Age-related tau burden and cognitive deficits are attenuated in *KLOTHO* KL-VS heterozygotes

Ira Driscoll, PhD[1,2,3], Yue Ma, PhD[2], Catherine L. Gallagher, MD[4,5], Sterling C. Johnson, PhD[1,2,4], Sanjay Asthana, MD[1,2,4], Bruce P. Hermann, PhD[1,2,5], Mark A. Sager, MD[1,2], Kaj Blennow, MD[6,7], Henrik Zetterberg, MD[6,7,8,9], PhD, Cynthia M. Carlsson, MD[1,2,4], Corinne D. Engelman, PhD[1,2,10], Dena B. Dubal, MD[11], PhD, and Ozioma C. Okonkwo, PhD[1,2,4]

1. Wisconsin Alzheimer's Disease Research Center, University of Wisconsin-Madison, Madison, WI, USA
2. Wisconsin Alzheimer's Institute, Madison, WI, USA
3. Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI, USA
4. Geriatric Research Education and Clinical Center, William S. Middleton VA Hospital, Madison, WI, USA
5. Neurology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
6. Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Göteborg, Sweden
7. Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
8. Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK
9. UK Dementia Research Institute at UCL, London, UK
10. Departments of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
11. Department of Neurology and Weill Institute for Neurosciences, University of California, San Francisco, CA, USA

Title Character Count (with spaces and punctuation): 90
Word Count (abstract): 245
Word Count (text): 1865
Number of References: 36
Number of Tables: 3
Number of Figures: 2

Corresponding Authors:
Dr. Ira Driscoll
idriscoll@medicine.wisc.edu; driscoli@uwm.edu
or
Dr. Ozioma Okonkwo
ozioma@medicine.wisc.edu

Statistical Analyses: Ira Driscoll and Ozioma Okonkwo

Search Terms: 1. Alzheimer's disease, 2. CSF, 3. Biomarkers, 4. Memory, 5. Executive function

Disclosure
Klotho is the subject of a pending international patent application held by the Regents of the University of California. All authors report no disclosures relevant to the manuscript.

ABSTRACT

Objective: To examine whether the functionally advantageous KL-VS variant of the *KLOTHO* gene attenuates age-related alteration in CSF biomarkers and cognitive function in a cohort of middle-aged and older adults enriched for Alzheimer's disease (AD) risk.

Methods: Non-demented adults from the Wisconsin Registry for Alzheimer's Prevention (WRAP) and the Wisconsin Alzheimer's Disease Research Center (W-ADRC) who underwent CSF sampling and neuropsychological testing (N=225, mean age = 63±8, 68% women), were genotyped for KL-VS status. Covariate-adjusted regression analyses examined relationships between age group (Younger vs. Older; mean split at 63 years), AD biomarkers, and performance on neuropsychological tests tapping memory and executive function, and whether these relationships differed by KL-VS status (non-carrier (KL-VS^{NC}) vs. heterozygote (KL-VS^{HET})).

Results: In the pooled analyses, older age was associated with higher levels of total tau (tTau), phosphorylated tau (pTau), tTau/Aβ42, and pTau/Aβ42 (all p's $\leq$ 0.002), as well as with poorer performance on all cognitive tests (all p's $\leq$ 0.001). In the stratified analyses, KL-VS^{NC} exhibited this age-related pattern of associations with CSF biomarkers (all *p's* $\leq$ .001), which were abated in KL-VS^{HET} (all *p's* $\geq$ 0.14). Similarly, KL-VS^{NC} exhibited age-related deficits in memory and executive function (all *p's* $\leq$0.003), which again were attenuated in KL-VS^{HET} (all *p's* $\geq$ 0.18).

Conclusion: Worse memory and executive function, and higher tau burden with age were attenuated in carriers of a functionally advantageous *KLOTHO* variant. KL-VS heterozygosity

seems to be protective against age-related cognitive and biomolecular alterations that increase risk for AD dementia.

Age is the single biggest risk factor for developing Alzheimer's disease (AD)[1]. Currently the sixth leading cause of death in the developed world[2], AD is a progressive, irreversible and debilitating neurological disorder of old age, clinically hallmarked by memory loss and neuropathologically by accumulation of plaques and tangles in the brain[3]. No effective treatments are currently available. With the mean population age steadily rising and the personal and socioeconomic costs of AD mounting, it is imperative to identify approaches that prevent, delay or treat the disease.

