biomarker (i.e., p-tau accumulation). Further confirmation of our findings is needed in larger samples given the collinearity between p-tau and t-tau.[36–38] It is important to note, however, that these and other reports of correspondence of p-tau and t-tau results are largely cross-sectional in nature, including our own previous report in this cohort, whereby we observed both lesser p-tau and t-tau burden at baseline in carriers of a functionally advantageous *KLOTHO* variant.[26]

The filamentous core of neurofibrillary tangles (NFTs), one of the two histopathologic hallmarks of AD, is composed of highly phosphorylated forms of tau.[39,40] In addition to being a key neuropathological feature of AD and central to NFT formation, p-tau accumulation is related to synaptic impairment, neuronal dysfunction, and impairment in mitochondrial transport.[41–43] There is substantial evidence that NFTs and neuropil threads are significantly lower in people without cognitive impairment compared to those with mild cognitive impairment (MCI) or AD, and further, that NFTs correlate with episodic memory performance,[43] although the exact mechanisms by which tau phosphorylation affects cognitive function are still under investigation. Our finding of attenuated age-related accumulation in CSF p-tau in KL-VS[HET] has direct implications for klotho as a potential target against age-related cognitive decline and AD neuropathology.

The role for *KLOTHO* in longevity[11,12,19–24] is well-established, and the evidence in support of preserved brain integrity and cognitive,[23,26,44–47] as well as slower cognitive decline,[45] in KL-VS[HET] within the context of aging is mounting. Moreover, KL-VS[HET] exhibits better memory[26,43] and better executive function[26,44] in conjunction with greater dorsolateral prefrontal cortex volume[44] and greater intrinsic connectivity in functional brain networks vulnerable

to deleterious effects of aging.[46] Although we previously reported that KL-VS[HET] in this cohort performed significantly better on tests of memory and executive function at baseline,[26] this favorable genotype-related outcome does not seem to extend to trajectories of memory and executive function performance with age.

Our sample is predominantly White, highly educated, and has a higher prevalence of both parental history of AD and *APOE* ε4 carriers than what is normally observed in the general population, which may potentially limit the generalizability of our findings. Another potential limitation is the arguably modest sample size of our CSF analyses. In addition, a recent study in bonobos and chimpanzees reported that while both sexes show an age-related decline in soluble alpha-klotho, females tend to have higher levels throughout life.[48] Although our findings could be influenced by 68% of our sample being female, sex was controlled for in all of our analyses. Nonetheless, the correlation between levels of soluble klotho protein in CSF and plasma across KL-VS variants should be established for males and females separately going forward and replicated in other cohorts. The foregoing caveats, however, should not undermine the unique strengths of our study, namely the longitudinal examination of multimodal AD-relevant outcomes using data from extensively characterized cohorts that have been followed prospectively for many years.

Overall our results suggest that KL-VS heterozygosity attenuates deleterious age-related changes in risk markers for AD, namely memory, executive function, and CSF p-tau. In addition to being the single greatest risk factor for AD, age is the most robust determinant of CSF biomarker changes and cognitive decline in the absence of diagnosis. Here, we offer a glimpse into how one genetic factor, *KLOTHO*, offers resilience against age-related changes in cognition and CSF tau. The importance of identifying factors that confer resilience is gaining increased recognition given the current lack of curative therapies for AD.[49] The identification of new genetic variants that modify AD risk will potentially uncover novel molecular targets. This line of research is poised to identify complementary pathways for curbing AD progression and delaying symptom onset.

CONFLICTS OF INTEREST

H.Z. has served at scientific advisory boards for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, and CogRx; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K.B. has served as a consultant, on advisory boards, or on data-monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). All other authors have no disclosures to report (see Supporting Information).

