

KLOTHO KL-VS and risk for dementia and cognitive impairment in postmenopausal women: The Women’s Health Initiative Memory Study

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HIGHLIGHTS

- No genotype-related differences in risk for incidence of cognitive impairment (MCI or dementia) in this cohort of older, post-menopausal women.
- The associations between KL-VS genotype and risk for MCI or dementia, while unaffected by HT assignment, were modified by *APOE* status.
- Our findings of better cognitive outcomes for KL-VS heterozygotes add support to the existing, albeit nascent, literature.
- We also report worse cognitive outcomes for homozygotes, which have largely been excluded from investigation to date because the genotype is infrequent.

INTRODUCTION

Klotho, a potent anti-aging protein, acts as a circulating hormone:

- extends lifespan, protects against neurodegeneration, and improves cardiovascular health
- strongly correlates with better health and better cognitive function¹⁻⁸

KLOTHO KL-VS heterozygotes (HET) have higher concentrations of circulating Klotho:

- longevity and resilience against late-life disorders^{1,2}
- protection against accumulation of core Alzheimer’s disease (AD) pathology (amyloid-β and tau)⁹⁻¹¹
- better cognition^{3,9,10}

The literature on KL-VS and cognition is nascent and equivocal. It remains largely unknown whether HET benefits extend to dementia.

AIMS

We investigated whether:

- 1) the risk for incident cognitive impairment [mild cognitive impairment (MCI), probable dementia (DEM), or MCI+DEM], and
- 2) the rate of decline in global or domain-specific cognition over follow-up (median 16.2 years)
- differ by genotype (HET; non-carriers=NC; homozygotes=HOM) in a sample of older, postmenopausal women.

METHODS

Participants

- The sample consisted of 3110 postmenopausal women from the Women’s Health Initiative Memory Study (WHIMS), ages 63-81 [M(SD) = 69.78 (3.71)] at enrollment.
- The median follow-up was 16.2 years.
- 1239 (~40%) women converted to MCI or DEM during follow-up.

Table 1. Sample Characteristics.