The long-standing belief that dementia, and the accumulation of its pathognomonic brain lesions, is an inevitable consequence of aging, however, has been challenged on multiple fronts. We now know that it is possible to age successfully[4], that not all individuals at genetic risk develop AD[5], and that even many individuals with AD neuropathology are able to maintain high levels of cognitive function well into old age[6,7]. This has shifted the focus from risk factors onto potentially protective or compensatory mechanisms, and thereby on prevention of disability and disease. [8]

Klotho is a transmembrane protein and longevity factor.[9,10] Two *KLOTHO* single nucleotide polymorphisms (rs9536314 and rs9527025) exist in perfect disequilibrium, and segregate together to form a functional haplotype, KL-VS, that modulates klotho secretion in humans.[11-13] Several recent meta-analyses indicate a significant association of KL-VS heterozygosity (KL-

VS^{HET}), which is the functionally advantageous *KLOTHO* genotype, with various favorable

outcomes.[14-18] Here we leverage data from at-risk, late-middle-aged adults from the Wisconsin

Registry for Alzheimer's Prevention (WRAP) and the Wisconsin Alzheimer's Disease Research

Center (W-ADRC) to examine whether *KLOTHO* confers resilience against age-related

changes in (1) cerebrospinal fluid (CSF) biomarkers of AD and (2) cognition. We predict that

the expected adverse effect of age on both cognitive performance and AD CSF biomarkers will

be attenuated in carriers of the functionally advantageous genotype of *KLOTHO,* i.e., KL-

VS^{HET}.

METHODS

Participants

The current sample is comprised of 225 cognitively normal adults (age range 45-65 at study

entry; 68% female) who are enrolled in either WRAP[19] or the W-ADRC's IMPACT

(**I**nvestigating **M**emory in **P**reclinical **A**D–**C**auses and **T**reatments) cohorts, both enriched for

AD risk based on family history[11]. Participants in this report were chosen based on availability

of genetic, CSF, and neuropsychological data and all were characterized as cognitively normal

based on standardized, multidisciplinary, consensus conferences diagnosis.[11,19]

Standard Protocol Approvals, Registrations, and Patient Consent

All study procedures are approved by the University of Wisconsin Institutional Review Board

and signed written consent was obtained from all participants

Data Availability Statement

Anonymized data will be shared by request from any qualified investigator to the

corresponding authors for purposes of replicating procedures and results.

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 PCR-based KASP genotyping assays. Quality

control procedures have been previously published[11,20] and are deemed satisfactory. As

expected based on HapMap and the literature[12,21,22], rs9536314 and rs9527025 were in perfect

linkage disequilibrium in our hands as well.

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, gently mixed, and centrifuged at 2,000g

for 10 minutes. Supernatants were frozen in 0.5mL aliquots in polypropylene tubes and stored

at −80°C. The samples were immunoassayed for Aβ42, total tau (tTau) and phosphorylated

tau181 (pTau) with INNOTEST ELISAs (Fujirebio, Ghent, Belgium), as previously

described.[11,23]

Neuropsychological Testing. Participants complete a comprehensive cognitive test battery that includes the National Alzheimer's Coordinating Center's Uniform Data Set. [24,25] The assessment spans five cognitive domains: episodic memory, attention, executive function, language, and visuospatial ability. Here we primarily focus on measures of episodic memory (Rey Auditory Verbal Learning Test, RAVLT)[26] and executive function (Trail Making Test, TMT, Parts A & B)[27] given their sensitivity to incipient AD,[28] and also because they were the tests that 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 whereas for the TMT we analyzed time to test completion.

Statistical Analyses

All analyses were done in SPSS, v. 26.0 (IBM, Armonk, NY). Participants were split into two age groups—Younger vs Older—for analytical purposes, using the mean age of 63 years. For the CSF biomarkers, a series of linear regression models that included terms for age, sex, *APOE* ε4, and parental history of AD were fitted to first ascertain the effect of age on the biomarkers. Then, the analyses were repeated after stratifying the sample by KL-VS genotype[29], to determine whether the deleterious effect of age differed as a function of KL-VS status (non-carriers (KL-VS[NC)]) vs. heterozygotes (KL-VS[HET])). For the cognitive measures, the same analytical strategy was adopted, with the inclusion of education as an additional covariate. Whenever possible, neuropsychological data corresponding to the visit at which lumbar puncture was performed was used; otherwise, analysis was restricted to neuropsychological test data available within one year of lumbar puncture (time interval = 0.16 (0.34) years).

