Amyloid-β positivity is less prevalent in cognitively unimpaired KLOTHO KL-VS heterozygotes

Noah Cook[a,b], Ira Driscoll[*,a,c], Julian M. Gaitán[a], Tobey J. Betthauser[a,c], Cynthia M. Carlsson[a,c,d], Sterling C. Johnson[a,c,d] , Sanjay Asthana[a,c,d], Henrik Zetterberg[e,f,g,h,i,j], Kaj Blennow[e,f], Gwendlyn Kollmorgen[k], Clara Quijano-Rubio[l], Dena B. Dubal[m], Ozioma C. Okonkwo[*,a,c,d]

[a.] Alzheimer's Disease Research Center and Department of Medicine, University of Wisconsin-Madison, Madison, WI, USA
[b.] Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[c.] Wisconsin Alzheimer's Institute, Madison, WI, USA
[d.] Geriatric Research Education and Clinical Center, William S. Middleton VA Hospital, Madison, WI, USA
[e.] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
[f.] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
[g.] Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK
[h.] UK Dementia Research Institute at UCL, London, UK
[i.] Hong Kong Center for Neurodegenerative Diseases, Clear Water Bay, Hong Kong, China
[j.] Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA
[k.] Roche Diagnostics GmbH, Penzberg, Germany
[l.] Roche Diagnostics International Ltd, Rotkreuz, Switzerland
[m.] Department of Neurology and Weill Institute for Neurosciences, University of California, San Francisco, CA, USA

Running Title: AT status varies by *KLOTHO*

*** Correspondence:**
Ozioma Okonkwo, PhD
Department of Medicine and Wisconsin Alzheimer's Disease Research Center
University of Wisconsin-Madison
600 Highland Avenue
Madison, WI 53792, USA
ozioma@medicine.wisc.edu

or

Ira Driscoll, PhD
Department of Medicine and Wisconsin Alzheimer's Disease Research Center
University of Wisconsin-Madison
600 Highland Avenue
Madison, WI 53792, USA

idriscoll@medicine.wisc.edu

Abstract

Background: Klotho, encoded by the *KLOTHO* gene, is an anti-aging and neuroprotective protein. Heterozygosity for *KLOTHO* KL-VS (KL-VS$_{HET}$) is protective against the accumulation of neuropathological hallmarks of Alzheimer's disease (AD), amyloid-β (Aβ) and tau.

Objective: To examine whether being positive for Aβ (A+) or tau (T+), or A/T status [positive for Aβ (A+T-), tau (A-T+), both (A+T+) or neither (A-T-)] vary by KL-VS and whether serum klotho protein levels vary based on A+, T+, or A/T status in a cohort enriched for AD risk.

Methods: The sample consisted of 704 cognitively unimpaired, middle-aged, and older adults, Mean$_{Age}$(SD)=64.9(8.3). Serum klotho was available for a sub-sample of 396 participants, Mean$_{Age}$(SD)=66.8(7.4). Covariate-adjusted logistic regression examined whether A+ or T+, and multinomial regression examined whether A/T status, varied based on KL-VS genotype. Covariate-adjusted linear regression examined whether serum klotho differed based on A+, T+, or A/T status.

Results: There was a non-significant trend for lower A+ prevalence in KL-VS$_{HET}$ (P=0.07), with no differences in T+ prevalence (P=0.53). KL-VS$_{HET}$ also had marginally lower odds of being A+T- (P=0.08). Serum klotho levels did not differ based on A+, T+, or A/T status in this sample (all Ps≥0.39).

Conclusion: KL-VS$_{HET}$ is associated with lower odds of being positive for Aβ, regardless of whether one is also positive for tau. Conversely, the likelihood of being tau positive did not differ based on KL-VS genotype. Our findings corroborate accumulating evidence that KL-VS$_{HET}$ confers resilience against Aβ accumulation and add to the growing *KLOTHO* literature in relation to AD.

Keywords: Alzheimer's disease, AD biomarkers, cerebrospinal fluid, klotho, positron emission tomography, tau

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder in the world, with the estimated costs expected to surpass $1 trillion by 2050 [1]. Efforts to diagnose or predict who will become demented later in life have steered research toward identifying reliable biomarkers that not only aid in early detection but also offer insights into the disease's pathophysiology. Among these biomarkers, amyloid-β (Aβ) and tau, detected through cerebrospinal fluid (CSF) or via positron emission tomography (PET), have garnered substantial attention as proxies for pathological changes occurring years before clinical symptoms manifest [2].

Klotho is an anti-aging, longevity, and neuroprotective protein encoded by the *KLOTHO* gene. Circulating throughout the body and brain, klotho regulates cognition <PMID 37400721, 35428698, 28793260, 24813892, 25673831, 37587231> and myriad of pathways, including insulin [3], fibroblast growth factor [4], and NMDA receptor [5-7] signaling. In humans, *KLOTHO* gene variants F352V and C370S segregate to form a KL-VS haplotype which modulates klotho secretion [7, 8]. Carrying one copy of the KL-VS haplotype ($KL\text{-}VS_{HET}$) is associated with higher systemic klotho levels [7, 9], longevity, resilience against disorders associated with older age [8, 10, 11], better cognition [6], greater cortical volume [9, 12] and better brain connectivity [13] in older adults. Literature also suggests that $KL\text{-}VS_{HET}$ may be protective against both *APOE* ε4-related Aβ accumulation [14] and age-related tau deposition [15].