	by COGNITIVE STATUS				by GENOTYPE			
	CU (N = 1871)	MCI (N =520)	DEM (N =719)	P	HOM (N=82)	NC (N=2238)	HET (N=790)	P
¹ Age (years): M±SD	69.1±3.3	70.5±0±3.8	70.9±3.8	<0.001	69.4±3.4	69.8±3.7	69.7±3.7	0.47
Education: N (%)				<0.001				0.72
• High school or less	485 (25.9)	180 (34.6)	203 (28.2)		22 (26.3)	628 (28.1)	218 (27.6)	
• Some college	727 (38.9)	206 (39.6)	254 (35.3)		29 (35.4)	866 (38.7)	292 (37.0)	
• College graduate	653 (34.9)	133 (25.6)	259 (36.0)		31 (37.8)	736 (32.9)	278 (35.2)	
• Missing	6 (0.3)	1 (0.2)	3 (0.4)		0 (0)	8 (0.4)	2 (0.3)	
Race/Ethnicity: N (%)				<0.001				0.34
• African American	124 (6.6)	68 (13.1)	59 (8.2)		6 (7.3)	175 (7.8)	70 (8.9)	
• Hispanic	35 (1.9)	14 (2.7)	17 (2.4)		1 (1.2)	55 (2.5)	10 (1.3)	
• Non-Hispanic white	1693 (90.5)	433 (83.3)	641 (89.2)		73 (89.0)	1989 (88.9)	705 (89.2)	
• Other/unknown	19 (1.0)	5 (1.0)	2 (0.3)		2 (2.4)	19 (0.9)	5 (0.6)	
HT Assignment: N (%)				<0.001				0.70
• E-alone intervention	321 (17.2)	112 (21.5)	139 (19.3)		16 (19.5)	424 (19.0)	129 (16.3)	
• E-alone control	357 (19.1)	125 (24.0)	129 (17.9)		13 (15.9)	434 (19.4)	164 (20.8)	
• E+P intervention	579 (30.9)	133 (25.6)	244 (33.9)		25 (30.5)	689 (30.8)	245 (31.0)	
• E+P control	614 (32.8)	159 (28.9)	207 (28.8)		28 (34.2)	691 (30.9)	252 (31.9)	
BMI (kg/m ²): M±SD	28.8±5.7	28.5±5.5	28.2±5.3	0.11	28.2±5.5	28.6±5.6	28.6±5.6	0.71
Diabetes: N (%)	113 (6.0)	52 (10.0)	54 (7.5)	0.006	4 (4.9)	166 (7.4)	49 (6.2)	0.38
Hypertension: N (%)	646 (34.5)	207 (39.8)	255 (35.5)	0.05	30 (36.6)	767 (34.3)	311 (39.4)	0.03
Stroke: N (%)	14 (0.8)	10 (1.9)	11 (1.5)	0.04	1 (1.2)	22 (0.1)	12 (1.5)	0.47
MI: N (%)	43 (2.3)	21 (4.0)	19 (2.6)	0.09	1 (1.2)	66 (3.0)	16 (2.0)	0.27
Coronary Revascularization, N(%)	35 (1.9)	16 (3.1)	16 (2.2)	0.28	2 (2.4)	50 (2.2)	15 (1.9)	0.83
Smoking History, N (%)				0.03				0.09
• Current	108 (5.8)	19 (3.7)	27 (3.8)		6 (7.3)	109 (4.9)	39 (4.9)	
• Former	732 (39.1)	214 (41.2)	259 (36.0)		36 (43.9)	838 (37.4)	333 (42.2)	
• Never	1014 (54.3)	279 (53.7)	421 (58.6)		38 (46.3)	1263 (56.6)	413 (52.3)	
• Missing	17 (0.9)	8 (1.5)	12 (1.7)		2 (2.4)	30 (1.3)	5 (0.6)	
Alcohol Intake, N (%)				0.001				0.33
• Never	210 (11.2)	66 (12.7)	104 (14.5)		4 (4.9)	290 (13.0)	86 (10.9)	
• Past	325 (17.4)	110 (21.2)	142 (19.8)		15 (18.3)	421 (18.8)	141 (17.9)	
• 0 drinks/week	628 (33.6)	190 (36.5)	217 (30.2)		29 (35.4)	739 (33.0)	267 (33.8)	
• 1 to <7 drinks/week	448 (23.7)	105 (20.2)	176 (24.5)		25 (28.1)	316 (23.1)	186 (23.5)	
• ≥ 7 drinks/week	360 (19.3)	46 (8.9)	70 (9.7)		11 (13.4)	251 (11.2)	104 (13.2)	
• Missing	14 (0.8)	3 (0.6)	10 (1.4)		0 (0)	21 (0.9)	6 (0.8)	
¹ <i>APOE</i> 4+: N (%)				<0.001				0.52
• ε4+	339 (18.8)	130 (26.3)	229 (33.2)		16 (19.5)	544 (24.3)	194 (24.6)	
• ε4-	1446 (80.3)	351 (70.9)	434 (62.9)		64 (78.1)	1611 (72.0)	556 (70.4)	
• Missing	71 (3.8)	25 (4.8)	29 (4.0)		2 (2.4)	83 (3.7)	40 (5.1)	
<i>KLOTHO</i> : N (%)				0.63				
• NC	1332 (71.2)	383 (73.7)	523 (72.7)					
• HET	492 (26.3)	121 (23.3)	177 (24.6)					
• HOM	47 (2.5)	16 (3.1)	19 (2.6)					

Abbreviations: *APOE*4+ = carrier of at least one copy of the *APOE* ε4 allele; BMI = body mass index; CI = confidence interval; DEM = probable dementia; E-alone = estrogen alone intervention; E+P = estrogen + progestin intervention; HT = hormone therapy; HET = *KLOTHO* KL-VS heterozygote; HOM = *KLOTHO* KL-VS homozygote; HR = hazard ratio; M = mean; MCI = mild cognitive impairment; MI = myocardial infarction; NC = *KLOTHO* KL-VS non-carrier; SD = standard deviation.