RESULTS

Sample Characteristics. Characteristics of the entire sample, and also stratified by KL-VS, are detailed in Table 1. Overall, participants were predominantly white (97%) and female (68%) with average age of 62.8±7.9 and education of 16.1±2.5 years. The sample is enriched for AD; 42% are *APOE* ε4 carriers, and 75% have a parental history of dementia. The MMSE scores ranged between 26 and 30, with an average of 29.3±0.86. When the sample was further stratified by KL-VS, there were no significant differences (all *p*'s > 0.3) in any of the above-mentioned characteristics between non-carriers (N=168) and heterozygotes (N=57).

Effect of age on CSF and cognitive measures. In the entire sample, Aβ42 did not differ significantly between the Younger and Older groups (*p* = 0.8). As expected, Older age was associated with both tau deposition and worse cognitive performance. Specifically, the Older group had significantly higher levels of CSF tTau and pTau (*all p*'s ≤ 0.001), as well as their respective ratios to Aβ42 (all *p*'s ≤ 0.002). Similarly, the Older group exhibited significantly worse cognitive performance across all neuropsychological measures of interest (all *p*'s ≤ 0.001).

Adverse effect of age on CSF and cognitive measures varies by KL-VS genotype. In the KL-VS[NC], the Older age group consistently exhibited the expected pattern of higher tau values (Table 2; Figure 1) and worse cognitive performance (Table 3; Figure 2) across measures (all *p*'s ≤ 0.003). In contrast, age-related differences in tau burden and cognitive performance were attenuated across the board in KL-VS[HET] (all *p*'s ≥ 0.1).

Because the sample size of KL-VS[NC] was about three times that of KL-VS[HET] (168 vs. 57), we repeated the foregoing analyses on a subsample of 57 KL-VS[NC] who were perfectly matched on sex and *APOE* status (*p*'s = 1) to the 57 KL-VS[HET] to rule out the possibility that our results were due to differences in sample size. Nearly identical pattern of results were seen in the matched sub-sample analyses compared to what was observed in the full sample, whereby Older KL-VS[NC] had more tau accumulation (*p*'s <0.01), worse executive function (*p*'s <0.04), and worse episodic memory (*p* = 0.01; with the exception of the RAVLT Long Delay measure (*p* = 0.21)), with abatement of these age-related differences in KL-VS[HET] (*p*'s > 0.14).

DISCUSSION

In this study, we report that well-established adverse associations of older age with cognition and CSF tau were mitigated in carriers of a functionally favorable KL-VS genotype in a late-middle-aged cohort enriched for AD risk. More specifically, expected age-related alterations in tTau, pTau, tTau/Aβ42, and pTau/Aβ42 were observed in KL-VS[NC], but abated in KL-VS[HET]. Similarly, whereas older KL-VS[NC] exhibited expectedly worse memory and executive function, KL-VS[HET] did not.

The role for *KLOTHO* in longevity[9,10,13,14-17] is well-established. There is mounting evidence in support of relationships between KL-VS heterozygosity and better brain integrity and cognitive performance during normal aging process[12,13,21,22,34,35]. For example, better global cognition is reported in heterozygotes compared to non-carriers in three independent cohorts of non-demented adults[12] as well as slower cognitive decline[34]. Moreover, KL-VS[HET] exhibit better executive function in conjunction with greater dorsolateral prefrontal cortex volume[22] and also show greater intrinsic connectivity in functional brain networks known to be

vulnerable to unfavorable effects of aging.[35] Our group has previously reported that KL-VS heterozygosity mitigated negative effects of *APOE* ε4 on Aβ burden in a late-middle-aged cohort at risk for AD[11]. These findings were confirmed in a recent meta-analysis combining data from 25 studies reporting that *APOE* ε4 carriers, who were also KL-VS heterozygotes, were at a reduced risk for the combined outcome of conversion to MCI or AD.[36] We add to the current state of the literature by demonstrating that the favorable effects of KL-VS[HET] extend to age-related tau burden and deficits in memory and executive function in a non-demented sample enriched for AD.

