

Original Article

Genetic Variants in *KLOTHO* Associate With Cognitive Function in the Oldest Old Group

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Abstract

Decline in cognitive abilities is a major concern in aging individuals. A potential important factor for functioning of the central nervous system in late-life stages is the *KLOTHO* (*KL*) gene. *KL* is expressed in various organs including the brain and is involved in multiple biological processes, for example, growth factor signaling. In the present study, 19 tagging gene variants in *KL* were studied in relation to 2 measures of cognitive function, a 5-item cognitive composite score and the Mini Mental State Examination, in 1,480 Danes 92–100 years of age. We found that heterozygotes for the previously reported KL-VS had poorer cognitive function than noncarriers. Two other variants positioned in the 5' end of the gene, rs398655 and rs562020, were associated with better cognitive function independently of KL-VS, and the common haplotype AG was associated with poorer cognition, consistently across two cognitive measures in two cohort strata. The haplotype effect was stronger than that of KL-VS. Two variants, rs2283368 and rs9526984, were the only variants significantly associated with cognitive decline over 7 years. We discuss an age-dependent effect of *KL* and the possibility that multiple gene variants in *KL* are important for cognitive function among the oldest old participants.

Keywords: *KLOTHO*—Dementia—Cognitive—Cognition—Longevity

The goddess Clotho, who in the ancient Greek mythology was known to spin the thread of life, has lend her name to a gene, which when mutated in mice, leads to multiple disorders resembling human aging and to a shortened life span (1). In addition, genetic *klotho* reduction in mice during embryogenesis has been shown to cause early postnatal death, hypomyelination (2), synaptic attrition (3), and cognitive impairment (4), suggesting that *klotho* is required for brain maturation. The mouse *Klotho* gene encodes a single-pass transmembrane protein that is predominantly expressed in the choroid plexus of the brain, distal tubule cells of the kidney, and parathyroid glands (5). The extracellular domain of the *klotho* protein is subject to ectodomain shedding, and as a result, the *klotho* protein exists in two forms: membrane and secreted *klotho* (5). The secreted

protein acts as a humoral factor with pleiotropic activities in the mouse, including not only maintenance of vascular integrity and inflammation (6) but also suppression of both growth factor signaling and oxidative stress, the latter with homology in human cells (7). Redundant mouse/human homology was found for the soluble form of *klotho*, which circulates in serum and cerebrospinal fluid throughout life and declines with aging (8–11). In parallel to the putative emergence of cognitive deficits, it is possible that *klotho* also fulfills important functions in the central nervous system in both early- and later-life stages.

The human homologue of the *KLOTHO* (*KL*) gene, is composed of five exons and covers more than 50kb on chromosome 13q12 (1). Variants in *KL* show a large degree of pleiotropic

associations including associations to carotid atherosclerosis (12); cardiovascular risk factors, such as fasting glucose, lipid levels, and blood pressure (13–18); kidney stones (19); and bone-related conditions (20–22). Additionally, age-related changes in the frequency of *KL* gene variants have been reported in several studies (15,18,23–26).