REFERENCES

1. Alzheimer's Association. 2018 Alzheimer's disease facts and figures. *Alzheimers Dement* 2018;14:367-429.
2. Van Cauwenberghe C, Van Broeckhoven C, Sleegers K. The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet Med*. 2016;18:421.
3. Briggs R, Kennelly SP, O'Neill D. Drug treatments in Alzheimer's disease. *Clin Med (Lond)*. 2016;16(3):247-253.
4. Lane CA, Hardy J, Schott JM. Alzheimer's disease. *Eur J Neurol*. 2018;25:59-70.
5. Hou Y, Dan X, Babbar M, et al. Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol* 2019;15:565-581.
6. Stern Y, Barnes CA, Grady C, Jones RN, Raz N. Brain reserve, cognitive reserve, compensation, and maintenance: operationalization, validity, and mechanisms of cognitive resilience. *Neurobiol Aging*. 2019;83:124-129.
7. Gallagher M, Okonkwo OC, Resnick SM, Jagust WJ, Benzinger TLS, Rapp PR. What are the threats to successful brain and cognitive aging? *Neurobiol Aging*. 2019;83:130-134.
8. Driscoll I, Resnick SM, Troncoso JC, An Y, O'Brien R, Zonderman AB. Impact of Alzheimer's pathology on cognitive trajectories in nondemented elderly. *Ann Neurol*. 2006;60(6):688-695.
9. Driscoll I, Troncoso J. Asymptomatic Alzheimer's disease: a prodrome or a state of resilience? *Curr Alzheimer Res*. 2011;8(4):330-335.
10. Singer B, Friedman E, Seeman T, Fava GA, Ryff CD. Protective environments and health status: cross-talk between human and animal studies. *Neurobiol Aging*. 2005;S26:S113-118.
11. Chateau MT, Araiz C, Descamps S, Galas S. Klotho interferes with a novel FGF-signaling pathway and insulin/Igf-like signalling to improve longevity and stress resistance in Caenorhabditis elegans. *Aging*. 2010;2:567-581.
12. Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone Klotho. *Science*. 2005;309:1829-1833.
13. Kim JH, Hwang KH, Park KS, Kong ID, Cha SK. Biological role of anti-aging protein klotho. *J Lifestyle Med*. 2015;5(1):1-6.
14. Kuang X, Chen YS, Wang LF, et al. Klotho upregulation contributes to the neuroprotection of ligustilide in an Alzheimer's disease mouse model. *Neurobiol Aging*. 2014;35(1):169-178.
15. Semba RD, Moghekar AR, Hu J, et al. Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. *Neurosci Lett*. 2014;558:37-40.
16. Dubal DB, Zhu L, Sanchez PE, et al. Life extension factor klotho prevents mortality and enhances cognition in hAPP transgenic mice. *J Neurosci*. 2015;35(6):2358-2371.
17. Erickson CM, Schultz SA, Oh JM, et al. KLOTHO heterozygosity attenuates APOE4-related amyloid burden in preclinical AD. *Neurology*. 2019;92(16):e1878-e1889.
18. Dubal DB, Yokoyama JS, Zhu L, et al. Life extension factor klotho enhances cognition. *Cell Rep*. 2014;7:1065-1076.
19. Arking DE, Krebsova A, Macek M, et al. Association of human aging with a functional variant of klotho. *Proc Natl Acad Sci USA*. 2002;99:856-861.
20. Cheikhi A, Barchowsky A, Sahu A, et al. Klotho: An elephant in aging research. *J Gerontol A Biol Sci Med Sci*. 2019;74(7):1031-1042.
21. Di Bona D, Accardi G, Virruso C, Candore G, Caruso C. Association of Klotho polymorphisms with healthy aging: a systematic review and meta-analysis. *Rejuvenation Res*. 2014;17:212-216.
22. Montesanto A, Dato S, Bellizzi D, Rose G, Passarino G. Epidemiological, genetic and epigenetic aspects of the research on healthy ageing and longevity. *Immunity Ageing*. 2012, 9:6-17.
23. Revelas M, Thalamuthu A, Oldmeadow C, et al. Review and meta-analysis of genetic polymorphisms associated with exceptional human longevity. *Mech Ageing Dev*. 2018;175:24-34.
24. Vo HT, Laszczyk AM, King GD. Klotho, the key to healthy brain aging? *Brain Plast*. 2018;3(2):183-194.
25. Belloy ME, Napolioni V, Han SS, Le Guen Y, Greicius MD, for the Alzheimer's Disease Neuroimaging Initiative. Associations of Klotho-KS heterozygosity with risk of Alzheimer's disease in individuals who carry APOE4. *JAMA Neurol*. 2020;77(7):849-862.
26. Driscoll I, Ma Y, Gallagher CL, Johnson SC, et al. Age-related tau burden and cognitive deficits are attenuated in KLOTHO KL-VS heterozygotes. *J Alzheimers Dis*. 2021;79(3):1297-1305.
27. Blacker D, Lee H, Muzikansky A, et al. Neuropsychological measures in normal individuals that predict subsequent cognitive decline. *Arch Neurol*. 2007;64(6):862-871.
28. Johnson SC, Koscik RL, Jonaitis EM, et al. The Wisconsin Registry for Alzheimer's Prevention: a review of findings and current directions. *Alzheimers Dement (Amst)*. 2018;10:130-142.
29. Darst BF, Koscik RL, Racine AM, et al. Pathway-specific polygenic risk scores as predictors of amyloid-beta deposition and cognitive function in a sample at increased risk for Alzheimer's disease. *J Alzheimers Dis*. 2017;55:473-484.
30. Palmqvist S, Zetterberg H, Blennow K, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol*. 2014;71:1282-1289.
31. Ma Y, Norton DL, Van Hulle CA, et al. Measurement batch differences and between-batch conversion of Alzheimer's disease cerebrospinal fluid biomarker values. *Alzheimers Dement (Amst)*. 2021;13(1):e12194.
32. Sager MA, Hermann B, La Rue A. Middle-aged children of persons with Alzheimer's disease: APOE genotypes and cognitive function in the Wisconsin Registry for Alzheimer's Prevention. *J Geriatr Psychiatry Neurol*. 2005;18(4):245-249.

