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Negative results

Klotho allele status is not associated with Aβ and *APOE* ε4–related cognitive decline in preclinical Alzheimer's disease

Tenielle Porter [a,b], Samantha C. Burnham [c,d], Lidija Milicic [a,b], Greg Savage [e], Paul Maruff [f,g], Yen Ying Lim [f], David Ames [h,i], Colin L. Masters [f], Ralph N. Martins [d], Stephanie Rainey-Smith [d], Christopher C. Rowe [j,k], Olivier Salvado [l], David Groth [m], Giuseppe Verdile [d,m], Victor L. Villemagne [f,j,k], Simon M. Laws [a,b,m,*]

[a] *Collaborative Genomics Group, Centre of Excellence for Alzheimer's Disease Research and Care, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia*
[b] *Cooperative Research Centre for Mental Health, Carlton South, Victoria, Australia*
[c] *CSIRO Health and Biosecurity, Parkville, Victoria, Australia*
[d] *Centre of Excellence for Alzheimer's Disease Research and Care, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia*
[e] *Department of Psychology, ARC Centre of Excellence in Cognition and its Disorders, Macquarie University, North Ryde, NSW, Australia*
[f] *The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia*
[g] *CogState Ltd., Melbourne, Victoria, Australia*
[h] *Academic Unit for Psychiatry of Old Age, St. Vincent's Health, The University of Melbourne, Kew, Victoria, Australia*
[i] *National Ageing Research Institute, Parkville, Victoria, Australia*
[j] *Department of Nuclear Medicine and Centre for PET, Austin Health, Heidelberg, Victoria, Australia*
[k] *Department of Medicine, Austin Health, The University of Melbourne, Heidelberg, Victoria, Australia*
[l] *CSIRO Health and Biosecurity/Australian e-Health Research Centre, Herston, Queensland, Australia*
[m] *School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia*

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ABSTRACT

The longevity gene Klotho (*KL*), specifically the functional *KL*-VS variant, has previously been associated with cognition and rates of cognitive decline. This study aimed to determine whether *KL*-VS associations with cognition were observable in preclinical Alzheimer's disease (AD). The study also aimed to determine whether there was a combined influence of *KL*-VS, neocortical amyloid-β (Aβ) burden, and carriage of the apolipoprotein E (*APOE*) ε4 allele on cognitive decline. This study involved 581 Aβ-imaged, cognitively normal older adults, enrolled in the Australian Imaging, Biomarkers and Lifestyle Study of Aging. Linear mixed effects models revealed no significant associations between *KL*-VS and cognitive decline independently or in combination with Aβ burden and *APOE* ε4 genotype. Overall, previous associations reported between *KL*-VS and cognitive decline are not observed at the preclinical stages of AD. Furthermore, the results do not support the hypothesis that *KL*-VS has a modifying effect on Aβ burden and *APOE* ε4–driven cognitive decline in preclinical AD.

1. Introduction

Klotho is a transmembrane protein most notably associated with aging-like phenotypes. Initial studies in mice reported that reduced klotho expression resulted in the observation of a number of phenotypes similar to those seen in human aging (Kuro-o et al., 1997). A number of studies (see Supplementary Information, Section S1) have validated these results in animal models and in humans. In the latter, protein levels in serum (Xiao et al., 2004) and cerebrospinal fluid (CSF) (Semba et al., 2014) decline with age and levels in plasma have been reported to be associated with reduced connectivity in brain regions impacted in aging and Alzheimer's disease (AD) (Yokoyama et al., 2017). Furthermore, decreased levels of CSF klotho have also been reported in AD (Semba et al., 2014).

A functional variant in the klotho (*KL*) gene, being the *KL*-VS variant (henceforth KL^{VS}), has been shown to be associated with the levels of secreted klotho (Arking et al., 2002). KL^{VS} heterozygosity

* Corresponding author at: Collaborative Genomics Group, School of Medical and Health Sciences, Edith Cowan University, 270 Joondalup Drive, Joondalup 6027, Western Australia, Australia. Tel.: +61 8 6304 5128; fax: +61 8 6304 2493.
E-mail address: s.laws@ecu.edu.au (S.M. Laws).
URL: http://www.mentalhealthcrc.com