Although studies have suggested that variations in the KL-VS genotype and alterations in klotho protein levels may influence neurodegenerative processes [7-16], to our knowledge, no studies have examined KL-VS genotype or circulating klotho in relation to being positive for Aβ or tau in both CSF and PET. Here we examine whether 1) being positive for amyloid-β (A+), tau (T+) or the combination thereof (A/T status) vary based on KL-VS genotype ($KL\text{-}VS_{HET}$ vs. non-carriers ($KL\text{-}VS_{NC}$)), and 2) whether serum klotho levels vary based on A+, T+, or A/T status.

Methods

Participants

Late middle-aged and older adults (N=704) from the Wisconsin Registry for Alzheimer's Prevention (WRAP) [17] and the Wisconsin Alzheimer's Disease Research Center (WADRC) [14] were included in the study (68% female; mean age (SD) = 64.9 (8.3)). They were deemed cognitively unimpaired based on their performance on a comprehensive battery of neuropsychological tests, absence of functional impairment and absence of neurological conditions that might impair cognition. Individuals were previously genotyped for *KLOTHO* and *APOE* and underwent either a lumbar puncture or PET [C-11] Pittsburgh compound B (PiB) and [F-18] MK-6240 (MK-6240) imaging, or both. A sub-sample of 396 individuals (66% female; mean age (SD) = 66.8 (7.4)) had serum klotho measurements. The entire cohort was enriched for AD risk based on the number of individuals carrying at least one *APOE* ε4 allele (36%) or having parental history of AD (70%).

Blood collection

Details of blood sampling have been previously published [18, 19]. Blood for serum analyses was collected following a 12-hour fast from food, caffeine, and alcohol into a 9mL vacutainer containing no anticoagulant or additive. The samples were centrifuged at 3000 rpm for 10 minutes at 4°C after being allowed to clot for no more than 30 minutes. The serum was then aliquoted into cryovials and frozen until assays were performed.

Serum klotho quantification

Soluble α-klotho was measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) as previously described [7, 19, 20]. In summary, recombinant human α-klotho protein was serially diluted to create a standard curve, and then serum was diluted four-fold with the immunoassay buffer. Diluted serum was then loaded in duplicate onto a plate that had been coated with affinity-purified anti-human klotho (67G3) mouse IgG monoclonal antibody. For each plate, control samples were used as a reference to ensure that inter-plate comparisons were correct. Following incubation for 1 hour at room temperature, the plates were then washed five times with washing buffer, horseradish peroxidase-conjugated anti-human klotho (100 µl, 91F1) mouse IgG monoclonal antibody was added, and then the plates were incubated for 30 minutes at room temperature. The addition of 100 µl of chromogenic substrate for 30 minutes at room temperature allowed for the visualization of the reaction, which was subsequently halted with 100 µl of 1 n H_2SO_4. The Spectramax 190 plate reader (Molecular Devices, Sunnyvale, CA, USA) was used to measure the absorbance at 450 nm and the SoftMax Pro software was used to determine the α-klotho levels (Molecular Devices). Samples with coefficient of variation (CV) above 10% were re-run. The final average CV for serum was 2.8%.

Genotyping

DNA was extracted from blood using the PUREGENE DNA Isolation Kit (Gentra Systems, Inc, Minneapolis, MN). DNA concentrations were quantified with ultraviolet spectrophotometry (DU 530 Spectrophotometer, Beckman Coulter, Fullerton, CA). Single nucleotide polymorphisms for *APOE* (rs429358 and rs7412) and *KLOTHO* (rs9536314 for F352V and rs9527025 for C370S) were genotyped by LGC Genomics (Beverly, MA) using competitive allele-specific PCR-based KASP genotyping assays [7]. Both rs9536314 and rs9527025 were in perfect linkage disequilibrium in the present sample. Quality control measures have been previously published [14-16, 21]. KL-VS homozygotes, carrying two copies of the KL-VS haplotype, were excluded from the analyses due to limited sample size (N = 8).

CSF assessment

Details of CSF collection and assessment have been previously published [22]. Briefly, samples were collected in the morning after an 8- to 12-hour fast using a Sprotte 24- or 25-gauge atraumatic spinal needle and 22 mL of fluid was collected via gentle extraction into polypropylene syringes and combined into a single 30 mL polypropylene tube. After gently mixing, samples were centrifuged to remove red blood cells or other debris; 0.5 mL CSF was aliquoted into 1.5-mL polypropylene tubes and stored at −80°C within 30 minutes of collection. All CSF samples were assayed at the Clinical Neurochemistry Laboratory, University of Gothenburg, using the same batch of reagents and under strict quality control procedures. $A\beta_{42}$, ptau181 and $A\beta_{40}$ levels in CSF were measured using Elecsys electrochemiluminescence immunoassays on the cobas e 601 analyzers (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) [23, 24].