There are three variants in the human *KL* gene that have been analyzed in numerous association studies. A common haplotype, KL-VS, consists of six sequence variants, two of which are located in exon 2 and result in the amino acid substitutions, F352V and C370S. Due to the presence of perfect linkage disequilibrium (LD) across the six KL-VS single-nucleotide polymorphisms (SNPs), the variant F352V (rs9536314) has often been used to tag the KL-VS haplotype. KL-VS influence both the functional trafficking and the catalytic activity of KL (15), and it has been associated with intelligence in individuals assessed both as tweens and as elderly adult (27). Recently, consistency was found in three independent cohorts of primarily Caucasians without dementia or cognitive complaints, where the KL-VS heterozygotes were found to have significantly increased cognitive Z-scores (28). Also, heterozygous carriers of the KL-VS haplotype had higher serum KL levels than noncarriers, which correlated with better cognition (28), thus supporting the relevance of KL in cognitive functioning. Another well-studied variant, C1818T (rs564481), is a synonymous variant located in the fourth exon, and it is therefore not likely to be functional by itself. However, the variant may, nevertheless, be a clinically relevant marker as it has been found to associate with cardiovascular risk factors such as fasting glucose, lipid levels, and blood pressure, although so far mainly in populations of Asian descent (13,16). The third variant, G395A (rs1207568) (19,22), is located in the promoter region and may indeed be a functional variant because it alters the DNA–protein affinity in cultures of human kidney cells (20). The interest in these two latter mentioned genetic variants has of course been driven not only by their association with biological conditions but also for their abundance in all three major population groups, that is, Asians, Africans, and Caucasians. In contrast, the KL-VS variant is very rare in the Asian populations (20,29,30). To our knowledge, C1818T (rs564481) and G395A (rs1207568) have not been reported previously in relation to cognitive function.

In this study, we first investigated whether the minor allele of the KL-VS variant, rs9536314 (F352V), is associated with a higher cognitive level and a slower cognitive decline in a large population of oldest old Danes. We also tested 19 tagging variants, of which no a priori hypothesis was applied for 18 variants in relation to cognition, thereby covering the majority of the genetic variation in *KL*. The study population comprised three birth cohorts of oldest old Danes, who were assessed by two panels of cognitive tests. In one cohort, the 1905 birth cohort, cognitive tests were assessed repeatedly with up to 7 years of follow-up, and in this cohort, the effect of the *KL* gene variants were studied both at a cross-sectional cognitive level and by the longitudinal change in cognition.

Materials and Methods

Subjects

The participants included in this study were drawn from three population-based nationwide surveys conducted at the University of Southern Denmark: the Danish 1905 birth cohort study (31), the Danish 1910 birth cohort study (S. Vestergaard, K.

Andersen-Ranberg, A. Skytthe, K. Christensen, J.-M. Robine, B. Jeune, unpublished data), and the Danish 1915 birth cohort study (32). The Danish 1905 birth cohort study is a prospective investigation of an entire Danish birth cohort. The survey was initiated in 1998, when the participants were 92–93 years old and followed by three follow-up studies of the participating survivors in 2000, 2003, and 2005. Of the 3,600 individuals still alive at intake, 2,262 participated and 1,651 provided either a blood spot sample or a cheek swap at their first assessment in 1998. The Danish 1910 and 1915 birth cohort studies include Danes born in 1910 and 1915, respectively, who were alive and living in Denmark on September 1, 2010. Among 400 invited participants from the 1910 birth cohort study, 273 participated and 176 provided blood samples. In the 1915 birth cohort study, 2,509 individuals were identified as eligible participants when they were 95 years old, 1,584 individuals participated and 1,165 individuals provided biological samples (32). Each of the surveys in the cohort studies comprises multidimensional face-to-face interviews and assessments of cognitive and physical functioning. Written informed consents were obtained from all participants, and all three surveys were approved by the Regional Scientific Ethical Committees for Southern Denmark.

Assessment of Cognitive Function

Cognitive functioning was assessed by using a five-component cognitive composite score (CCS) and the Mini Mental State Examination (MMSE) (33). The five-component cognitive composite measures were originally selected to represent tasks that are sensitive to normative age changes, and which could be reliably and briefly assessed by lay interviewers. The cross-sectional decline in cognitive function was estimated to approximately two and a half standard deviation (SD) from age 45 to 90 years (34). The specific tasks included a fluency task, which involved the number of animals an individual could name in a 1-minute interval, forward and backward digit span, and immediate and delayed recall of a 12-item list. The CCS was computed separately for each cohort by taking the sum of the five standardized measures, separately from each cohort (33). The widely used MMSE ranges from 0 to 30 and can be graded as severely impaired for scores between 0 and 17, mildly impaired for scores between 18 and 23, and normal for scores between 24 and 30.