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(KL^{VS+}) is associated with high levels of klotho in serum (Dubal et al., 2014; Yokoyama et al., 2017), improved longevity (Arking et al., 2002; Di Bona et al., 2014), and healthy aging (Di Bona et al., 2014) when compared to noncarriers (KL^{VS-}). Carriage of KL^{VS+} has been reported to be associated with higher levels of cognitive performance in young adults (Morar et al., 2017) and the elderly (Dubal et al., 2014), slower cognitive decline (de Vries et al., 2017; Deary et al., 2005), and increased brain volume in the frontal lobe (de Vries et al., 2017), in particular, the prefrontal cortex (Yokoyama et al., 2015). Although findings have been inconsistent (see Supplementary Information, Section S1), it should be noted that these associations are not observed in a gene dosage–dependent manner, with homozygosity (KL^{VS++}) associated with reduced life expectancy and a negative influence on aging phenotypes (Arking et al., 2002, 2005).

Variations in klotho expression and sequence are of particular interest in AD, as aging is considered the greatest risk factor for the development of the disease. AD-associated cognitive decline can be observed in the asymptomatic stages of the disease, particularly those at greatest risk of AD, that is, those with early pathological brain changes (Lim et al., 2016) such as the accumulation of neocortical amyloid-β (Aβ), a feature included in the National Institute on Aging-Alzheimer's Association recommended criteria for preclinical AD (Sperling et al., 2011). Furthermore, cognitive decline at this preclinical AD stage can be genetically influenced, for example, by the ε4 allele of the apolipoprotein E (*APOE)* gene, the Val66Met (rs6265) variant in brain-derived neurotrophin factor *(BDNF)* gene and variation in the kidney and brain expressed protein *(KIBRA)* gene (Lim et al., 2015a,b; Porter et al., 2018). Considering the results from previous studies and biological evidence for associations between KL^{VS} and cognitive performance, it was hypothesized that in cognitively normal (CN) older adults, KL^{VS+} would be more resilient to cognitive decline when compared to KL^{VS-}. As such, it was aimed to determine whether KL^{VS} influences measures of cognitive decline in CN older adults through analysis of 7.5 years of longitudinal cognitive and neuroimaging assessment data from the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study of Ageing. Furthermore, we sought to investigate the interactional effects of KL^{VS} and factors known to modify preclinical AD–related cognitive decline, specifically Aβ burden and *APOE* ε4 status.

2. Material and methods

This study was undertaken in the AIBL Study of Aging focusing on a CN subset (n = 581) of brain-imaged participants (Ellis et al., 2009). Participants underwent Aβ imaging and cognitive testing, methodology for which is described in detail in Supplementary Material. Positron emission tomography standardized uptake value ratios were calculated for all participants, with these then classified as low ($A\beta^{low}$) or high ($A\beta^{high}$) based on tracer-specific thresholds of ≥1.4, ≥1.05, and ≥0.55 for PiB, florbetapir, and flutemetamol, respectively (Rowe et al., 2013). Three cognitive composite scores such as global cognition, verbal episodic memory (Burnham et al., 2015), and pre-Alzheimer's cognitive composite (Donohue et al., 2014), were calculated as described in Supplementary Material, with 18-month follow-up data over 7.5 years utilized. For this study, *APOE* carrier status was defined as the presence (*APOE* ε4+ve) or absence (*APOE* ε4-ve) of the *APOE* ε4 allele. Multiple *KL* single nucleotide polymorphisms were genotyped to confirm results and ensure single nucleotide polymorphisms were in 100% linkage disequilibrium. KL^{VS} homozygotes (KL^{VS++}) were not included in this study (AIBL frequency, 3.9%) with all analyses performed comparing KL^{VS+} and KL^{VS-}. Linear mixed models were performed in R studio (Rstudio Team, 2015) Version 0.98.1103 for Macintosh (RStudio Team, 2015) as implemented in the "nlme" package.

3. Results

Table 1 reports demographic information for the cohort investigated here, stratified by the KL^{VS} genotype. Depressive symptomology, as measured by the Geriatric Depression Scale, was the only variable significantly different between the 2 groups. However, due to the mean Geriatric Depression Scale values for each group being well below the threshold for clinical relevance, the difference was not further investigated. When investigating rates of cognitive decline between groups stratified by the *KL* genotype (KL^{VS+} n = 152, KL^{VS-} n = 429), no significant differences were observed in any cognitive composite measures (Supplementary Table 1).

Although significant differences between group rates of decline were observed as a result of the interaction between Aβ burden and the KL^{VS} genotype, they reflect differences driven by Aβ burden status with no modification by KL^{VS} (Supplementary Table 2). That is, $A\beta^{low}/KL^{VS+}$ significantly differed from $A\beta^{high}/KL^{VS-}$ but $A\beta^{high}/KL^{VS+}$ did not differ significantly from $A\beta^{high}/KL^{VS-}$ (Supplementary Table 2). This pattern of results was observed for all cognitive composites.