PET imaging

Detailed imaging methods for WRAP/WADRC including radiopharmaceutical production, acquisition protocols, and image reconstruction, processing, and quantification of PiB and MK-6240 PET data are detailed elsewhere [25-27]. Briefly, cortical amyloid burden was assessed using PiB PET imaging at 0-70 minutes after injection of a target dose of 15 mCi. Mean cortical PiB distribution volume ratios (DVRs) were estimated using the Logan method with cerebellar gray matter as a reference region [28]. For MK-6240, standard uptake value ratios (SUVRs) were calculated from a 20-minute dynamic acquisition (4 × 5-minute frames) beginning 70 minutes after bolus injection using the inferior cerebellum grey matter as a reference region [29].

Group classifications based on Aβ and tau

For CSF analytes, A+ and T+ were defined based on $A\beta_{42}/A\beta_{40}$ ratio greater than 0.046 pg/mL and pTau181 greater than 24.8 pg/mL, respectively. Derivations of these cut-off points have been previously published [22]. For PET imaging, A+ was based on previously defined global DVR threshold of >1.19 [30, 31]. The threshold for being T+ was established by setting the MK-6240 SUVR threshold for the medial temporal lobe (MTL) at 2 standard deviations above the mean of the A- group (MTL SUVR >1.17) [29]. MTL was chosen because it is the earliest region to display neurofibrillary tangle (NFT) formation in AD [32].

If an individual only had CSF measurements or only had PET imaging, their A/T status was determined based on the previously defined measurement threshold for the available modality (PET or CSF). If individuals had both CSF measurements and PET imaging, they would be considered positive for Aβ or tau if their measurements for either modality were above the predefined thresholds. Based on these thresholds, participants were classified into four groups to determine their A/T status: A-T-, A+T-, A-T+, and A+T+.

Statistical analyses

All analyses were performed using the "nnet" and "rms" package in R version 4.3.0 [33]. Sample characteristics were compared across A/T status using analysis of variance (ANOVA) for continuous measures and chi-square for categorical measures. Characteristics from the sub-sample of individuals who had a serum klotho measurement were compared to the entire sample using t-test for continuous measures and chi-square for categorical measures. Logistic regression models examined whether A+ or T+ varied based on KL-VS genotype. Multinomial regression models were applied to investigate whether A/T status, with A-T- as the reference group, varied based on KL-VS genotype. Odds ratios were calculated to quantify the strength of the above-mentioned relationships. Covariates included age at CSF collection or age at PET imaging if participant did not have CSF data, self-reported sex, *APOE* ε4 allele carrier status and parental history of AD.

In the sub-sample with available serum klotho measurements, linear regression models controlling for age at serum collection, self-reported sex, *APOE* ε4 carrier status, parental history of AD and difference in age between serum collection and CSF sampling or PET imaging, examined whether serum klotho protein levels differed as a function of A+ and T+, or A/T status. Serum klotho measurements were log-transformed to improve model fit. ANOVAs

assessed if the covariates had any significant influence on the model, and variance inflation factors were calculated to check for co-linearity.

Results

Sample characteristics

Table 1 details characteristics of the entire sample (N = 704) and for each A/T group separately. The average age at biomarker (CSF or PET) collection was 64.9 ± 8.3 years and 68% of the sample identified as female. The sample was predominantly white (94%) with 70% of the sample reporting parental history of AD and 36% carrying at least one copy of the *APOE* ε4 allele. There were significant differences between the four A/T groups with regard to age at biomarker collection, *APOE* ε4 carrier status, and parental history of AD (all *P*s < 0.001); A-T- was significantly younger compared to all other groups, and A+T- and A+T+ had a higher percentage of *APOE* ε4 carriers and parental history of AD compared to the other two groups. Similar relationships were observed in the sub-sample of participants with just PET and no CSF (Supplementary Table 1A), just CSF and no PET (Supplementary Table 1B) and those with available serum klotho (Supplementary Table 1C) measurements. Other than the sub-sample of individuals with serum klotho being significantly older than the entire sample (*P* < 0.001), there were no observed differences between the entire sample and the sub-sample (all *Ps* ≥ 0.24).

A+ prevalence based on KL-VS genotype

Participants who were KL-VS$_{HET}$ were approximately 36% less likely (OR = 0.64) to be A+ compared to KL-VS$_{NC}$ (Table 2) in both the unadjusted (OR 95% C.I. = 0.44-1.00, *P* = 0.05) and adjusted (OR 95% C.I. = 0.40-1.03, *P* = 0.07) models. Similar trends were observed for the sub-sample where A+/- was determined by PET only (Supplementary Table 2A). However, this association was not observed in the sub-sample with Aβ measurements obtained by CSF alone (Supplementary Table 2B).

T+ prevalence based on KL-VS genotype

The prevalence of being T+ did not differ between KL-VS$_{HET}$ and KL-VS$_{NC}$ (see Table 2, adjusted *P* = 0.53) nor in the sub-samples where T+/- was determined by PET only (Supplementary Table 2A) or CSF alone (Supplementary Table 2B).

