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Association of *Klotho*-VS Heterozygosity With Risk of Alzheimer Disease in Individuals Who Carry *APOE4*

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Editorial

Supplemental content

IMPORTANCE Identification of genetic factors that interact with the apolipoprotein e4 (*APOE4*) allele to reduce risk for Alzheimer disease (AD) would accelerate the search for new AD drug targets. *Klotho*-VS heterozygosity (*KL*-VS^{HET+} status) protects against aging-associated phenotypes and cognitive decline, but whether it protects individuals who carry *APOE4* from AD remains unclear.

OBJECTIVES To determine if *KL*-VS^{HET+} status is associated with reduced AD risk and β-amyloid (Aβ) pathology in individuals who carry *APOE4*.

DESIGN, SETTING, AND PARTICIPANTS This study combined 25 independent case-control, family-based, and longitudinal AD cohorts that recruited referred and volunteer participants and made data available through public repositories. Analyses were stratified by *APOE4* status. Three cohorts were used to evaluate conversion risk, 1 provided longitudinal measures of Aβ CSF and PET, and 3 provided cross-sectional measures of Aβ CSF. Genetic data were available from high-density single-nucleotide variant microarrays. All data were collected between September 2015 and September 2019 and analyzed between April 2019 and December 2019.

MAIN OUTCOMES AND MEASURES The risk of AD was evaluated through logistic regression analyses under a case-control design. The risk of conversion to mild cognitive impairment (MCI) or AD was evaluated through competing risks regression. Associations with Aβ, measured from cerebrospinal fluid (CSF) or brain positron emission tomography (PET), were evaluated using linear regression and mixed-effects modeling.

RESULTS Of 36 530 eligible participants, 13 782 were excluded for analysis exclusion criteria or refusal to participate. Participants were men and women aged 60 years and older who were non-Hispanic and of Northwestern European ancestry and had been diagnosed as being cognitively normal or having MCI or AD. The sample included 20 928 participants in case-control studies, 3008 in conversion studies, 556 in Aβ CSF regression analyses, and 251 in PET regression analyses. The genotype *KL*-VS^{HET+} was associated with reduced risk for AD in individuals carrying *APOE4* who were 60 years or older (odds ratio, 0.75 [95% CI, 0.67-0.84]; $P = 7.4 \times 10^{-7}$), and this was more prominent at ages 60 to 80 years (odds ratio, 0.69 [95% CI, 0.61-0.79]; $P = 3.6 \times 10^{-8}$). Additionally, control participants carrying *APOE4* with *KL*-VS heterozygosity were at reduced risk of converting to MCI or AD (hazard ratio, 0.64 [95% CI, 0.44-0.94]; *P* = .02). Finally, in control participants who carried *APOE4* and were aged 60 to 80 years, *KL*-VS heterozygosity was associated with higher Aβ in CSF (β, 0.06 [95% CI, 0.01-0.10]; *P* = .03) and lower Aβ on PET scans (β, −0.04 [95% CI, −0.07 to −0.00]; *P* = .04).

CONCLUSIONS AND RELEVANCE The genotype *KL*-VS^{HET+} is associated with reduced AD risk and Aβ burden in individuals who are aged 60 to 80 years, cognitively normal, and carrying *APOE4*. Molecular pathways associated with *KL* merit exploration for novel AD drug targets. The *KL*-VS genotype should be considered in conjunction with the *APOE* genotype to refine AD prediction models used in clinical trial enrichment and personalized genetic counseling.

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Klotho (KL) is a transmembrane protein and longevity factor implicated in reducing aging-associated phenotypes and cognitive decline.[1,2] Two *KL* missense variants (F352V [rs9536314] and C370S [rs9527025]), in perfect linkage disequilibrium, form a functional haplotype known as *KL*-VS. Specifically, heterozygosity for *KL*-VS (*KL*-VS^{HET+} status) has been shown to increase serum levels of KL and exert protective effects on healthy aging and longevity when compared with individuals who are homozygotes for the major or minor alleles (*KL*-VS^{HET-}).[2-5] It currently remains unclear if *KL*-VS^{HET+} status also provides protection against aging-associated neurodegenerative disorders, such as Alzheimer disease (AD).

The apolipoprotein E4 (*APOE4*) allele is the strongest genetic risk factor for late-onset AD.[6] The most established pathogenic effect of *APOE4* is β-amyloid (Aβ) accumulation in the brain, which correlates with decreased Aβ in the cerebrospinal fluid (CSF).[7,8] Brain Aβ accumulation likely represents a central early step in AD pathogenesis[9]; Aβ accumulates before symptom onset in individuals during early old age (60-80 years) before it reaches plateau levels and individuals convert to experiencing mild cognitive impairment (MCI) and/or AD.[10-12] Over this age range, Aβ accumulation and correlated cognitive decline are most prominent in individuals who carry *APOE4*.[13-16] Similarly, during this time, *APOE4* is most strongly associated with AD risk.[17-19] In the search for new AD drug targets, it is thus critical to identify genetic factors that interact with *APOE4* to reduce risk for AD by lowering Aβ burden.[20]

Two recent studies evaluated whether *KL*-VS^{HET+} status confers protection against AD in individuals who were cognitively normal. One cohort study[21] (N = 309; mean age, 61 years) showed that *KL*-VS^{HET+} status reduced Aβ burden in individuals who carry *APOE4*. The second cohort study[22] (N = 581; mean age, 71 years) showed that *KL*-VS^{HET+} did not protect against cognitive decline, and this was not modulated by *APOE4* status. Here, we test on a larger scale and across the age span older than 60 years whether *KL*-VS^{HET+} status is associated with reduced risk for AD and conversion to MCI or AD. We also reevaluate in larger samples the putative protective association of *KL*-VS^{HET+} status with Aβ burden assessed by CSF and positron emission tomography (PET) scanning measures. Similar to the prior studies, we stratified analyses by *APOE4* status to determine if the associations of *KL*-VS with outcome measures are specific to individuals who carry *APOE4*. Because the role of *APOE4* in AD is most pronounced between age 60 to 80 years and genetic risk varies importantly in relatively younger individuals (60-80 years) compared with older individuals (≥80 years),[23] we also tested the hypothesis that the associations of *KL*-VS^{HET+} status with AD risk would differ between those aged 60 to 80 years and those older than 80 years.