Finally, no significant modification of the effects of *APOE* ε4 status on cognition was observed as a result of KL^{VS}. This was observed in both the $A\beta^{low}$ and $A\beta^{high}$ samples. All composites recorded no significant differences between any group's rates of decline in the $A\beta^{low}$ sample (n = 331; Table 2). In addition, in the $A\beta^{high}$ sample (n = 250), significant differences in rates of decline

Table 1
Demographic information

	Overall n = 581	KL^{VS+} n = 152	KL^{VS-} n = 429	*p*
Age [years], mean (SD)	70.87 (6.45)	70.76 (6.81)	70.93 (6.26)	0.772
Female, n (%)	321 (55.25)	84 (55.26)	237 (55.24)	0.999
Years of Education, n (%)				0.82
0-8	46 (7.94)	12 (7.95)	34 (7.94)	
9-12	211 (36.44)	53 (35.10)	158 (36.92)	
13-15	121 (20.90)	29 (19.21)	92 (21.50)	
15+	201 (34.72)	57 (37.75)	144 (33.64)	
Premorbid IQ [FSIQ], mean (SD)	108.00 (7.24)	108.26 (6.83)	107.90 (7.37)	0.604
Depressive Symptoms [GDS], mean (SD)	1.06 (1.27)	1.27 (1.45)	0.96 (1.18)	0.028
APOE ε4 carriage, n (%)	160 (27.54)	49 (32.24)	111 (25.87)	0.161
High amyloid-β burden, n (%)	250 (43.03)	60 (39.47)	190 (44.29)	0.350

Baseline demographic and clinical characteristics of all imaged cognitively normal adults in the AIBL study and stratified by KL^{VS} genotype (KL^{VS+} and KL^{VS-}). *p*-values represent statistical significance when comparing the KL^{VS} genotype.
Key: *APOE*, apolipoprotein E; GDS, Geriatric Depression Scale; FSIQ, Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) Full Scale Intelligence Quotient; SD, standard deviation.

Table 2
Mean slopes for cognitive composites in cognitively normal adults stratified by amyloid-β status and *APOE* ε4 interaction

	APOE ε4-ve KL^{VS+}	*APOE* ε4-ve KL^{VS-}	*APOE* ε4+ve KL^{VS+}	*APOE* ε4+ve KL^{VS-}
	β	β	β	β
$A\beta^{low}$	n = 71	n = 205	N = 21	n = 34
Global	0.055	0.047	0.011	0.057
Verbal episodic memory	0.066	0.046	0.008	0.056
PACC	0.030	0.033	−0.043	0.111
$A\beta^{high}$	n = 32	n = 113	N = 28	n = 77
Global	0.001	0.032	−0.033	−0.085[a,b]
Verbal episodic memory	−0.017	0.042	−0.025	−0.070[b]
PACC	0.063	0.124	−0.026	−0.222[a,b]

Mean slopes for cognitive composites (presented in SD/year) in cognitively normal participants with low amyloid-βburden ($A\beta^{low}$; n = 331) or high amyloid-β burden ($A\beta^{high}$; n = 250).
Key: *APOE*, apolipoprotein E; PACC, pre-Alzheimer's cognitive composite; KL^{VS+}, KL^{VS} heterozygosity; KL^{VS-}, noncarriers.
[a] $p < 0.05$ when comparing to the *APOE* ε4-ve KL^{VS+} group.
[b] $p < 0.05$ when comparing to the *APOE* ε4-ve KL^{VS-} group, φ $p < 0.05$ when comparing to the *APOE* ε4+ve KL^{VS+} group.

were only observed between the *APOE* ε4+ve/KL^{VS-}group and the 2 *APOE* ε4-ve groups.

4. Discussion

The results reported here suggest that KL^{VS} has no influence on cognitive decline in preclinical AD in the AIBL Study of Ageing. These findings fail to support the hypothesis of this study being that KL^{VS+} would confer increased resilience to cognitive decline as compared to KL^{VS-}. Independent of Aβ and *APOE* ε4 status, KL^{VS} was not associated with longitudinal measures of global cognition, the pre-Alzheimer's cognitive composite, or verbal episodic memory. In addition, KL^{VS} did not influence cognitive decline driven by high Aβ burden and modified by *APOE* ε4 carrier status.