A/T status based on KL-VS genotype

The distribution of A/T groups for the entire sample and stratified by KL-VS genotype is depicted in Figure 1A-C. KL-VS$_{HET}$ had a non-significant trend for lower odds of being A+T- compared to KL-VS$_{NC}$ (Table 3; adjusted OR 95% C.I. = 0.32-1.04, *P* = 0.08). Compared to A-T-, there was no association between KL-VS genotype and odds of being A-T+ (adjusted OR 95% C.I. = 0.42-1.84, *P* = 0.73) or odds of being A+T+ (adjusted OR 95% C.I. = 0.38-1.35, *P* = 0.30). Similar overall trends were observed for the sub-samples in which A/T status was determined by PET only (Supplementary Table 3A) or CSF alone (Supplementary Table 3B).

Serum klotho protein levels based on A+, T+ and A/T status

Serum klotho levels did not differ between A+ and A- nor between T+ and T- (Table 4), in the adjusted models (all *P*s ≥ 0.41). Serum klotho levels also did not differ based on A/T status in the adjusted models (all *P*s ≥ 0.42) (see Table 5 and Figure 2). Males had significantly lower serum

klotho levels compared to females across all models (all *P*s ≤ 0.001). Older age, carrying at least one copy of the *APOE* ε4 allele and parental history of AD were not associated with lower serum klotho levels in our sample (all *P*s ≥ 0.25).

Discussion

Overall, our results indicate that having the functionally advantageous KL-VS$_{HET}$ variant is associated with lower odds of being positive for Aβ, regardless of tau. Odds for being A+/T- compared to A-T- were also marginally lower for KL-VS$_{HET}$. Our current findings align with emerging evidence implicating genetic variants, including *KLOTHO* KL-VS, in susceptibility to AD-related pathology [34]. The lower prevalence of Aβ positivity among heterozygotes hints at potential underlying mechanism related to Aβ accumulation or clearance, warranting further examination. Albeit nascent, the literature supports the potential pathway related to klotho overexpression in KL-VS$_{HET}$ [19] and the fact that klotho overexpression regulates the Aβ transporters which clear β-amyloid from the brain to the blood and prevent accumulation [35].

The prevalence of tau positivity did not differ based on KL-VS genotype in our sample, regardless of Aβ. The absence of an association between the KL-VS genotype and tau pathology in this cohort challenges the hypothesis of a direct relationship between *KLOTHO* and tau accumulation in the absence of cognitive impairment [36]. However, it should be noted that these findings are in general agreement with the two recently published studies which reported that the percentage of those who were positive based on CSF pTau did not significantly differ between KL-VS heterozygotes and non-carriers [15] and no association between KL-VS genotype and CSF tau concentrations [37]. Our findings suggest a potential interplay between klotho biology and tau-related mechanisms, which may not be dependent on KL-VS genotype in cognitively unimpaired individuals. Although the exact nature of the relationship between the circulating klotho and tau pathology is still unknown, we do know that it involves several biological processes that reduce neuronal damage, such as growth factor functions [38], insulin regulation [3], calcium signaling [39] and reactive oxygen species regulation [40, 41].

We found no significant differences in serum klotho levels in our sample based on A+, T+, or A/T status, which adds another layer of complexity to understanding the relationships between *KLOTHO* KL-VS genotype, circulating klotho protein levels and core AD biomarkers. The consistent levels of serum klotho across all A/T groups imply that, in this cohort of cognitively unimpaired individuals, albeit many of whom are at risk for AD either based on their *APOE* ε4 status or family history, systemic klotho concentrations may not directly correlate with Aβ or tau pathologies. One factor potentially contributing to the absence of discernible differences in serum klotho levels based on A/T status could be attributed to klotho sampling.

The serum rather than CSF measurements might underscore a crucial distinction in the compartmentalization and dynamics of klotho distribution between peripheral circulation and the central nervous system. As a transmembrane protein expressed predominantly in the kidneys and the choroid plexus [42], it is possible that klotho accessibility in serum does not faithfully mirror its concentrations within the central nervous system [19]. For example, our group recently reported that klotho concentrations were significantly higher in CSF compared to serum across all KL-VS genotypes [19]. The same study also found that the mean serum klotho

concentrations, after controlling for age and sex of participants, did not significantly differ between KL-VS$_{HET}$ and KL-VS$_{NC}$ in a sample of 1116 cognitively unimpaired middle-aged and older adults [19]. Currently the number of participants with available CSF klotho in the WRAP and WADRC cohorts is limited; both studies continue to collect data prospectively and should be able to address potential differences between CSF and serum klotho in the future. Moreover, given that the sub-sample of individuals with serum klotho measurements was significantly older compared to the entire sample, it is likely that the lesser variability and a smaller range of serum klotho levels contributed to the lack of expected groups differences. Moreover, the interplay between klotho and AD pathology might involve multifaceted mechanisms that extend beyond a direct correlation between serum klotho levels and Aβ or tau accumulation. The diverse functions of Klotho in cellular processes, including anti-oxidative stress [43], modulation of synaptic plasticity [44] and regulation of neuronal survival pathways [6] could all serve as indirect pathways through which *KLOTHO* ultimately influences AD pathogenesis, outside of the mere modulation of klotho levels.