Key Points

Question Does *Klotho*-VS heterozygosity protect against Alzheimer disease (AD) in individuals who carry *APOE4*?

Findings In this study, associations were evaluated across 22 AD cohorts (n = 20 928), 3 longitudinal cohorts (n = 3008), and 4 cohorts collecting β-amyloid measurements (cerebrospinal fluid, n = 556; brain, n = 251). In individuals who carry *APOE4*, *Klotho*-VS heterozygosity was associated with reduced AD risk and more favorable β-amyloid profiles in the brain and cerebrospinal fluid of older control participants. *Klotho*-VS heterozygosity was also associated with reduced AD conversion risk in individuals who carry *APOE4*.

Meaning Pathways associated with *KL* merit exploration for novel AD drug targets, and the *KL*-VS genotype should be considered in conjunction with *APOE* genotype to refine prediction models used in clinical trial enrichment.

Methods

Ascertainment of Genotype and Phenotype Data

Twenty-two late-onset AD cohorts with genotype data were used for case-control analyses (**Table 1**).[24-38] Ascertainment and collection of genotype and phenotype data for each cohort are summarized in the eMethods in the Supplement and described in detail elsewhere.[38] The National Alzheimer Coordinating Center's Alzheimer's Disease Center data sets 1 through 7 (NACC [ADC1-7]) and the Alzheimer's Disease Neuroimaging Initiative (ADNI) and Religious Orders Study and Memory and Aging Project (ROSMAP) longitudinal cohorts provided data on the age at MCI or AD diagnosis and were used in conversion-risk analyses. Genotyping was performed using various high-density single-nucleotide variant (formerly single-nucleotide polymorphism) microarrays across cohorts (eTable 1 in the Supplement). Participants or their caregivers provided written informed consent in the original studies.

The current study protocol was granted an exemption by the Stanford University institutional review board because the analyses were carried out on deidentified, off-the-shelf data. Further informed consent was therefore not required.

The ADNI cohort provided longitudinal measures of Aβ42 in CSF and Aβ aggregates in the brain from florbetapir PET[24] (with sample and image processing described elsewhere[39,40]). For Aβ levels on PET scans, we investigated standardized uptake value ratios (referenced to the cerebellum) in a set of brain regions (composite regions of interest: parietal, temporal, frontal, and cingulate cortices) commonly affected by amyloid pathology.[41,42] Associations with CSF Aβ42 were also evaluated in 3 cross-sectional cohorts that are available through National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site (NIAGADS). The cohorts' genetic data and CSF measures were made publicly available on NIAGADS as part of the data sharing associated with an article by Cruchaga et al.[43] Both the genetic data and CSF measures were processed in the Cruchaga et al article[43] and made available under their processed format. All data were collected between September 2015 and September 2019.

The conversion and Aβ analyses used cohorts that are largely overlapping with the main case-control analysis. Thus, these should be considered supportive rather than fully independent analyses.

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Table 1. Demographics of Cohorts Used in the Alzheimer Disease Case-Control Regression Analysis

Cohort		Diagnosis			Age, mean (SD) [%]				Age, No. (%), y			
									60-80 y		≥80 y	
Name	Participants after quality control, No.	Type	No.	Female, No. (%)	At death	At last visit	At examination[a]	At onset[b]	All	*APOE4+*	All	*APOE4+*
ACT	2132	CN	1604	881 (54.9)	83.6 (5.7) [56.9]	80.3 (5.9) [43.1]	NA	NA	630	144 (22.9)	974	199 (20.4)
		AD	528	342 (64.8)	86.0 (3.5) [0.6]	NA	83.1 (5.2) [20.6]	80.7 (6.6) [78.8]	232	138 (59.5)	296	99 (33.4)
NACC												
ADC1	1790	CN	404	243 (60.1)	85.5 (8.6) [38.4]	78.0 (8.4) [61.6]	NA	NA	196	68 (34.7)	208	36 (17.3)
		AD	1386	770 (55.6)	83.5 (6.3) [1.40]	NA	79.7 (8.7) [7.4]	72.4 (7.2) [91.3]	1155	830 (71.9)	231	122 (52.8)
ADC2	705	CN	105	72 (68.6)	86.1 (7.0) [19.0]	78.8 (9.4) [81.0]	NA	NA	54	19 (35.2)	51	8 (15.7)
		AD	600	317 (52.8)	NA	NA	77.2 (7.5) [1.5]	72.9 (7.0) [98.5]	518	370 (71.4)	82	30 (36.6)
ADC3	1036	CN	380	238 (62.6)	88.8 (8.1) [20.0]	77.6 (8.5) [80.0]	NA	NA	209	59 (28.2)	171	26 (15.2)
		AD	656	368 (56.1)	99.0) [0.2]	NA	80.4 (8.8) [4.9]	74.3 (8.1) [95.0]	512	367 (71.7)	144	56 (38.9)
ADC4	629	CN	325	200 (61.5)	86.9 (8.2) [19.4]	77.8 (7.6) [80.6]	NA	NA	174	57 (32.8)	151	26 (17.2)
		AD	304	164 (53.9)	NA	NA	72.5 (0.7) [0.7]	73.4 (7.0) [99.3]	257	173 (67.3)	47	7 (14.9)
ADC5	807	CN	498	336 (67.5)	89.0 (6.4) [20.3]	80.2 (8.3) [79.7]	NA	NA	222	58 (26.1)	276	52 (18.8)
		AD	309	170 (55.0)	NA	NA	NA	73.4 (7.3) [100]	259	193 (74.5)	50	21 (42.0)
ADC6	535	CN	253	182 (71.9)	86.8 (8.6) [20.6]	77.6 (7.9) [79.4]	NA	NA	149	52 (34.9)	104	20 (19.2)
		AD	282	154 (54.6)	NA	NA	NA	73.7 (7.6) [100]	233	161 (69.1)	49	14 (28.6)
ADC7	1035	CN	601	395 (65.7)	84.1 (8.4) [9.0]	76.5 (7.4) [91.0]	NA	NA	404	132 (32.7)	197	52 (26.4)
		AD	434	236 (54.4)	NA	NA	NA	72.3 (7.6) [100]	371	262 (70.6)	63	29 (46.0)
ADDNEURO	239	CN	115	64 (55.7)	NA	78.5 (7.2) [100]	NA	NA	77	23 (29.9)	38	9 (23.7)
		AD	124	77 (62.1)	NA	NA	79.8 (6.6) [9.7]	73.3 (6.9) [90.3]	101	67 (66.3)	23	9 (39.1)
ADNI	724	CN	291	149 (51.2)	84.0 (0.3)	78.2 (6.8) [99.7]	NA	NA	188	62 (33.0)	103	22 (21.4)
		AD	433	183 (42.3)	NA	NA	75.2 (6.6) [100]	NA	340	251 (73.8)	93	49 (52.7)
ADOD	72	CN	72	0	NA	70.3 (5.3) [100]	NA	NA	69	17 (24.6)	3	1 (33.3)
		AD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GenADA	1371	CN	687	436 (63.5)	NA	74.3 (7.1) [100]	NA	NA	545	131 (24.0)	142	34 (23.9)
		AD	684	398 (58.2)	NA	NA	85.2 (6.4) [2.2]	73.7 (6.7) [97.8]	576	390 (67.7)	108	51 (47.2)
NIA-LOAD	1693	CN	718	443 (61.7)	85.9 (5.9) [2.9]	74.8 (7.8) [97.1]	NA	NA	556	190 (34.2)	162	36 (22.2)
		AD	975	631 (64.7)	NA	NA	80.3 (8.0) [0.9]	72.2 (6.7) [99.1]	881	705 (80.0)	94	38 (40.4)
MAYO	1738	CN	1079	557 (51.6)	NA	73.3 (4.3) [100]	NA	NA	1079	301 (27.9)	NA	NA
		AD	659	387 (58.7)	NA	NA	73.8 (4.9) [100]	NA	659	442 (67.1)	NA	NA
MAYO2	122	CN	62	28 (45.2)	83.0 (7.7) [100]	NA	NA	NA	19	2 (10.5)	43	5 (11.6)
		AD	60	39 (65.0)	83.9 (5.5) [100]	NA	NA	NA	60	33 (55.0)	NA	NA
MIRAGE	481	CN	211	116 (55.0)	NA	71.6 (7.4) [100]	NA	NA	184	74 (40.2)	27	9 (33.3)
		AD	270	168 (62.2)	NA	NA	73.4 (6.1) [1.90]	70.6 (6.6) [98.1]	252	163 (64.7)	18	8 (44.4)