Previous animal (Dubal et al., 2014, 2015; Laszczyk et al., 2017; Leon et al., 2017; Nagai et al., 2003) and human (de Vries et al., 2017; Deary et al., 2005; Dubal et al., 2014; Morar et al., 2017) studies have identified an association between klotho levels or KL^{VS+} and increased levels or improvements in cognitive function. In particular, in mouse models of AD, increasing klotho expression reduced cognitive decline (Dubal et al., 2015), whereas klotho deficiency further impaired cognition (Nagai et al., 2003). These results could be due to an effect of klotho on Aβ toxicity. In primary neuronal cultures of mice and rats, those overexpressing klotho have been reported to be more resistant to Aβ toxicity when compared to the wild type (Masso et al., 2015). Furthermore, a study in mice reported klotho's influence on cognition could be in part due to its regulation of hippocampal synaptic plasticity (Li et al., 2017).

Despite the in vivo evidence of the protective effects of klotho in mouse models, no such benefits are evident in human cohort studies, where either no significant effect of KL^{VS} or an actual detrimental effect of KL^{VS+} has been reported (Mengel-From et al., 2016; Morar et al., 2017; Yokoyama et al., 2015). Furthermore, it has been previously reported that *APOE* and KL^{VS} independently influence cognitive decline. De Vries et al. reported independent effects of both *APOE* and KL^{VS} on cognition; however, these effects were lost after combining the genes (de Vries et al., 2017).

It has been suggested previously that the cognitive advantage observed as a result of carriage of KL^{VS+} may only be present in very late life (de Vries et al., 2017). The average ages of the participants investigated in the present study, ∼70 years of age (yoa), however, are in line with the ages observed in studies where significant KL^{VS+} associations with cognition have been reported [58-85 yoa (Dubal et al., 2014), ∼70 yoa (de Vries et al., 2017), ∼79 yoa (Deary et al., 2005)]. Furthermore, in AD, significant decline in cognitive performance occurs with age. The lack of significant associations reported in this study could be due to an overwhelming effect of preclinical AD pathology on cognition during the ages at which the influence of klotho on cognition is most pronounced. With respect to statistical power, the present study was similarly powered to previous studies reporting on cognition. The largest of these from Dubal et al (2014) reported a cross-sectional association between VS carriage and global cognition in their study that had ∼89% power to see their effect size of 0.3. The present study observed no cross-sectional differences in cognition (data not shown) although had ∼85% power to detect a similar 0.3 effect size.

The study reported herein utilized a well-characterized longitudinal cohort of preclinical AD to explore the independent and interactional influence of KL^{VS} on measures of cognitive decline. However, there are acknowledged limitations of the present study. Due to the absence of tau imaging or CSF data, this study defined preclinical AD based on National Institute on Aging-Alzheimer's Association-recommended criteria (Sperling et al., 2011) and not updated criteria that include amyloidosis, tau, and neurodegeneration. Participation in the AIBL Study is on a voluntary basis rather than through random participant selection. This may result in higher levels of education than typically observed in a community-based study. This high level of education, particularly in the CN subset of the cohort, investigated in this study, could reduce the ability to discern subtle influences of KL^{VS} on cognition and cognitive decline mediated by an elevated Aβ and *APOE* ε4 carriage.

The study reported here is the first to investigate the combinatory effect of KL^{VS}, Aβ, and *APOE* ε4 on preclinical AD–related cognitive decline. The results presented do not support the previous findings of associations between KL^{VS} and cognitive decline, in particular, decline in global cognition and verbal episodic memory. Furthermore, the results imply no interactional influence of KL^{VS}, Aβ, and *APOE* ε4 on longitudinal cognitive measures.

Disclosure

CLM is an advisor to Prana Biotechnology Ltd and a consultant to Eli Lilly. PM is a full-time employee of Cogstate Ltd. DA has served on scientific advisory boards for Novartis, Eli Lilly, Janssen, and Pfizer Inc. RNM is a consultant to Alzhyme. CCR has served on scientific advisory boards for Bayer Pharma, Elan Corporation, GE Healthcare and AstraZeneca, has received speaker honoraria from Bayer Pharma and GE Healthcare, and has received research support from Bayer Pharma, GE Healthcare, Piramal Lifesciences and Avid Radiopharmaceuticals. VLV served as a consultant for Bayer Pharma and received research support from a NEDO grant from Japan. All other authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.12.014.

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