Genotyping and Quality Control

DNA from 1905 birth cohort participants was extracted either from blood spots using the QIAamp DNA Mini and Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol or from whole blood using a salting out method (35).

DNA from participants in the 1910 and 1915 cohorts was extracted from dried blood spots as described previously (36).

KL genotype data for the 1905 birth cohort were drawn from a previous study investigating the known genetic variation of a large number of candidate aging-related genes (24). Tagging SNPs with a minor allele frequency of at least 5% and covering the majority of the common variation in the coding region plus 5,000 bp upstream and 1,000 bp downstream of the gene were selected and genotyped using the Illumina GoldenGate platform (Illumina, San Diego, CA). Genotype data for a total of 19 *KL* tagging SNPs in 1,088 individuals were available for the present study.

For the 1910 and 1915 birth cohorts, *KL* genotype data were drawn from an existing genome wide data set previously acquired

using the Illumina HumanOmniExpress BeadChips (Illumina) (36). Eight of the 19 tagging SNPs in the *KL* region were present in this data set and passed the quality control criteria: rs398655, rs562020, rs495392, rs2283368, rs9526984, rs2320762, rs657049, and rs648202. Two additional SNPs, rs9527024 and rs683907, were used as proxies because they were in perfect LD with the initially selected tagging SNPs, rs9536314 and rs564481, respectively. The pairwise LD estimates were obtained using the SNAP database (Broad Institute, MIT) based on the 1000 Genomes pilot 1 data set and the CEU population panel (37). Genotype data were retrieved for a total of 405 study participants, who were all older than 96 years of age.

Statistical Analyses

Statistical analyses were performed using STATA 10.1 (StataCorp, College Station, TX). The influence of *KL* variants were assessed by linear regression analysis applying an additive genetic model and using intake measures adjusted for age, sex, and time (birth year from 1905 coded as 0, 5, and 10). Data were analyzed in the combined 1905, 1910, and 1915 cohort group with 1 *df* on genotypes recoded 0, 1, and 2, where 0 are homozygotes for the major allele, 1 are heterozygotes, and 2 are homozygotes of the minor allele. Additionally, stratified analyses were performed by including the 1905 birth cohort and the 1910–1915 cohorts independently. Both the CCS and the MMSE were analyzed as continuous variables. Subsequently, the *KL-VS* genotypes were analyzed as categorical variables to test a nonadditive model. Post hoc linear regression analyses were performed on selected SNPs using the additive genetic model with and without the inclusion of an interaction term. Haplotypes of rs562020 and rs398655 were analyzed on the combined cohort using linear regression analyses. Interactions were studied in categories of low versus high cognition and presence or absence of the rs562020 and rs398655 AG haplotype and the *KL-VS* genotypes. Odds ratios were calculated using logistic regression adjusted for age, sex, and birth year.

The 1905 cohort participants were assessed by cognitive measures in 1998, 2000, 2003, and 2005. A random effects model was applied to this cohort to perform analyses of the associations between *KL* variants and both the intercept and the slope of cognitive functioning separately within the 1905 birth cohort because this cohort was assessed repeatedly with a follow-up time of up to 7 years. By using a random effects model, we considered the time from intake and the fact that some participants were assessed two to four times. The annual linear slope of decline was estimated for both CCS and MMSE among the 1905 cohort participants. Analyses included additive genetic modes with genotypes coded as 0, 1, and

2 as written above for regression analysis. Sex and time from intake were included as covariates. The intercept was defined as the level of the cognitive functioning at the age of 93 as described in detail elsewhere (38).