(continued)

Table 1. Demographics of Cohorts Used in the Alzheimer Disease Case-Control Regression Analysis (continued)

Cohort		Diagnosis			Age, mean (SD) [%]				Age, No. (%), y			
									60-80 y		≥80 y	
Name	Participants after quality control, No.	Type	No.	Female, No. (%)	At death	At last visit	At examination[a]	At onset[b]	All	*APOE4+*	All	*APOE4+*
OHSU	316	CN	226	120 (53.1)	85.6 (7.1) [100]	NA	NA	NA	42	16 (38.1)	184	34 (18.5)
		AD	90	58 (64.4)	NA	NA	88.5 (9.5) [4.4]	86.8 (7.2) [95.6]	16	9 (56.3)	74	26 (35.1)
ROSMAP	1379	CN	821	579 (70.5)	87.2 (6.8) [56.4]	84.7 (6.9) [43.6]	NA	NA	171	40 (23.4)	650	104 (16.0)
		AD	558	411 (73.7)	88.2 (2.6) [1.1]	NA	83.8 (6.6) [98.9]	NA	163	88 (54.0)	395	131 (33.2)
TGEN2	946	CN	334	163 (48.8)	80.0 (8.7) [100]	NA	NA	NA	182	44 (24.2)	152	28 (18.4)
		AD	612	410 (67.0)	83.2 (6.6) [85.1]	NA	NA	74.3 (7.1) [14.9]	526	359 (68.3)	86	48 (55.8)
UPITT	1664	CN	682	436 (63.9)	NA	75.6 (6.2) [100]	NA	NA	546	117 (21.4)	136	17 (12.5)
		AD	982	628 (64.0)	NA	NA	76.7 (7.8) [11.7]	72.6 (6.4) [88.3]	844	523 (62.0)	138	56 (40.6)
UM/VU/MSSM	1198	CN	642	410 (63.9)	76.8 (10.7) [10.0]	73.2 (6.9) [90.0]	NA	NA	531	135 (25.4)	111	19 (17.1)
		AD	556	358 (64.4)	83.8 (9.0) [6.1]	NA	81.3 (10.4) [2.9]	72.6 (7.3) [91.0]	453	310 (68.4)	103	49 (47.6)
WASHU	316	CN	127	81 (63.8)	NA	76.4 (8.5) [100]	NA	NA	92	31 (33.7)	35	4 (11.4)
		AD	189	108 (57.1)	NA	NA	NA	74.7 (7.5) [100]	146	91 (62.3)	43	17 (39.5)
Total	20 928	CN	10 237	6129 (59.9)	84.5 (7.6) [25.4]	76.4 (7.6) [74.6]	NA	NA	6319	1772 (28.0)	3918	741 (18.9)
		AD	10 691	6377 (59.6)	83.3 (6.6) [6.0]	NA	78.0 (7.6) [19.4]	73.5 (7.5) [74.6]	8554	5925 (69.3)	2137	860 (40.2)

Abbreviations: ACT, Adult Changes in Thought; ADC1-7, Alzheimer's Disease Center data sets 1 through 7; ADDNEURO, European Collaboration for the Discovery of Novel Biomarkers for Alzheimer's Disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; ADOD, ADNI Department of Defense; GenADA, Multi-Site Collaborative Study for Genotype-Phenotype Associations in Alzheimers Disease; NA, not applicable; NACC, National Alzheimer Coordinating Center; NIAGADS, National Institute on Aging and Genetics of Alzheimer's Disease Data Storage Site; NIA-LOAD, National Institute on Aging Genetics Initiative for Late-Onset Alzheimer's Disease; MAYO, Mayo Clinic Alzheimer's Disease Genetics Studies; MAYO2, Mayo RNaseq Study; MIRAGE, Multi-institutional Research on Alzheimer Genetics Epidemiology; OHSU, Oregon Health and Science University study; ROSMAP, Rush University Religious Orders Study/Memory and Aging Project; TGEN2, Translational Genomics Research Institute Series 2; UM/VU/MSSM, University of Miami/Vanderbilt University/Mt. Sinai School of Medicine Studies; UPITT, University of Pittsburgh Study; WASHU, Washington University Study.