Statistical Analyses. Survival analyses examined genotype differences in risk for incident cognitive impairment. Analyses were repeated after stratifying by hormone therapy (HT) (intervention vs. placebo) assignment or by *APOE*4+ status (ε4 allele carriers). We also examined genotype differences in change in global and domain-specific cognition from baseline to the most recent assessment and respective rates of age-related decline.

RESULTS

Table 2. The relationship between risk for DEM, MCI or MCI+DEM incidence and *KLOTHO* KL-VS genotype is modified by *APOE*4 status (*APOE*4 +/- status * KL-VS genotype).

	<i>KLOTHO</i> KL-VS	<i>APOE</i> 4 Status	Association Measure	P
DEM	NC	<i>APOE</i> 4 (0)	-----	
	HET	<i>APOE</i> 4 (1)	0.30 (0.20)	0.13
	HOM	<i>APOE</i> 4 (1)	0.004 (0.64)	0.99
	HET	<i>APOE</i> 4 (2)	1.30 (0.52)	0.01
MCI	HOM	<i>APOE</i> 4 (2)	1.68 (1.07)	0.17
	NC	<i>APOE</i> 4 (0)	-----	
	HET	<i>APOE</i> 4 (1)	-0.24 (0.25)	0.34
	HOM	<i>APOE</i> 4 (1)	0.82 (0.67)	0.22
MCI or DEM	HET	<i>APOE</i> 4 (2)	1.64 (0.79)	0.04
	HOM	<i>APOE</i> 4 (2)	2.67 (1.11)	0.02
	NC	<i>APOE</i> 4 (0)	-----	
	HET	<i>APOE</i> 4 (1)	-0.23 (0.15)	0.13
	HOM	<i>APOE</i> 4 (1)	0.53 (0.46)	0.25
	HET	<i>APOE</i> 4 (2)	1.36 (0.43)	0.002
	HOM	<i>APOE</i> 4 (2)	2.03 (0.76)	0.008

Notes: Bolded *P*-values significant at P < 0.05; ----- = reference group.
Abbreviations: *APOE* 4 (0) = carrier of 0 copies of *APOE* ε4 allele; (1) = carrier of a single copy of *APOE* ε4 allele; (2) = carrier of both copies of *APOE* ε4 allele; DEM = probable dementia; HET = *KLOTHO* KL-VS heterozygote; HOM = *KLOTHO* KL-VS homozygote; MCI = mild cognitive impairment; NC = *KLOTHO* KL-VS non-carrier.

Table 3. Rates of age-related decline in global and domain-specific cognition by *KLOTHO* KL-VS.

	<i>KLOTHO</i> KL-VS	Association Measure	95% CI	P
Global Cognition	NC	-----		
	HET	-0.15 (1.10)	-2.32, 2.01	0.89
	HOM	2.95 (1.36)	-0.28, 5.62	0.03
	HOM	2.95 (1.36)	-0.28, 5.62	0.03
Verbal Knowledge: PMA Vocabulary	NC	-----		
	HET	0.12 (0.29)	-0.45, 0.69	0.67
	HOM	0.27 (0.72)	-1.14, 1.68	0.71
	HOM	0.27 (0.72)	-1.14, 1.68	0.71
Letter Fluency (F, A, S)	NC	-----		
	HET	-0.15 (0.48)	-1.09, 0.80	0.76
	HOM	1.87 (1.39)	-0.86, 4.60	0.18
	HOM	1.87 (1.39)	-0.86, 4.60	0.18
Semantic Fluency (vegetables, fruits)	NC	-----		
	HET	-0.27 (0.27)	-0.81, 0.26	0.31
	HOM	-0.76 (0.62)	-1.97, 0.46	0.22
	HOM	-0.76 (0.62)	-1.97, 0.46	0.22
Figural Memory: BVRT	NC	-----		
	HET	0.12 (0.14)	-0.16, 0.40	0.41
	HOM	0.0009 (0.33)	-0.65, 0.65	0.99
	HOM	0.0009 (0.33)	-0.65, 0.65	0.99
Verbal Memory: CVLT	NC	-----		
	HET	-0.71 (0.29)	-1.27, -0.15	0.01
	HOM	-0.48 (0.71)	-1.86, 0.91	0.50
	HOM	-0.48 (0.71)	-1.86, 0.91	0.50
Digits Forward	NC	-----		
	HET	-0.04 (0.09)	-0.21, 0.13	0.64
	HOM	0.05 (0.32)	-0.58, 0.67	0.89
	HOM	0.05 (0.32)	-0.58, 0.67	0.89
Digits Backward	NC	-----		
	HET	0.04 (0.08)	-0.12, 0.20	0.64
	HOM	0.07 (0.26)	-0.45, 0.58	0.79
	HOM	0.07 (0.26)	-0.45, 0.58	0.79
Spatial Rotational Ability: Card Rotations	NC	-----		
	HET	-1.42 (1.07)	-3.52, 0.68	0.18
	HOM	-4.47 (2.22)	-8.82, -0.12	0.04
	HOM	-4.47 (2.22)	-8.82, -0.12	0.04