The literature suggests that circulating klotho differs based on sex [20, 45]. In our sample, serum klotho concentrations were higher in females compared to males, which is largely in alignment with the literature [19, 20, 45, 46]. This finding, however, stands in contrast to one study reporting no sex differences in serum klotho [47]. The differences in findings could likely be attributed to the inclusion criteria, whereby the study reporting no sex differences included individuals who were 75+ years of age, resulting in a sample quite older (Mean$_{Age}$ = 85 years) than most other studies. Still, klotho is neuroprotective [6] and compared to males, females live longer by more than five years on average in the United States [48]. It is plausible that higher klotho levels in females are reflected in longevity compared to their male counterparts [49], which may in turn add to the higher lifetime AD risk in females relative to males given that older age is the strongest risk factor for AD [1]. Alternatively, it may be that klotho is overexpressed in response to the higher AD risk [14, 16].

Our finding of no significant relationship between age and serum klotho levels stands in contrast to a couple published studies to date on the topic [10, 13]. Notably, our cohort was approximately a decade younger, with average age of 65 in comparison to their samples with a mean age of 78 [10] and 74 [13]. In addition, it is important to consider the age range of each sample given that KL-VS$_{HET}$, which is directly related to serum klotho levels [19], seems to exert an age-dependent effect with an advantage present in those 80 years of age or older [8]. The age range of our cohort was 50-84 years with 95% of the sample below age 80, in contrast to the age ranges of 19-109 years [10] and 55-90 years [13], with 40% and 35% of participants being older than 80, respectively. Given the considerable differences in both average age and age range across the studies and the documented age-dependent effect of klotho [8, 10, 12], it is likely that our cohort being on average considerably younger with majority of participants below the age of 80, precluded the discernible age-related decline in serum klotho levels.

Our study is not without limitations. The majority of our cohort is white, and college educated. This limits the generalizability of our findings and makes it imperative to diversify the study population in future investigations. Another potential limitation is the generally small sample size of the subgroups after splitting the sample by A/T status. One final drawback of the current study is that it is cross-sectional. Since the WRAP and WADRC studies are prospective and

ongoing, however, it will be possible to investigate the trajectories of change in klotho protein levels as function of change in Aβ and tau in the future.

Still, to our knowledge, the present study is the first to, based on KL-VS genotype, examine 1) the odds of being positive for core AD biomarkers and 2) circulating klotho levels in those positive for core AD biomarkers by CSF or PET. Our key finding is that having the KL-VS_{HET} genotype is associated with lower odds of being positive for Aβ. This finding adds to the growing literature suggesting that *KLOTHO* KL-VS_{HET} confers resilience against Aβ accumulation specifically [14] and deleterious effects of aging or AD-associated pathology in general [12, 15, 16, 50]. Collectively, our findings point to seemingly multifaceted relationships between *KLOTHO* gene, circulating klotho protein levels and core AD biomarkers. This is not entirely surprising given the complexities inherent in both AD pathology and klotho biology, which necessitate comprehensive investigations encompassing various facets of their respective regulation, including genetic variations, post-translational modifications and tissue-specific expression patterns. Integrating multi-modal approaches, such as combining serum and CSF measurements alongside genetic profiling, might offer a more comprehensive understanding of the relationships. Such lines of inquiry have the potential to uncover complementary pathways that may be instrumental in delaying AD symptom onset or disease progression.

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Conflict of Interest
All authors have no conflict of interest directly related to this study.

Data Availability
The data that support the findings of this study are available from the corresponding author upon reasonable requests and an approval by the Executive Committees of the Wisconsin Registry for Alzheimer's Prevention and the Wisconsin Alzheimer's Disease Research Center.

Footnotes
Disclosures
Klotho is the subject of an international patent issued and held by the Regents of the University of California.

The NeuroToolKit is a panel of exploratory prototype assays designed to robustly evaluate biomarkers associated with key pathologic events characteristic of AD and other neurological disorders, used for research purposes only and not approved for clinical use (Roche Diagnostics International Ltd). Elecsys β-amyloid (1–42) CSF and Elecsys Phospho-Tau (181P) CSF assays are approved for clinical use. All other product names and trademarks are the property of their respective owners.

S.C.J. has served on advisory boards for ALZPath and Enigma Biosciences. K.B. has served as a consultant, on advisory boards, or on data-monitoring committees for Acumen, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; has served at data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, which is a part of the GU Ventures Incubator Program (outside submitted work). H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche, 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). B.B.B has received precursor and reagents from AVID Radiopharmaceuticals and has served as an advisor to Cognito Therapeutics, Merry Life, and New Amsterdam. G.K. is a full-time employee of Roche Diagnostics GmbH. C. Q_R. is a full-time employee of Roche Diagnostics International Ltd.). D.B.D. has consulted for Unity Biotechnology and S.V. Health Investors. All other authors have no relevant disclosures to report.