[a] Age at examination represents a mixture of age types; when multiple data were available for a participant, the youngest age was taken to approximate age at onset.

[b] Age at onset refers to the first onset of cognitive symptoms as reported by the participant or informant and generally precedes clinical diagnosis.

[c] Cohort data were available through NIAGADS, the NACC, AMP-AD Knowledge Portal, the Database of Genotypes and Phenotypes, Rush Alzheimer's Disease Center at Rush University, and the Image & Data Archive powered by Laboratory of Neuro Imaging. Cohorts included the ACT, ADC1-7 (for which phenotype data are managed by the NACC), ADDNEURO, ADNI, ADOD, GenADA, NIA-LOAD, MAYO, MAYO2, MIRAGE, OHSU, ROSMAP, TGEN2, UM/VU/MSSM, UPITT, and WASHU.

Genetic Data Quality Control and Processing

Genetic data underwent standard quality control (Plink version 1.9 [the Laboratory of Biological Modeling and the Purcell Lab]), imputation, and ancestry determination (SNPweights version 2.1 [T. H. Chan School of Public Health at Harvard University]; eFigure 1 in the Supplement).[44-46] To obtain the largest and most homogeneous sample, only non-Hispanic individuals of Northwestern European ancestry were selected. Principal component analysis of genotyped single-nucleotide variants was performed to obtain principal components that capture population substructure (eFigure 2 in the Supplement). Participants' relatedness was estimated from identity-by-descent analysis. If samples were from related individuals (identity-by-descent value ≥0.25; ie, second-degree relatives), only a single participant per relatedness cluster was used. Detailed descriptions of processing procedures and inclusion criteria are in the eMethods and eTable 2 in the Supplement.

Statistical Analyses

We evaluated the association of *KL*-VS^{HET+} status with (1) relative risk for AD, (2) absolute risk of converting from being cognitively normal to having MCI or AD, and (3) Aβ levels. All analyses were stratified by groups who carried *APOE4* (*APOE*-24/34/44) and did not carry *APOE4* (*APOE*-22/23/33). Associations with AD risk and Aβ were evaluated across 3 age ranges: 60 years and older, 60 to 80 years, and 80 years and older. The full sample of those 60 years and older represents the primary analyses. The groups aged 60 to 80 years and 80 years or older were used to test the secondary hypothesis that outcomes of *KL*-VS status differ across age. Associations with conversion risk were evaluated in the full sample of individuals 60 years and older, whereas age stratification was not needed in these time-to-event analyses. We also evaluated the formal interaction of *APOE4* with *KL*-VS^{HET+} status in analyses that additionally included *APOE4* and *APOE4* × *KL*-VS^{HET} interactions as model covariates. Outcomes were evaluated per cohort and combined using inverse-variance-weighted meta-analysis. In all models, we adjusted the outcome measure for sex and the first 3 genetic principal components. For associations with AD risk and Aβ, we also adjusted for age, even within age-stratified groups, to account for remaining age-associated outcomes. Associations were considered significant at a threshold *P* value of less than .05 (2-tailed).

A schematic overview of all analyses is provided in eFigure 3 in the Supplement. The association between *KL*-VS^{HET+} status and AD risk was evaluated using logistic regression analysis under a case-control design. When multiple age data were available, we prioritized age at onset (AAO) above age at examination, which was itself prioritized above age at death in affected individuals, and we prioritized age at death above age at last examination in control participants (Table 1). This priority ranking is consistent with prior AD studies[34,38] and reflects the reasoning that AAO best marks the advent of pathological changes, while age at death in control participants marks the total time spent without cognitive impairment. Association between *KL*-VS^{HET+} status and absolute risk of conversion to MCI or AD, accounting for death as a competing risk, was evaluated using competing risk regression.[47,48] In competing risk regression, we also adjusted for years of education, which was available for most participants in cohorts with conversion data. Participants were required to be cognitively normal at baseline and have at least 3 years of follow-up.[49-51] Conversions were defined as the first clinical diagnosis of MCI or AD, while participants who were cognitively normal and did not convert or die were censored. Association testing with Aβ levels was restricted to control participants, as in prior studies.[21,22] Associations between *KL*-VS^{HET+} status and Aβ measures in the ADNI study were evaluated by linear mixed-effects analysis to take into account the correlation between multiple measurements within each participant, additionally adjusting for diagnosis and participant as a random effect. The diagnosis term dealt with reversions from having MCI to being cognitively normal. Associations with Aβ CSF in the Cruchaga et al[43] sample were evaluated by means of multiple linear regression, additionally adjusting for cohort (eMethods in the Supplement).

To evaluate and quantify potential cohort bias, case-control and conversion risk analyses were repeated using mega-analyses that included the cohort as a covariate. To evaluate potential bias attributable to the heterogeneity in age information across different cohorts (Table 1), case-control analyses were repeated using only cases that had AAO data available (n = 7994). To increase the reliability of age at diagnosis, conversion risk analyses were repeated requiring 4 and 5 years of minimal follow-up.[49-51] In addition, we performed regression analyses to validate whether the association of *APOE4* with risk for AD differs across age groups (60-80 years vs ≥80 years) and if *APOE4* status affects AAO. All analyses were performed in R version 3.6.0 (nlme, metaphor, and cmprsk packages; R Foundation for Statistical Computing) between April 2019 and December 2019. Additional details for model/inclusion criteria are in the eMethods in the Supplement.