Although topographic evolution of tauopathy in the brain is the basis for Braak neuropathological staging of AD[30], which in turn strongly associates with cognitive impairment[30-32], there is considerable interindividual heterogeneity and significant diagnostic overlap with neuropathological findings in cognitively unimpaired individuals at autopsy[33]. Age is not only the greatest risk factor for clinical AD, but also the most robust determinant of AD biomarker changes and cognitive decline in the absence of manifest disease. Together, the literature underscores the importance of identifying factors that confer resilience. Here, we offer a glimpse into how one genetic factor, *KLOTHO*, offers resilience against age-related changes in cognition and tau deposition.

KL-VS[HET] may confer resilience by leading to higher circulating klotho levels <Dubal 2014; Yokoyama 2015> or changing its functions. In mouse studies, elevating klotho levels extends lifespan <Kuroso 2005>, enhances cognition in aging mice <Leon Cell Reports 2017>, and increases resilience to AD-related toxicity <Dubal 2015>. In future studies, it will be interesting to assess whether klotho protein levels in the serum and csf of individuals associate with measures of AD and preclinical disease. It is interesting to speculate that KL-VS[HET]

individuals could be biologically younger and thus show resilience to age-induced cognitive and tau changes.

One potential limitation of our study is that a large majority of participants are white and highly educated, limiting the generalizability of our findings. Participants were selected for family history of AD and many are *APOE* ε4 carriers, resulting in the prevalence of both traits being higher in this cohort than what is normally observed in the general population. Moreover, given the cross-sectional nature of the present study, we cannot ascertain causality in the observed effects. This last limitation, however, is addressable in future publications as both WRAP and W-ADRC cohorts are prospective and continuing to collect longitudinal CSF and cognitive data.

Overall, our results suggest that KL-VS heterozygosity may attenuate deleterious effects of aging on AD risk. With aging of the US population and current lack of curative therapies, AD is poised to become a public health crisis. Our results suggest that, KL-VS heterozygosity may be protective against age-related cognitive impairment and accumulation of tau burden in CSF. Identification of new genetic variants that modify AD risk will bring to light novel molecular pathways and accelerate the search for druggable targets. This line of research is poised to identify complementary pathways for curbing the disease progression and delaying symptom onset.

Acknowledgment

REFERENCES

1. Hou Y, Dan X, Babbar M, *et al.* Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol* 2019;15:565–581.
2. Alzheimer's Association. 2018 Alzheimer's disease facts and figures. *Alzheimers Dement* 2018;14:367–429.
3. Van Cauwenberghe C, Van Broeckhoven C, Sleegers K. The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet Med* 2016;18:421.
4. 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.
5. 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.
6. 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.

7. Driscoll I, Troncoso J. Asymptomatic Alzheimer's disease: a prodrome or a state of resilience? *Curr Alzheimer Res* 2011;8(4):330-335.
8. 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.
9. Chateau MT, Araiz C, Descamps S, Galas S. Klotho interferes with a novel FGF-signalling pathway and insulin/Igf-like signalling to improve longevity and stress resistance in Caenorhabditis elegans. *Aging* 2010;2:567–581.
10. Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone Klotho. *Science* 2005;309:1829–1833.
11. Erickson CM, Schultz SA, Oh JM, et al. KLOTHO heterozygosity attenuates APOE4-related amyloid burden in preclinical AD. *Neurology* 2019;92(16):e1878-e1889.
12. Dubal DB, Yokoyama JS, Zhu L, et al. Life extension factor klotho enhances cognition. *Cell Rep* 2014;7:1065–1076.
13. 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.
14. 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.
15. 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.
16. 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.
17. 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.
18. Vo HT, Laszczyk AM, King GD. Klotho, the Key to Healthy Brain Aging? *Brain Plast* 2018;3(2):183-194.
19. 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.
20. 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.
21. Arking DE, Atzmon G, Arking A, Barzilai N, Dietz HC. Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. *Circ Res* 2005;96:412–418.
22. 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.
23. 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.
24. 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.