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Tables

Table 1. Sample background characteristics.

	Entire Sample (N=704)	A-T- (N=499)	A+T- (N=84)	A-T+ (N=43)	A+T+ (N=78)	*P*
Age Mean (SD)	64.9 (8.3)	63.4 (8.1)	67.2 (6.9)	69.6 (8.2)	69.3 (7.6)	**<0.001**
Female N (%)	475 (68)	331 (66)	56 (67)	33 (77)	55 (71)	0.49
***APOE* ε4+** N (%)	256 (36)	140 (28)	51 (61)	13 (30)	52 (67)	**<0.001**
White N (%)	664 (94)	470 (94)	80 (95)	41 (95)	73 (94)	0.99
PH of AD N (%)	490 (70)	341 (68)	61 (73)	23 (54)	65 (83)	**<0.001**
KL-VS$_{HET}$ N (%)	190 (27)	145 (29)	16 (19)	11 (26)	18 (23)	0.20

Abbreviations: AD = Alzheimer's disease; *APOE* ε4+ = Carrier of at least one *APOE* ε4 allele; A-T- = amyloid-β negative and tau negative; A+T- = amyloid-β positive and tau negative; A-T+ = amyloid-β negative and tau positive; A+T+ = amyloid-β positive and tau positive; KL-VS$_{HET}$ = *KLOTHO* KL-VS heterozygote; PH = parental history of AD (at least one parent with AD diagnosis); SD = standard deviation.

Table 2. Odds ratios for A+ and T+ by KL-VS genotype.

		A- (N=542)	A+ (N=162)	T- (N=583)	T+ (N=121)
KL-VS$_{NC}$	N (%)	386 (75)	128 (25)	422 (82)	92 (18)
KL-VS$_{HET}$	N (%)	156 (82)	34 (18)	161 (85)	29 (15)
Unadjusted Model OR (95% C.I.)		1 (Ref.)	0.66 (0.43, 1.00)	1 (Ref.)	0.83 (0.52, 1.30)
	P	-	**0.05**	-	0.41
Adjusted Model OR (95% C.I.)		1 (Ref.)	0.64 (0.40, 1.03)	1 (Ref.)	0.85 (0.52, 1.40)
	P	-	**0.07**	-	0.53

Abbreviations: A- = negative for β-amyloid by CSF and PET; A+ = positive for β-amyloid by CSF or PET; C.I. = confidence interval; KL-VS$_{HET}$ = *KLOTHO* KL-VS heterozygote; KL-VS$_{NC}$ = *KLOTHO* KL-VS non-carrier; OR = odds ratio; Ref. = reference group; T- = negative for tau by CSF and PET; T+ = positive for tau by CSF or PET.

Table 3. Odds ratios for each A/T group by KL-VS genotype.

Model	A/T Status	OR (95% C.I.)	*P*
Unadjusted	A-T-	1 (Ref.)	-
	A+T-	0.57 (0.32, 1.02)	**0.06**
	A-T+	0.84 (0.41, 1.71)	0.63
	A+T+	0.73 (0.42, 1.28)	0.28
Adjusted	A-T-	1 (Ref.)	-
	A+T-	0.58 (0.32, 1.06)	**0.08**
	A-T+	0.88 (0.42, 1.84)	0.73
	A+T+	0.72 (0.38, 1.35)	0.30

Abbreviations: A-T- = amyloid-β negative and tau negative; A+T- = amyloid-β positive and tau negative; A-T+ = amyloid-β negative and tau positive; A+T+ = amyloid-β positive and tau positive; C.I. = confidence interval; OR = odds ratio; Ref. = reference group.

Table 4. Serum klotho levels based on Aβ or tau positivity.

	amyloid-β					tau			
Coefficient	**Estimate**	**S.E.**	**t**	***P***	**Coefficient**	**Estimate**	**S.E.**	**t**	***P***
Intercept	6.83	0.15	45.07	**<0.001**	**Intercept**	6.80	0.15	46.64	**<0.001**
A+	0.03	0.04	0.86	0.39	**T+**	0.001	0.04	0.02	0.99
$Age_{(klotho)}$	-0.002	0.002	-0.82	0.41	**$Age_{(klotho)}$**	-0.001	0.002	-0.61	0.54
Female	0.10	0.03	3.37	**0.001**	**Female**	0.10	0.03	3.39	**<0.001**
***APOE* ε4+**	-0.04	0.03	-1.18	0.24	***APOE* ε4+**	-0.03	0.03	-0.94	0.35
PH of AD	-0.01	0.03	-0.40	0.69	**PH of AD**	-0.01	0.03	-0.30	0.76
$Age_{(klotho)} - Age_{(bio)}$	0.003	0.004	0.72	0.47	**$Age_{(klotho)} - Age_{(bio)}$**	0.002	0.004	0.63	0.53

Abbreviations: $Age_{(bio)}$ = age at biomarker collection; $Age_{(klotho)}$ = age at serum klotho collection; *APOE* ε4+ = carrier of at least one *APOE* ε4 allele; A+ = positive for β-amyloid by CSF or PET; PH of AD = parental history of AD (at least one parent with AD diagnosis); S.E. = standard error; t = t-test statistic; T+ = positive for tau by CSF or PET.