Results

KL-VS Heterozygosity and AD Risk per *APOE4* Status

We evaluated the association of *KL*-VS^{HET+} status with AD risk by meta-analyzing across 22 AD cohorts (Table 1). We investigated 3 different age ranges, stratified by *APOE4* status (**Table 2**). While *KL*-VS^{HET+} status is associated with decreased risk for AD in participants who carry *APOE4* across the entire age range of those 60 years and older (odds ratio [OR], 0.75 [95% CI, 0.67-0.84]; $P = 7.4 \times 10^{-7}$), the outcome was driven mainly by the group aged 60 to 80 years (OR, 0.69 [95% CI, 0.61-0.79]; $P = 3.6 \times 10^{-8}$), with no significant association observed in the group 80 years and older (OR, 0.99 [95% CI, 0.77-1.27]; *P* = .94). There was no association found in any *APOE4*-negative group. The interaction between *KL*-VS^{HET+} status and *APOE4* status for AD risk in the group aged 60 to 80 years was significant and protective (OR, 0.76 [95% CI, 0.66-0.89]; $P = 3.9 \times 10^{-4}$). Forest plots in eFigure 4 in the Supplement show high cohort homogeneity of *KL*-VS^{HET+} status association patterns in individuals who carry *APOE4*.

Table 2. Association of *Klotho*-VS Heterozygosity (*KL*-VS^{HET+}) Status With Alzheimer Disease Status in Age and Apolipoprotein E4 (*APOE4*) Strata[a]

Group	Association between *KL*-VS^{HET+} and AD risk by *APOE4* status				Interaction between *KL*-VS^{HET+} and AD risk by *APOE4* status			
	Control participants with KL-VS^{HET+} status, No./total No. (%)	Participants with AD with KL-VS^{HET+} status, No./total No. (%)	Odds ratio (95% CI)	*P* value	Control participants with KL-VS^{HET+} status, No./total No. (%)	Participants with AD with KL-VS^{HET+} status, No./total No. (%)	Odds ratio (95% CI)	*P* value
60-80 y								
APOE4+	528/1737 (30.4)	1475/5883 (25.1)	0.69 (0.61-0.79)	3.6×10^{-8}	1694/6189 (27.3)	2137/8478 (25.2)	0.73 (0.61-0.87)	5.1×10^{-4}
APOE4−	1166/4452 (26.2)	662/2595 (25.5)	0.98 (0.87-1.11)	.79	NA	NA	NA	NA
≥80 y								
APOE4+	187/713 (26.2)	218/826 (26.4)	0.99 (0.77-1.27)	.94	972/3772 (25.9)	552/2053 (26.9)	0.92 (0.69-1.24)	.61
APOE4−	796/3090 (25.8)	339/1253 (27.1)	1.09 (0.93-1.28)	.28	NA	NA	NA	NA
Full sample								
APOE4+	724/2488 (29.1)	1707/6752 (25.3)	0.75 (0.67-0.84)	7.4×10^{-7}	2704/10 103 (26.8)	2718/10 631 (25.5)	0.76 (0.66-0.89)	3.9×10^{-4}
APOE4−	1997/7670 (26.0)	1015/3906 (26.0)	1.01 (0.91-1.11)	.91	NA	NA	NA	NA

Abbreviations: AD, Alzheimer disease; HET+, heterozygous; NA, not applicable.

[a] This Table shows the results of meta-analyses including cohorts with a minimal sample size of 50 that had both affected individuals and control participants.

In sensitivity analyses, results were highly consistent when cohorts were combined through mega-analysis (eTable 3 in the Supplement). Additionally, given that 25.4% of cases did not have AAO data provided (Table 1), we repeated analyses using only affected individuals with AAO data and all control participants (eTables 4 and 5 in the Supplement). Despite smaller sample sizes, the protective association of *KL*-VS^{HET+} status with AD in individuals carrying *APOE4* was even more pronounced and remained strongest in the group of individuals who carried *APOE4* and were between 60 and 80 years (meta-analysis; OR, 0.64 [95% CI, 0.55-0.74]; $P = 4.0 \times 10^{-9}$). In addition, we confirmed that, as expected, the association between *APOE4* positivity and AD risk was stronger in those aged 60 to 80 years (OR, 5.79 [95% CI, 5.38-6.23]) compared with those 80 years or older (OR, 2.97 [95% CI, 2.63-3.35]; $P < 2.2 \times 10^{-16}$). Participants who carried *APOE4* also had reduced AAO (mean [SD] age, 72.0 [6.7] years) compared with participants who did not carry *APOE4* (mean [SD] age, 76.1 [8.1] years; $P < 2.2 \times 10^{-16}$).

KL-VS^{HET+} Status and Risk of Conversion to MCI or AD in Individuals Stratified by *APOE4* Status