25. 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.
26. Schmidt M. Rey Auditory Verbal Learning Test: A Handbook. Torrance, CA: Western Psychological Services; 1996.
27. Reitan R, Wolfson D. The Halstead-Reitan Neuropsychological Test Battery: Theory and clinical interpretation. *Tucson: Neuropsychology Press*; 1993.
28. 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.
29. Behrens G, Winkler TW, Gorski M, Leitzmann MF, Heid IM. To stratify or not to stratify: power considerations for population-based genome-wide association studies of quantitative traits. *Genet Epidemiol* 2011;35:867–879.
30. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 1991;82(4):239-59.
31. Duyckaerts C, Bennecib M, Grignon Y, Uchihara T, He Y, Piette F, Hauw JJ. Modeling the relation between neurofibrillary tangles and intellectual status. *Neurobiol Aging 2*1997;18(3):267-273.
32. Bennett DA, Schneider JA, Arvanitakis Z, Kelly JF,Aggarwal NT, Shah RC, Wilson RS. Neuropathology of older persons without cog-nitive impairment from two community-based studies. *Neurology* 2006;66:1837–1844.
33. 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.
34. 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.
35. 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.
36. 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 inindividuals who carry APOE4. JAMA Neurol 2020;Epub ahead of print.

Table 1. Background characteristics of study participants

VARIABLE	TOTAL SAMPLE (N =225)	KL-VS^{NC} (N=168)	KL-VS^{HET} (N=57)	*p*
Age	62.85 (7.99)	62.99 (7.99)	61.81(8.16)	0.34
Education	16.09 (2.51)	16.06 (2.49)	16.19 (2.59)	0.73
MMSE	29.31 (0.86)	29.32 (0.89)	29.30 (0.86)	0.93
Females (%)	68	68	65	0.62
White (%)	97	98	96	0.34
***APOE* ε4+ (%)**	42	43	37	0.38
KL-VS^{HET} (%)	25	-	-	-
Parental history of AD (%)	75	74	75	0.88

Abbreviations: KL-VS$_{NC}$ = KL-VS non-carriers; KL-VS$_{HET}$ = KL-VS heterozygotes; MMSE = Mini-Mental State Examination score; *APOE* ε4+ = *APOE* ε4 carrier

Table 2. Association between CSF AD biomarkers and age across KL-VS strata

CSF MEASURE	AGE GROUP	KL-VS[NC]			KL-VS[HET]		
		M (SE)	F	p	M (SE)	F	p
Aβ42	<63	718.59 (22.88)	0.001	0.98	740.77 (35.18)	0.51	0.48
	≥63	719.17 (20.47)			705.44 (39.96)		
tTau	<63	275.74 (16.12)	16.40	<0.001	292.59 (31.39)	0.71	0.40
	≥63	364.99 (14.42)			333.61 (35.66)		
pTau	<63	39.21 (1.82)	11.45	0.001	38.46 (2.78)	1.41	0.24
	≥63	47.64 (1.63)			43.12 (3.15)		
tTau/Aβ42	<63	0.39 (0.04)	16.27	<0.001	0.40 (0.06)	2.25	0.14
	≥63	0.58 (0.03)			0.54 (0.07)		
pTau/Aβ42	<63	0.06 (0.004)	12.54	0.001	0.05 (0.005)	2.28	0.14
	≥63	0.08 (0.004)			0.07 (0.006)		

Abbreviations: KL-VS^{NC} = KL-VS non-carriers; KL-VS^{HET} = KL-VS heterozygotes; Aβ42 = β-amyloid42; tTau = total tau; pTau = phosphorylated tau

Table 3. Association between cognitive function and age across KL-VS strata

COGNITIVE MEASURE	AGE GROUP	KL-VS^{NC}			KL-VS^{HET}		
		M (SE)	**F**	**p**	**M (SE)**	**F**	**p**
RAVLT Trials 1-5	**<63**	52.77 (0.91)	19.43	<0.001	49.84 (1.41)	1.01	0.32
	≥63	47.19 (0.82)			48.21 (1.61)		
RAVLT Long Delay	**<63**	10.69 (0.36)	8.86	0.003	10.23 (0.45)	0.69	0.41
	≥63	9.22 (0.32)			9.89 (0.51)		
TMT A (time)	**<63**	23.66 (0.97)			9.89 (0.51)		
	≥63	27.80 (0.87)	9.24	0.003	25.36 (1.36)	0.02	0.88
TMT B (time)	**<63**	53.69 (2.67)			57.95 (4.46)		
	≥63	69.48 (2.39)	18.01	<0.001	68.31 (5.06)	1.81	0.18