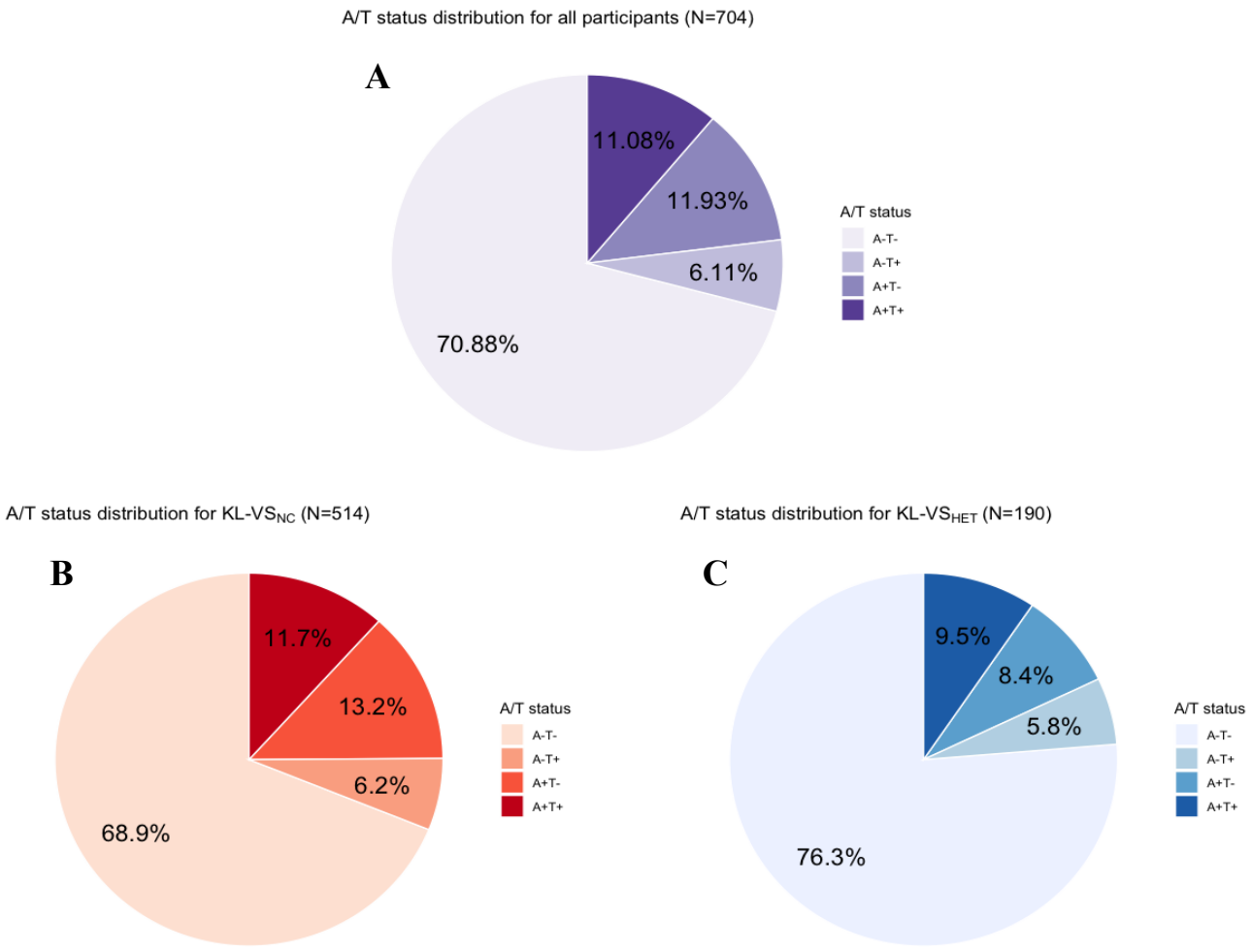
Table 5. Serum klotho levels based on A/T status.

Coefficients		Estimate	S.E.	t	*P*
Intercept	A-T-	6.82	0.15	44.15	**<0.001**
	A+T-	0.02	0.04	0.38	0.70
	A-T+	-0.05	0.06	-0.82	0.42
	A+T+	0.03	0.05	0.70	0.48
Female		0.10	0.03	3.36	**<0.001**
Age$_{(klotho)}$		-0.002	0.002	-0.66	0.51
***APOE* ε4+**		-0.04	0.03	-1.16	0.25
PH of AD		-0.01	0.03	-0.36	0.72
Age$_{(klotho)}$ – Age$_{(bio)}$		0.003	0.004	0.70	0.48

Abbreviations: Age$_{(bio)}$ = age at biomarker collection; Age$_{(klotho)}$ = age at serum klotho collection; *APOE* ε4+ = Carrier of at least one *APOE* ε4 allele; A-T- = amyloid-β negative and tau negative; A+T- = amyloid-β positive and tau negative; A-T+ = amyloid-β negative and tau positive; A+T+ = amyloid-β positive and tau positive; PH of AD = parental history of AD (at least one parent with AD diagnosis); S.E. = standard error; t = t-statistic.