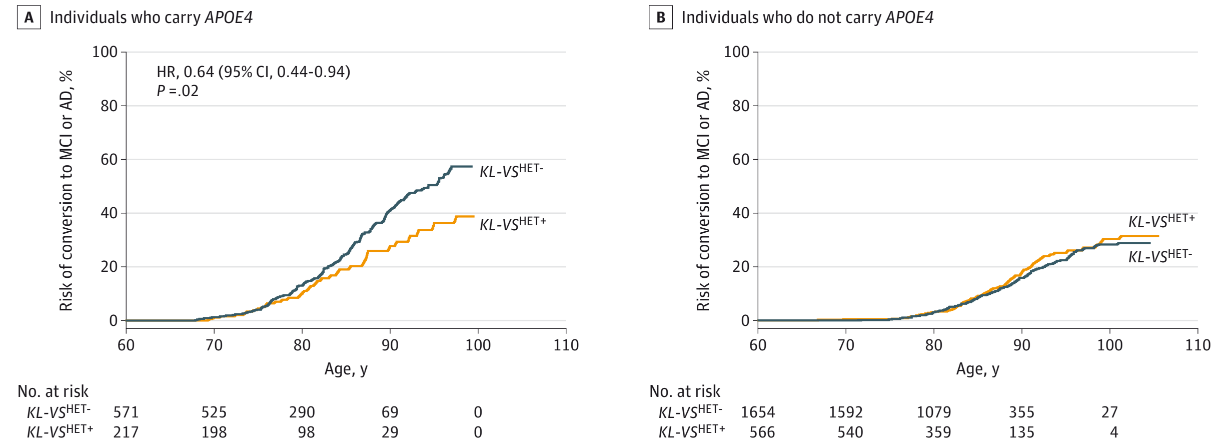
We then assessed the association of *KL*-VS^{HET+} status with risk for conversion to MCI or AD. Meta-analysis across the 3 investigated cohorts (eTable 6 in the Supplement) showed a significant protective association of *KL*-VS^{HET+} status with conversion risk in those who carry *APOE4* (hazard ratio [HR], 0.64 [95% CI, 0.44-0.94]; *P* = .02) but not in participants who did not carry *APOE4* (HR, 1.06 [95% CI, 0.81-1.37]; *P* = .69; eTable 7 in the Supplement). The interaction between *KL*-VS^{HET+} status and *APOE4* status was significant and protective (HR, 0.62 [95% CI, 0.39-1.00]; *P* = .048). **Figure 1** shows the cumulative conversion risk across the age span, where the protective association of *KL*-VS^{HET+} status in the group with *APOE4* begins around 77 years of age. Forest plots in eFigure 5 in the Supplement and cumulative risk plots in eFigure 6 in the Supplement show that these association and interaction patterns are consistent across all 3 cohorts. In sensitivity analyses, these findings remained consistent when evaluated through mega-analysis and after requiring minimum follow-up times of 4 or 5 years (eTable 8 in the Supplement).

We additionally evaluated the association of *KL*-VS^{HET+} status with conversion from being cognitively normal or having MCI to having AD (eTables 9 and 10 and eFigure 7 in the Supplement). The *KL*-VS^{HET+} status reduced conversion risk in the group carrying *APOE4* (HR, 0.81 [95% CI, 0.66-1.00]; *P* = .047) but not in the group without *APOE4* (HR, 1.12 [95% CI, 0.78-1.61]; *P* = .99). These outcomes were consistent for a minimum of 4 years and 5 years of follow-up. The interaction of *KL*-VS^{HET+} status with *APOE4* status was protective but significant only for patients with a minimum of 5 years of follow-up (HR, 0.68 [95% CI, 0.49-0.95]; *P* = .02; eTable 9 in the Supplement).

KL-VS^{HET+} Status and Aβ in Control Participants Aged 60 to 80 Years Stratified by *APOE4* Status

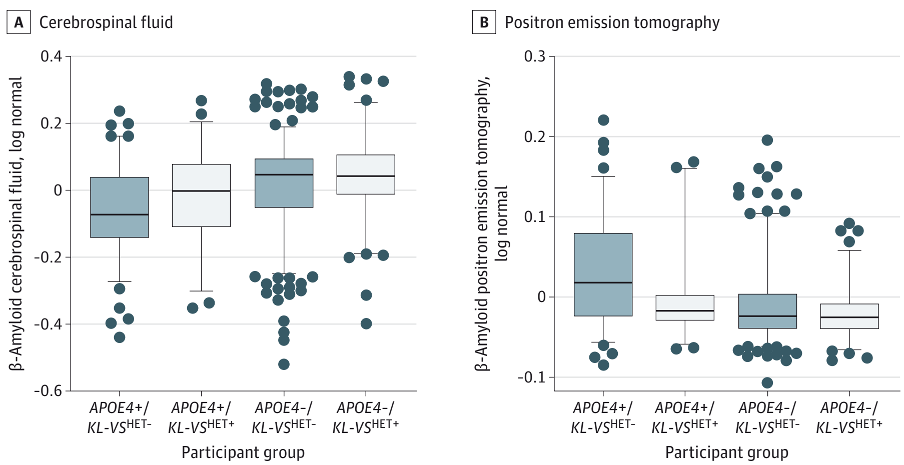
Similar to AD risk analyses, we evaluated whether there was an age-dependent association of *KL*-VS^{HET+} status with Aβ CSF levels. In the age range of 60 to 80 years, *KL*-VS^{HET+} status was significantly associated with increased Aβ CSF levels in control participants carrying *APOE4* (β, 0.06 [95% CI, 0.01-0.10], *P* = .03) but not in control participants without *APOE4* (β, 0.04 [95% CI, −0.02 to 0.09]; *P* = .22; **Figure 2**A). In the full age range (≥60 years), this association was not significant in control participants carrying *APOE4* (β, 0.02 [95% CI, −0.03 to 0.06]; *P* = .50) or control participants without *APOE4* (β, 0.02 [95% CI, −0.03 to 0.07]; *P* = .44; eFigure 8 in the Supplement). Forest plots in eFigure 9 in the Supplement show consistent associations for both cohorts in those aged 60 to 80 years who carried *APOE4*. Finally, we evaluated the association of *KL*-

Figure 1. Risk of Conversion to Mild Cognitive Impairment or Alzheimer disease by *Klotho*-VS Heterozygosity Status, Stratified by *APOE4* Status



A, Individuals with apolipoprotein E4 (*APOE4*). The outcome of *KL*-VS^{HET+} status, as determined from competing risk regression analysis meta-analyzed across 3 independent cohorts, is significant in individuals who carry *APOE4* (hazard ratio, 0.64 [95% CI, 0.4-0.94]; *P* = .02). B, Individuals without *APOE4* (hazard ratio, 1.06 [95% CI, 0.81-1.37]; *P* = .69). AD indicates Alzheimer disease; HET+, heterozygosity; HET-, nonheterozygosity; MCI, mild cognitive impairment.