Notes: bolded *P*-values significant at P < 0.05; ----- = reference group.
Abbreviations: BVRT = Benton Visual Retention Test; CI = confidence interval; CVLT = California Verbal Learning Test; HET = *KLOTHO* KL-VS heterozygote; HOM = *KLOTHO* KL-VS homozygote or non-carrier; HR = hazard ratio; HT = hormone therapy; PMA = Primary Mental Abilities.

CONCLUSIONS

•We found no significant *KLOTHO* KL-VS genotype differences in risk for cognitive impairment in a nationwide, geographically diverse sample of older, postmenopausal women.

•Also, no significant genotype-related differences were observed for change in global cognition over ~16 years of follow-up.

•HOM had a significantly steeper rate of global cognitive decline compared to NC.

•Domain-specific cognitive performance significantly differed by genotype with respect to both change from baseline to last available assessment and associated rates of age-related decline.

•Spatial rotational ability declined at a significantly faster rate in HOM than NC.

•Verbal memory decline was significantly slower in HET compared to NC.

Together, our observations provide novel insights into genetic risks for pathological age-related cognitive decline in females.

- Additional research is needed on new genetic variants that modify risk and may bring to light novel molecular targets for future therapeutic trials, potentially identifying complementary pathways for curbing disease progression and delaying symptom onset.

REFERENCES

1. Arking DE, Krebsova A, Macek M, Sr., et al. Association of human aging with a functional variant of klotho. Proc Natl Acad Sci U S A 2002;99:856-861.
2. 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.
3. Dubal DB, Yokoyama JS, Zhu L, et al. Life extension factor klotho enhances cognition. Cell Rep 2014;7:1065-1076.
4. Cheikhi A, Barchowsky A, Sahu A, et al. Klotho: An Elephant in Aging Research. J Gerontol A Biol Sci Med Sci 2019;74:1031-104219.
5. 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.
6. Montesanto A, Dato S, Bellizzi D, Rose G, Passarino G. Epidemiological, genetic and epigenetic aspects of the research on healthy ageing and longevity. Immun Ageing 2012;9:6.
7. 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.
8. Vo HT, Laszcyk AM, King GD. Klotho, the Key to Healthy Brain Aging? Brain Plast 2018;3:183-194.
9. Erickson CM, Schultz SA, Oh JM, et al. KLOTHO heterozygosity attenuates APOE4-related amyloid burden in preclinical AD. Neurology 2019;92:e1878-e1889.
10. Driscoll I, Ma Y, Gallagher CL, et al. Age-Related Tau Burden and Cognitive Deficits Are Attenuated in KLOTHO KL-VS Heterozygotes. J Alzheimers Dis 2021;79:1297-1305.
11. Driscoll I, Ma Y, Lose SR, et al. AD-associated CSF biomolecular changes are attenuated in KL-VS heterozygotes. Alzheimers Dement (Amst) 2022;14:e12383

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ACKNOWLEDGMENTS

Data used in this analysis were obtained from the Women's Health Initiative (WHI) program, sponsored by the National Heart, Lung, and Blood Institute (NHLBI). The authors acknowledge the contributions and dedication of the study participants, and the dedicated staff at the participating WHI Clinical Centers and Coordinating Centers.