Results

Study Population and Genotypes

The study population comprised three cohorts of oldest old participants born in 1905, 1910, or 1915. Descriptives of the cohorts, mean age, assessment date, and mean cognitive performance CCS and MMSE, are presented in Table 1. The estimated annual linear slope of decline for CCS and MMSE among the 1905 cohort is described in detail elsewhere (38). Descriptives of the 19 *KL* tagging SNPs in the 1905 birth cohort and the 10 equivalent SNPs or proxy SNPs in the 1910 and 1915 birth cohorts are shown in Table 2. All gene variants, except rs397703 and rs1207362, were in Hardy–Weinberg equilibrium ($p > .05$).

Cross-sectional Analysis of *KL* Variants and Cognitive Function

Carriers of the functional *KL-VS* variant, as determined by either rs9536314 or the proxy rs9527024, had a poorer cognitive function than noncarriers when assessed using the MMSE (β : $-0.59, p = .046$). This association was, however, only borderline significant and not reflected in the CCS (β : $-0.06, p = .74$). Of note, those homozygous for the *KL-VS* variant ($N = 38$) had a better cognitive function than noncarriers, thus we subsequently applied a restricted model suggested by Dubal and coworkers that leaves out the homozygous *KL-VS* carriers from the analysis. This model was slightly better than the additive model, as heterozygous carriers of *KL-VS* had 1/10 of a *SD* poorer cognitive function in the CCS (β : $-0.40, p = .06$) and 1/7 of a *SD* poorer cognitive function in the average MMSE (β : $-0.81, p = .02$) than noncarriers.

In contrast, two variants, rs398655 and rs562020, that are located in the 5' end of the gene region were found to associate with both MMSE and CCS. The rs398655 variant is located upstream from the coding region, and carriers of the rs398655 C allele had better cognitive function than noncarriers both on the CCS (β : $0.35, p = .008$) and the MMSE (β : $0.64, p = .003$). Regarding the other variant, rs562020, which is located in the 5' end of the first intron, carriers of the A allele were found to have better cognitive function than noncarriers based on both the CCS (β : $0.30, p = .03$) and the MMSE (β : $0.54, p = .02$; Table 3).

The effects of the rs398655 and rs562020 variants were investigated using post hoc statistical regression models both including

Table 1. Descriptives in the 1905 Birth Cohort at Baseline in 1998 and Follow-up in 2000, 2003, and 2005. The 1910 Birth Cohort and the 1915 Birth Cohort at Baseline in 2010

Assessment Year	1905 Birth Cohort				1910 Birth Cohort	1915 Birth Cohort
	1998	2000	2003	2005	2010	2010
Age, y (SD)	93.14 (0.31)	95.26 (0.31)	97.63 (0.28)	99.64 (0.29)	100.25 (0.32)	95.26 (0.29)
Number of individuals	1651	901	383	182	162	158
Completed CCS	1578	786	274	109	162	158
Mean CCS (SD)	0.23 (3.47)	0.19 (4.15)	0.21 (3.51)	0.30 (3.72)	-0.55 (3.65)	0.84 (3.48)
Completed MMSE	1583	774	292	138	159	158
Mean MMSE (SD)	21.77 (5.78)	21.18 (6.27)	20.27 (6.60)	19.86 (6.36)	21.32 (6.57)	23.70 (4.98)

Notes: CCS = cognitive composite score; MMSE = Mini Mental State Examination; SD = standard deviation.

Table 2. Genotype Distribution and MAF Estimations for the 1905 Birth Cohort and Combined for the 1910 and 1905 Birth Cohorts