33. Weintraub S, Salmon D, Mercaldo N, et al. The Alzheimer's Disease Centers' Uniform Data Set (UDS): the neuropsychologic test battery. *Alzheimer Dis Assoc Disord*. 2009;23(2):91-101.

34. Schmidt M. Rey Auditory Verbal Learning Test: A Handbook. *Western Psychological Services*; 1996.

35. Reitan R, Wolfson D. *The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation*. Neuropsychology Press; 1993.

36. Blom ES, Giedraitis V, Zetterberg H, et al. Rapid progression from mild cognitive impairment to Alzheimer's disease in subjects with elevated levels of tau in cerebrospinal fluid and the APOE epsilon4/epsilon4 genotype. *Dement Geriatr Cogn Disord*. 2009;27(5):458-464.

37. Sämgård K, Zetterberg H, Blennow K, Hansson O, Minthon L, Londos E. Cerebrospinal fluid total tau as a marker of Alzheimer's disease intensity. *Int J Geriatr Psychiatry*. 2010;25(4):403-410.

38. Wattmo C, Blennow K, Hansson O. Cerebro-spinal fluid biomarker levels: phosphorylated tau (T) and total tau (N) as markers for rate of progression in Alzheimer's disease. *BMC Neurol*. 2020;20(1):10.

39. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA*. 1986;83:4913-4917.

40. Goedert M, Wischik CM, Crowther RA, Walker JE, Klug A. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proc Natl Acad Sci USA*. 1988;85:4051-4055.

41. Stoothoff WH, Johnson GVW. Tau phosphorylation: physiological and pathological consequences. *Biochim Biophys Acta (BBA) - Mol Basis Dis*. 2005;1739(2-3):280-297.

42. Lee G, Leugers CJ. Tau phosphorylation: physiological and pathological consequences. In Teplow DB (ed). *Tau and Tauopathies. In: Progress in Molecular Biology and Translational Science*. Academic Press 2012;107:263-293.

43. Mitchell TW, Mufson EJ, Schneider JA, et al. Parahippocampal tau pathology in healthy aging, mild cognitive impairment, and early Alzheimer's disease. *Ann Neurol*. 2002;51(2):182-189.

44. Yokoyama JS, Sturm VE, Bonham LW, et al. Variation in longevity gene KLOTHO is associated with greater cortical volumes. *Ann Clin Transl Neurol*. 2015;2:215-230.

45. de VriesCF, Staff RT, Harris Se, et al. Klotho, APOEepsilon4, cognitive ability, brain size, atrophy and survival: a study in Aberdeen Birth Cohort of 1936. *Neurobiol Aging*. 2017;55:91-98.

46. Yokoyama JS, Marx G, Brown JA, et al. Systemic klotho is associated with KLOTHO variation and predicts intrinsic cortical connectivity in healthy human aging. *Brain Imaging Behav*. 2017;11(2):391-400.

47. Neitzel J, Franzmeier N, Rubinski A, et al. KL-VS heterozygosity is associated with lower amyloid-dependent tau accumulation and memory impairment in Alzheimer's disease. *Nat Commun*. 2021;12(1):3825.

48. Behringer V, Stevens JMG, Deschner T, Sonnweber R, Hohmann G. Aging and sex affect soluble alpha klotho levels in bonobos and chimpanzees. *Front Zool*. 2018;15:35.

49. Okonkwo OC, Vemuri P. Stemming the Alzheimer tsunami: introduction to the special issue on reserve and resilience in Alzheimer's disease. *Brain Imaging Behav*. 2017;11(2):301-303.

50. Kuro-o M, Matsumura Y, Aizawa H, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*. 1997;390(6655):45-51.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.