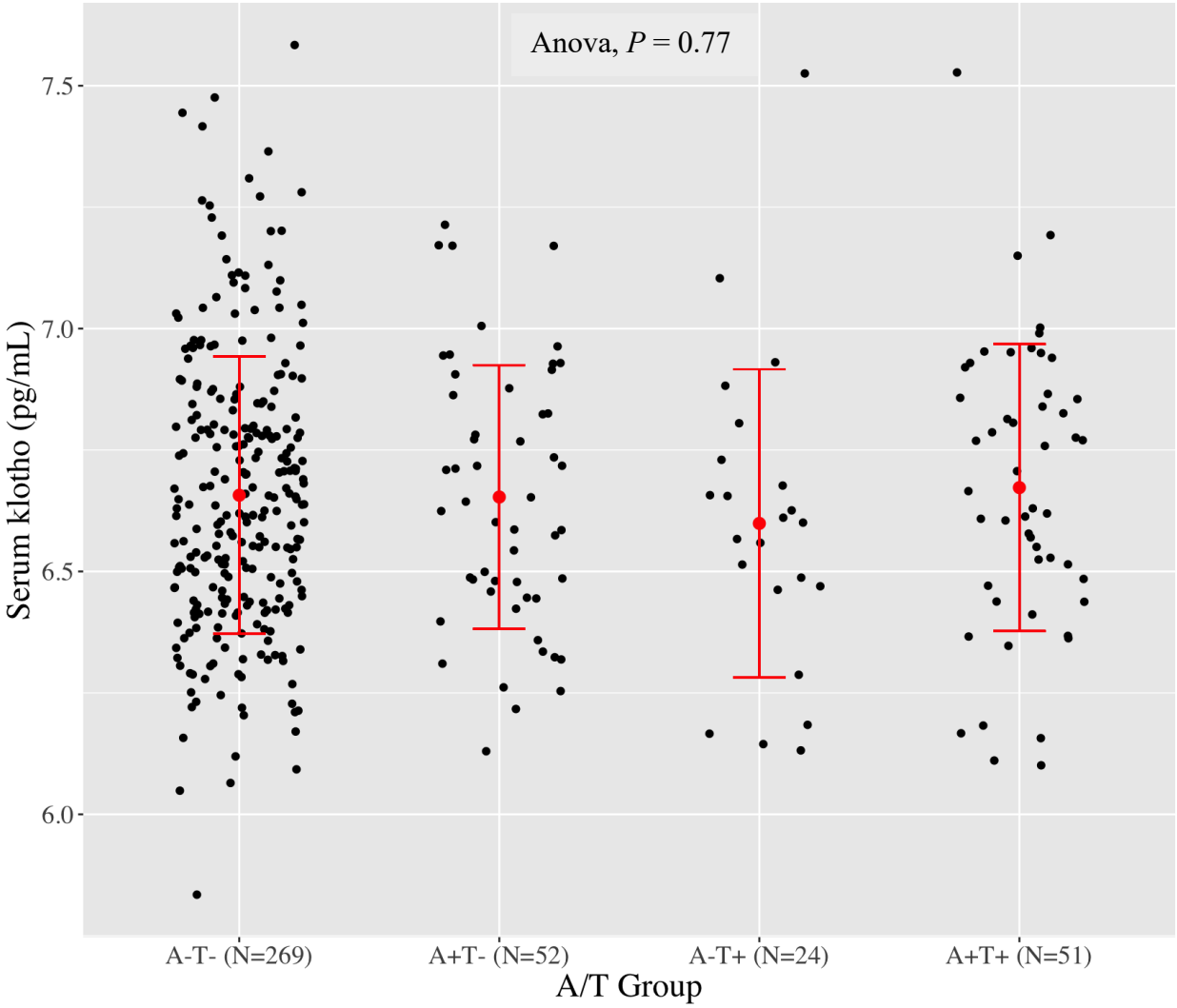
Figures

Figure 1A-C. Visual representation of A/T distribution for A) the entire sample, B) KL-VS$_{NC}$ and C) KL-VS$_{HET}$.



Abbreviations: A-T- = amyloid-β negative and tau negative; A+T- = amyloid-β positive and tau negative; A-T+ = amyloid-β negative and tau positive; A+T+ = amyloid-β positive and tau positive; KL-VS$_{HET}$ = *KLOTHO* KL-VS heterozygote; KL-VS$_{NC}$ = *KLOTHO* KL-VS non-carrier.

Figure 2. Serum klotho levels do not significantly differ across A/T status.



*Serum klotho values were log-transformed.
Abbreviations: A-T- = amyloid-β negative and tau negative; A+T- = amyloid-β positive and tau negative; A-T+ = amyloid-β negative and tau positive; A+T+ = amyloid-β positive and tau positive; pg/mL = picogram per milliliter.