SNPs	Position*	Variant Location	1905 Birth Cohort		1910 and 1905 Birth Cohorts Combined	
			Major/Minor Allele	MAF	Major/Minor Allele	MAF
rs397703 ⁵	33,587,329	Upstream gene variant	A/G	0.21	—	—
rs398655	33,587,652	Upstream gene variant XM_005266617: missense variant H585Q	A/C	0.43	A/C	0.43
rs562020	33,592,070	Intron 1 variant	G/A	0.34	G/A	0.32
rs495392	33,592,193	Intron 1 variant	C/A	0.29	C/A	0.28
rs385564	33,592,409	Intron 1 variant	C/G	0.28	—	—
rs575536	33,592,777	Intron 1 variant	G/A	0.27	—	—
rs576404	33,593,100	Intron 1 variant	C/A	0.44	—	—
rs2283368	33,593,270	Intron 1 variant	A/G	0.11	A/G	0.13
rs9526984	33,609,937	Intron 1 variant	A/G	0.09	A/G	0.08
rs1207362 ⁵	33,612,839	Intron 1 variant	C/A	0.25	—	—
rs2320762	33,617,174	Intron 1 variant	A/C	0.36	A/C	0.37
rs1888057	33,622,695	Intron 1 variant	G/A	0.20	—	—
rs657049	33,622,817	Intron 1 variant	A/G	0.28	A/G	0.31
rs683907 [†]	33,624,175	Intron 1 variant	—	—	A/G	0.41
rs687045	33,624,889	Intron 1 variant	A/G	0.44	—	—
rs9536314 [†]	33,627,138	Missense F352V exon 2	A/C	0.15	—	—
rs9527024 [†]	33,627,693	Intron 1 variant	—	—	G/A	0.16
rs9527026	33,628,239	Synonymous variant K385K	G/A	0.16	—	—
rs522796	33,630,055	Intron 3 variant	A/G	0.41	—	—
rs564481	33,634,983	Synonymous variant H589H exon 4	G/A	0.44	—	—
rs648202 [‡]	33,635,463	Synonymous variant A749A exon 4	G/A	0.15	G/A	0.15

Notes: MAF = minor allele frequency; SNP = single-nucleotide polymorphism.

*Genomic position according to genome build GRCh37/hg19 on chromosome 13.

[†]Proxy for KL-VS variant.

[‡]Proxy for rs564481.

⁵Not in Hardy-Weinberg equilibrium.

and leaving out an interaction term. When repeating the analyses conditioning on the KL-VS variant, the associations between the rs398655 variant or the rs562020 variant and MMSE or CCS remained virtually unchanged, suggesting that these variants associate to cognition independently of KL-VS. Also, there was no interaction between the KL-VS variant and the variants rs398655 and rs562020. However, there was significant interaction between rs562020 and rs398655 ($p < .004$), but the two variants are only in partial LD ($D' = 0.62$, $R^2 = .27$). Thus, haplotype analyses were carried out, and as illustrated in the combined analysis (all) in Table 4, the analyses revealed that the haplotype, rs398655/rs562020 AG, that is, the haplotype not carrying any of the minor alleles, was significantly associated with poorer CCS equivalent to 1/5 of a SD ($\beta: -0.76$, $p = .001$) and 1/5 of a SD poorer MMSE ($\beta: -1.10$, $p = .003$). Thus, the haplotype effect was larger than that of KL-VS in the oldest old participants. Also, the haplotype association was strikingly consistent as the associations remained statistical significant across two cognitive measures in the two cohort strata (Table 4). As displayed in Table 5, more individuals are cognitive impaired among carriers of the AG haplotype than noncarriers equivalent to an increased risk (odds ratio) of 45% for being severely impaired (MMSE < 18) and an 37% higher risk of having a CCS lower than the mean (CCS < 0.21) among noncarriers of the KL-VS variant (wild type). The genotype-restricted analyses not only illustrate the effect of the AG haplotype but also show that the AG haplotype is not an underlying effect of the KL-VS variant. In contrast, the risk of the AG haplotype was much smaller and did not reach significance among heterozygotes KL-VS carriers.