Figure 2. Association of *Klotho*-VS Heterozygosity Status with β-Amyloid Levels in Control Participants 60 to 80 Years Old, Stratified by *Apolipoprotein E4* (*APOE4*) Status



A, Measured by cerebrospinal fluid samples. B, Measured by positron emission tomography imaging. Box plot error bars show the 95th-percentile range. Gray circles indicate values outside of the 95th percentile range. Meta-analyses between Alzheimer's Disease Neuroimaging Initiative and Cruchaga et al[43] samples were significant in participants who carry *APOE4* (leftmost pairs in each graph; cerebrospinal fluid, β, 0.06 [95% CI, 0.01-0.10]; *P* = .03; positron emission tomography, β, −0.04 [95% CI, −0.07 to −0.00]; *P* = .04). HET+ indicates heterozygosity; HET-, nonheterozygosity.

VS^{HET+} status with Aβ findings on PET in an AD-relevant brain composite region of interest. Findings were highly consistent with those for CSF levels; that is, *KL*-VS^{HET+} status significantly decreased Aβ on PET in the group who were positive for *APOE4* and aged 60 to 80 years (β, −0.04 [95% CI, −0.07 to 0.00]; *P* = .04; Figure 2B) but not in those aged 60 to 80 years who did not carry *APOE4* (β, 0.00 [95% CI, −0.02 to 0.01]; *P* = .69) or either of the other groups aged 60 years or older (eFigure 8 in the Supplement).

Additional Analyses

In addition to comparing participants with *KL*-VS^{HET+} status vs *KL*-VS^{HET-} status, we contrasted individuals with *KL*-VS^{HET+} status vs those who did not carry *KL*-VS (eTables 11-15 in the Supplement). Results were highly consistent with the main analyses but had slightly reduced effect sizes. Because *KL*-VS homozygosity (*KL*-VS^{HOM}) has been associated with negative outcomes on life span,[2] brain-aging resilience,[52] and cognition,[4] we also evaluated individuals with *KL*-VS^{HOM} status compared with those who did not carry *KL*-VS (eTables 16-19 and eFigure 10 in the Supplement). In individuals who carry *APOE4*, results were consistent, with *KL*-VS^{HOM} status increasing risk, but only conversion risk from being cognitively normal or having MCI to having AD reached nominal significance. There were no significant results in participants who did not carry *APOE4*. Finally, given the biological ambiguity of individuals who carry *APOE24* (both risk-increasing and decreasing alleles), we repeated analyses excluding these participants (eTables 20-24 in the Supplement). Again, results were highly consistent with the main analyses.

Discussion

Our results demonstrate that *KL*-VS^{HET+} status was associated with reduced AD risk in individuals who carried *APOE4*, and this was so mostly between 60 and 80 years. In this age range, *KL*-VS^{HET+} status was also associated with lower Aβ burden in individuals who are cognitively normal and carry *APOE4*. Additionally, starting close to 80 years of age, control participants who carried *APOE4* and had *KL*-VS^{HET+} status were at reduced risk of converting to MCI or AD.

To our knowledge, the current study is the largest to date to evaluate a heterozygous genetic association with AD risk. Specifically, we hypothesized that *KL*-VS^{HET+} status would reduce risk of AD in those who carried *APOE4*. Furthermore, given that the genetic risk for AD attributable to *APOE4* is higher between 60 and 80 years of age,[17-19] which was confirmed in our case-control analysis in which the OR for *APOE4* was almost 2-fold higher in the group 60 to 80 years old (OR, 5.8) compared with those 80 years or older (OR, 3.0), we hypothesized that the protective association of *KL*-VS^{HET+} status in those with *APOE4* would be strongest in the 60-year to 80-year age range. We showed that protective outcomes of *KL*-VS^{HET+} status on AD risk in those who carry *APOE4* was highly significant across the entire age range older than 60 years but was considerably stronger between the ages of 60 and 80 years and was not detectable in the ages 80 years and older. This age-specific interaction of *KL*-VS^{HET+} status with *APOE4* is also consistent with recent work that showed how genome-wide risk for AD differs between 60 and 80 years and those older than 80 years.[43] The largest (to our knowledge) prior *APOE4*-stratified genome-wide association study of AD did not stratify by age and only evaluated additive genetic effects and so would not have picked up the *KL*-VS^{HET+} status outcome identified here.[53]

We then evaluated the association of *KL*-VS^{HET+} status with conversion risk. In individuals who carry *APOE4*, *KL*-VS^{HET+} status reduced risk of conversion from cognitively normal status to MCI or AD with a hazard ratio of approximately 0.65 and from cognitively normal status or MCI to AD with a hazard ratio of about 0.80. This suggests that the protective nature of *KL*-VS^{HET+} status is stronger in control participants and diminishes in affected individuals who have already developed MCI. Ascertainment differences across cohorts represent a source of bias, but findings were consistent for both mega-analyses and meta-analyses. Additionally, by restricting our analyses to participants with a minimal follow-up time of 3, 4, or 5 years, we could increase confidence in the age at diagnosis.[49-51] For each model that required 5 or more years of minimal follow-up, we obtained significant results for *KL*-VS^{HET+} status in the *APOE4*-positive groups and interactions of *KL*-VS^{HET+} status with *APOE4*. Lastly, we could add years of education as a covariate in the conversion models, allowing us to account for MCI or AD risk mitigation attributable to possible differences in cognitive reserve.[54]

Notably, the difference in conversion risk between participants who had *KL*-VS^{HET+} status vs those with *KL*-VS^{HET-} status who carried *APOE4* became apparent around 80 years of age. There are no prior reports on MCI or AD conversion risk attributable to having *KL-VS*$^{HET+}$ status to compare our findings with. However, Porter et al[22] examined individuals who were cognitively normal with a mean age of 71 years and reported there was neither an association of *KL*-VS^{HET+} status with longitudinal measures of global cognition nor a modifying association with *APOE4* status. Other studies that evaluated the association of *KL*-VS^{HET+} status with measures of cognition in control participants did not directly investigate interactions with *APOE4* but did observe protective associations that were more pronounced closer to 80 years of age.[3,5,55] Overall, our findings appear consistent with prior literature, but further studies need to evaluate the interaction of age, *APOE4*, and *KL*-VS^{HET+} status on cognition in control populations.