Abbreviations: KL-VS^{NC} = KL-VS non-carriers; KL-VS^{HET} = KL-VS heterozygotes; RAVLT = Rey Auditory Verbal Learning Test; TMT = Trail Making Test
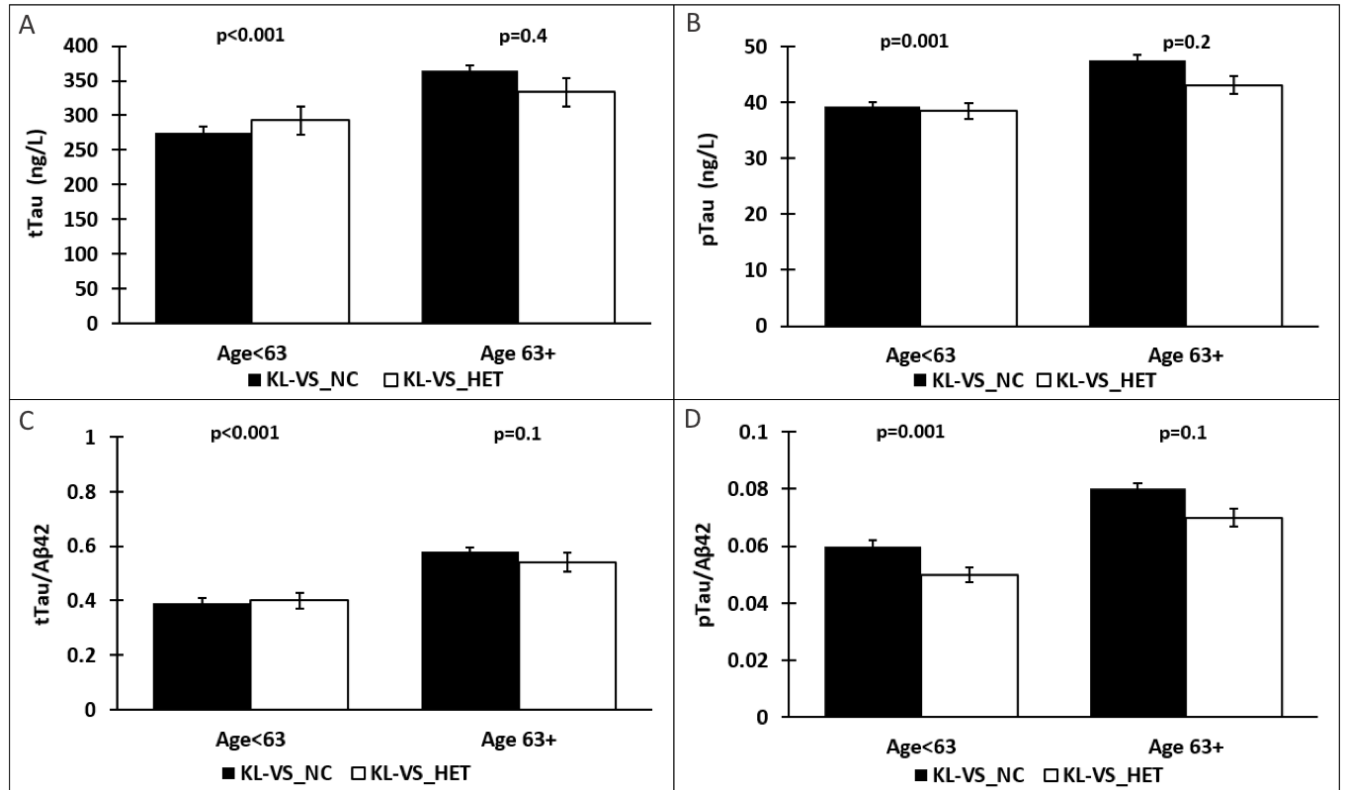
Figure 1. Age differentially associates with CSF tau measures as a function of KL-VS status. Bar graphs depicting group differences in tau between Younger (black) and Older (white) individuals. Among KL-VS[NC], Older age was associated with worse levels of A) total tau (tTau), B) phosphorylated tau (pTau), and their respective ratios to Aβ42 (C & D). This age-related pattern of associations with CSF tau biomarkers was abated in KL-VS[HET].