Longitudinal Analysis of KL Variants and Cognitive Function

The effect of KL variants on change of cognitive functioning was studied among the 1905 birth cohort participants, who were 92–93 years of age when entering the study and were followed until their 100th year birthday. No significant associations with cognitive decline were observed for the KL-VS variant, the rs398655 variant, or the rs562020 variant. However, there was a tendency for the rs398655C allele to be associated with a steeper rate of cognitive decline as compared to noncarriers (slope: 0.08, p value: .08). Similarly, a more rapid cognitive decline was observed for carriers of the rs22833368 G allele compared with noncarriers with respect to the CCS (slope: -0.19 , p value: .008). In addition, carriers of the rs9526984 G allele declined more rapidly than noncarriers when assessed on the MMSE (slope: -0.38 , p value: .02; Table 6).

Discussion

The KL gene has recently been proposed to be relevant for normal cognitive functioning. In the present study, most of the common genetic variation in KL was tagged using 19 polymorphic variants of which 10 variants were studied across 3 cohorts of oldest old. Here, we observed several associations between cognitive function and the minor alleles of gene variants in the coding region and upstream of KL, which indicates that KL may be relevant in cognitive function among the oldest old participants. Our most noteworthy results are the consistent association with cognition across two measures of

Table 3. Cross-sectional Association Study of KL Gene Variants and CCS and MMSE at Intake of the Participants in the 1905, 1910, and 1915 Birth Cohorts

SNP	1905 Birth Cohort			1910 and 1915 Birth Cohorts			All			
	CCS		MMSE	CCS		MMSE	CCS		MMSE	
	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value
rs397703*	0.13 (0.19)	.51	0.48 (0.31)	.13	—	—	—	—	—	—
rs398655*	0.41 (0.15)	.006	0.77 (0.25)	.002	0.13 (0.26)	.61	0.30 (0.44)	.50	0.35 (0.13)	.008
rs62020	0.21 (0.16)	.19	0.44 (0.26)	.09	0.54 (0.28)	.05	0.82 (0.47)	.08	0.30 (0.14)	.03
rs495392	0.20 (0.17)	.24	0.50 (0.28)	.07	0.20 (0.29)	.49	-0.02 (0.50)	.97	0.21 (0.15)	.16
rs385564	-0.02 (0.16)	.92	-0.67 (0.27)	.01	—	—	—	—	—	—
rs575536	-0.07 (0.17)	.70	0.41 (0.28)	.14	—	—	—	—	—	—
rs576404	0.02 (0.15)	.89	0.24 (0.24)	.32	—	—	—	—	—	—
rs2283368	0.44 (0.23)	.06	0.04 (0.38)	.91	0.14 (0.37)	.71	0.68 (0.63)	.29	0.36 (0.20)	.07
rs9526984	0.31 (0.26)	.24	-0.14 (0.43)	.74	-1.08 (0.46)	.02	-1.52 (0.79)	.06	-0.04 (0.23)	.85
rs1207362	-0.04 (0.17)	.80	-0.06 (0.27)	.84	—	—	—	—	—	—
rs2320762	0.09 (0.15)	.56	-0.05 (0.25)	.83	-0.62 (0.27)	.02	-1.06 (0.45)	.02	-0.10 (0.13)	.46
rs1888057	0.23 (0.19)	.23	0.28 (0.32)	.38	—	—	—	—	—	—
rs657049	0.23 (0.17)	.18	0.11 (0.28)	.70	-0.26 (0.28)	.35	-0.43 (0.47)	.36	0.10 (0.15)	.50
rs687045	-0.15 (0.15)	.32	-0.08 (0.25)	.75	—	—	—	—	—	—
rs9536314†/rs9527024	0.07 (0.21)	.75	-0.64 (0.34)	.06	-0.40 (0.34)	.24	-0.47 (0.59)	.42	-0.06 (0.18)	.74
rs9527026	0.07 (0.21)	.75	-0.48 (0.34)	.16	—	—	—	—	—	—
rs522796	0.07 (0.15)	.65	0.31 (0.25)	.22	—	—	—	—	—	—
rs564481/rs683907	-0.13 (0.15)	.36	-0.01 (0.25)	.97	0.49 (0.26)	.06	1.05 (0.46)	.02	0.02 (0.13)	.86
rs648202	0.29 (0.22)	.17	0.79 (0.35)	.03	-0.17 (0.39)	.66	-0.48 (0.66)	.47	0.19 (0.19)	.33

Notes: CCS = cognitive composite score; MMSE = Mini Mental State Examination; SE = standard error; SNP = single-nucleotide polymorphism. Analyses were conducted using a linear regression model and a genetic additive model adjusted for age, sex, and time (year of birth). Significant results are in italic ($p < .05$).