We observed significant protective interactions between *APOE4* status and *KL*-VS^{HET+} status for both risk of AD and risk of conversion, whereas *KL*-VS^{HET+} status had no association with outcome in individuals who did not carry *APOE4*. This suggests that *KL*-VS interacts with aspects of AD pathology that are more pronounced in those who carry *APOE4*, such as Aβ accumulation during the presymptomatic phases of the disease. Our analyses of Aβ CSF and PET in control participants with *APOE4* between ages 60 and 80 years indeed confirmed reduced Aβ burden attributable to *KL*-VS^{HET+} status. Erickson et al[21] reported similar results, in that those with *KL*-VS^{HET+} status did not display the commonly expected difference in Aβ burden (in CSF levels and on PET scanning) between control participants with *APOE4* vs without *APOE4*, but participants who were *KL*-VS^{HET-} did. All brain areas that we investigated in the composite region of interest also displayed consistent results in the study by Erickson et al. While Porter et al[22] reported there was no association of *KL*-VS^{HET+} status with cognition, they did not directly evaluate associations with Aβ. In that study,[22] participants were classified as having low or high amounts of Aβ based on brain Aβ levels on PET scans. When we considered ratios of participants with low and high Aβ amounts, as reported in Table 2 of their article,[22] we could derive risk estimates associated with high levels of Aβ for those with *KL*-VS^{HET+} status and *APOE4* (OR, 0.59) and without *APOE4* (OR, 0.82). These are similar to our finding that *KL*-VS^{HET+} status reduced Aβ on PET in those who carry *APOE4*. Overall, our findings associating *KL*-VS^{HET+} status with Aβ appear consistent with results in 2 prior, independent studies.

Reduced Aβ burden attributable to *KL*-VS^{HET+} status in control participants with *APOE4* between ages 60 and 80 years may provide an explanation for the age shift between our case-control and conversion findings. The AD risk attributable to *KL*-VS^{HET+} status in those who carry *APOE4* was lower between ages 60 and 80 years, where the age for cases mainly represented AAO (mean age, 72 years). Protective associations of *KL*-VS^{HET+} status with conversion risk became apparent around 77 years of age, roughly indicating a 5-year shift between the onset of symptoms and a formal diagnosis or conversion. Abnormal Aβ levels in control participants can precede conversion by 5 to 10 years,[10] suggesting that *KL*-VS^{HET+} status may delay conversion by reducing Aβ levels. Currently, there is an increasing need to identify risk factors that improve prognostication of AD conversion risk.[56] These risk factors can be used to stratify patients into high-risk groups who can be recruited into clinical prevention trials to increase their statistical power and efficiency. The *APOE4* allele is a major genetic risk factor used for AD trial enrichment.[57] Our results suggest that for prevention trials, it will help to further select control participants who have *KL*-VS^{HET-} status and *APOE4* (70% of the sample), who appear more likely to convert to AD. On an interesting, related matter, *KL*-VS^{HET+} status has been associated with increased serum levels of KL,[3,52] while *KL*-VS^{HOM} has conversely been associated with decreased serum levels of KL.[52] Both studies further found direct correlations between systemic KL levels and cognitive performance in mice[3] and brain aging resilience in humans.[52] Additionally, CSF levels of KL were shown to be lower in individuals with AD vs age-matched participants who were cognitively normal.[58] Combined with our findings that *KL*-VS^{HET+} status is consistently associated with reductions (and *KL*-VS^{HOM} with increases) in AD conversion risk, this suggests that systemic KL levels may serve as a promising biomarker to help identify those who are positive for *APOE4* and at higher risk for developing AD.

Currently, there is no known mechanism by which *KL*-VS interacts with *APOE4* to modulate Aβ levels. Interestingly, *KL* expression is regulated by amyloid precursor protein (APP).[59] Furthermore, 3 enzymes linked to APP cleavage (a disintegrin and metalloproteinase domain-containing proteins 10 and 17 [ADAM10 and ADAM17] and β-secretase 1 [BACE1]) also cleave KL in the cell membrane leading to shedding of KL's extracellular domain.[60-62] In AD mouse models, therapies aimed at increasing *KL* expression or KL cleavage were shown to reduce Aβ burden through autophagy-mediated clearance and confer neuroprotection through increased expression of *ADAM10*.[63,64] Overall, this raises the intriguing possibility of an interaction between *APOE4*, *KL*-VS, and the molecular APP processing machinery that produces Aβ. Other studies, in animal models and humans, indicate that *KL*-VS^{HET+} status confers resilience to brain-aging and cognitive aging,[4,52,65,66] which may also contribute to protective associations against AD. Although lacking direct validation, our findings may also suggest that individuals with *KL*-VS^{HET+} status are biologically younger than those who have *KL*-VS^{HET-} status. Indeed, previous studies reported both a slowed epigenetic age for individuals with *KL*-VS heterozygosity[67] and a direct correlation between telomerase activity and *KL* expression.[68] Notably, *KL*-VS^{HET+} status showed an age-specific association with AD here, which is in line with prior findings on life span trajectories.[2,69] Future studies will need to explore these promising research avenues.

Limitations

One limitation for our analyses is the variability in age and diagnosis ascertainment across cohorts. However, we repeated all tests using both meta-analyses and mega-analyses. We also performed sensitivity analyses, including only individuals with AD who had AAO data available. Our findings were highly consistent across all models and displayed little to no heterogeneity, making it unlikely that the results were affected by cohort bias. The null findings in the groups 80 years and older may, however, also be attributable to limited sample sizes in this age stratum.

Conclusions

Overall, our findings suggest that *KL*-VS^{HET+}, possibly by increasing systemic KL levels, is associated with a protective outcome against AD that manifests in participants who carry *APOE4* and are cognitively normal between the ages of 60 and 80 years. Our work paves the way for biological validation studies to elucidate the molecular pathways by which *KL*-VS and *APOE* interact. Information on *KL*-VS status should also prove useful in further refinement of genetic risk profiles for both clinical trial enrichment and personalized genetic counseling.

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