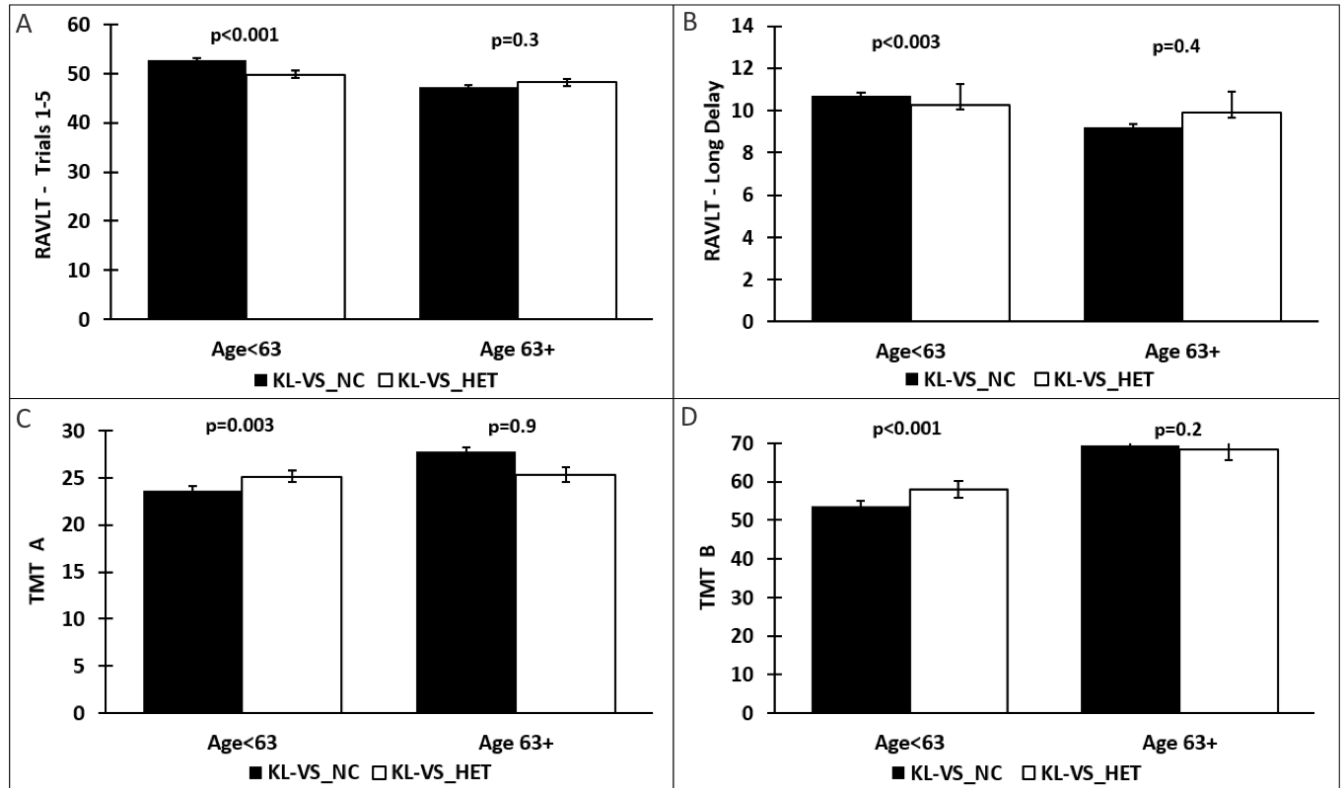*Aβ42 = β-amyloid42; tTau = total tau; pTau = phosphorylated tau.

Figure 2. Age differentially associates with measures of episodic memory and executive function as a function of KL-VS status. Bar graphs depicting group differences in cognition between Younger (black) and Older (white) individuals. KL-VS[NC] exhibited age-related deficits in memory (A & B) and executive function (C & D), which were attenuated in KL-VS[HET].
*RAVLT = Rey Auditory Verbal Learning Test (total trials); TMT = Trail Making Test (time in seconds).