*Upstream of the KL gene.

†Tagging the KL-VS variant.

Table 4. Cross-sectional Haplotype Study With CCS and MMSE at Intake of the Participants in the 1905, 1910, and 1915 Birth Cohort

Haplotypes	1905 Birth Cohort			1910 and 1915 Birth Cohorts			All					
	CCS		MMSE	CCS		MMSE	CCS		MMSE			
	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value		
AG	-0.63 (0.26)	.01	-0.95 (0.42)	.03	-1.08 (0.44)	.01	-1.48 (0.75)	.049	-0.76 (0.22)	.001	-1.10 (0.37)	.003
AA	-0.19 (0.22)	.38	-0.10 (0.36)	.78	-0.55 (0.39)	.15	-0.41 (0.65)	.53	-0.28 (0.19)	.13	-0.19 (0.31)	.54
CG	0.22 (0.22)	.32	0.59 (0.35)	.10	-1.08 (0.37)	.003	-1.28 (0.63)	.04	-0.12 (0.19)	.51	0.07 (0.31)	.83
CA	0.14 (0.21)	.51	0.42 (0.35)	.23	0.08 (0.37)	.82	0.25 (0.63)	.69	0.13 (0.19)	.48	0.36 (0.31)	.24

Notes: CCS = cognitive composite score; MMSE = Mini Mental State Examination; SE = standard error. Haplotypes were estimated from rs398655 and rs562020. Analyses were conducted using a linear regression model and a model adjusted for age, sex, and time (year of birth). Significant results are in italic ($p < .05$).

cognitive performance, CCS and MMSE, of the upstream positioned rs398655 variant, and the rs562020 variant, situated in the far 5' end of the first intron of *KL*. Also, it is noteworthy that the effect of the combined AG haplotype of these variants was larger than the effect of the most intensively studied variant in *KL*, the KL-VS variant, in the oldest old participants. In addition, the rs2283368 and rs9526984 variants were significantly associated with the slope of decline, but these were also restricted to only one of the two cognitive measures.

In the present study, the KL-VS variant was associated with poorer cognitive performance on the MMSE scale, and this was further supported when applying the Dubal and coworkers model restricted to heterozygous KL-VS variant carriers and noncarriers, which led the results in the CCS to become borderline significant. Nonetheless, these results are contrary to those found recently by Dubal and coworkers (28). We cannot rule out that the results in the present study may simply be chance findings, but we must emphasize that both CCS, MMSE, and Dubal and coworkers cognitive tests are combined tests that share elements of working memory in addition to specific elements, that is, speeded tasks in CCS and basic orientation tasks in MMSE. Another explanation for the difference in the two studies is that the association is age dependent; this is supported by the notion that our observations were made among individuals aged 92 years and older, whereas the initially report by Dubal and coworkers was observed among middle-aged and elderly individuals with a maximum age of 85 years. Secondly, Dubal and coworkers reported a potential regression towards the mean with age, a tendency that could continue to the oldest old ages thereby supporting the discordance between the two studies (28). Thirdly, an age dependent effect of the KL-VS variant has analogously been observed in longevity studies (26). Thus, the *KL* gene variant may exemplify that a genetic effect can be age dependent not only for mortality but also for aging phenotypes such as cognitive function.

The novel finding that the rs562020 variant is associated with cognitive performance could be of substantial interest because the variant is situated in a potential regulatory element. Also, rs562020 is not in LD with other variants with obviously functional effects according to the HaploReg V2 database (Broad Institute, MIT). Additionally, rs562020 uniquely modifies a binding site for the zinc-finger transcriptional repressor protein, RP58, which in mice is a key regulator of neuronal migration (39). The rs398655 variant may likewise be important in gene regulation, but it seems more obvious that the association is caused by one or several other variants in LD with rs398655. Many of these variants were not investigated in the present study and are both located in enhancer elements and/or modify one or several transcriptional binding sites. An alternative hypothesis is that rs398655 causes a missense variation, H585Q, in a yet only hypothetically predicted transcript (XM_005266617) that expectedly encodes an uncharacterized protein LOC101927403 (NCBI database). Similarly, the variant G395A (rs1207568), which is important in other age-related phenotypes, is predicted to be a missense variant, P85S, in the same transcript, but in the present study, it was not found to be associated with cognition (by proxy rs397703). The complexity of the *KL* gene variants, including rs398655 and rs562020, that is, whether they themselves are functional or whether they are proxies for functional variants, is difficult to deduce. One way of understanding the complexity is by functional studies that characterize the regulatory elements of the *KL* gene as well as the possible role of the hypothetical protein LOC101927403. Another inform came from our haplotype analyses that suggest a combined role of rs398655 and rs562020

Table 5. Summary Statistics of the Proportion of Cognitive Impaired Participants in the Combined Cohort

	Haplotype AG Absent		Haplotype AG Present	
	% Severely Impaired (N)		% Severely Impaired (N)	
WT	15% (275)		20% (772)	1.45 (1.00–2.13)
Heterozygous KL-VS	22% (25)		22% (314)	1.05 (0.49–2.25)
Homozygous KL-VS	0% (3)		18% (34)	
OR (95% CI)	1.72 (0.78–3.80)		1.21 (0.88–1.67)	

	Haplotype AG Absent		Haplotype AG Present	
	% With Low Cognition on the CCS (N)		% With Low Cognition on the CCS (N)	
WT	42% (275)		49% (772)	1.37 (1.03–1.80)
Heterozygous KL-VS	53% (25)		50% (314)	0.88 (0.46–1.66)
Homozygous KL-VS	0% (3)		32% (34)	
OR (95% CI)	1.61 (0.85–3.03)		1.05 (0.80–1.36)	

Notes: CCS = cognitive composite score; CI, confidence interval; MMSE = Mini Mental State Examination; OR = odds ratio; WT = wild type. The upper table displays the percentage of severely impaired individuals (MMSE < 18), and the lower table displays the percentage of individuals with a cognition (CCS) lower than the mean (CCS < 0.21). Total numbers are in brackets. The groups are stratified by the presence or absence of the AG, rs398655, and rs562020 haplotype and by the KL-VS genotype. Homozygous KL-VS are not included in OR calculations. OR are adjusted for sex, age, and birth year and presented in columns or rows with reference to the KL WT or absence of the AG haplotype.

Table 6. The Longitudinal Association Study of KL Gene Variants Associated With CCS and MMSE by Random Effect Models at Intercept 93 Years of Age and Yearly Decline (Slope) for Participants in the 1905 Birth Cohort

SNP	CCS Baseline		CCS Slope		MMSE Baseline		MMSE Slope	
	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value	Coefficient (SE)	p Value
rs397703*	0.10 (0.19)	.60	-0.11 (0.06)	.07	0.47 (0.31)	.14	-0.17 (0.12)	.14
rs398655*	0.41 (0.15)	.007	-0.08 (0.05)	.08	0.80 (0.25)	.001	-0.08 (0.09)	.40
rs562020	0.17 (0.16)	.29	-0.04 (0.05)	.46	0.42 (0.26)	.11	0.10 (0.10)	.32
rs495392	0.14 (0.17)	.41	-0.08 (0.06)	.13	0.54 (0.28)	.05	-0.07 (0.10)	.48
rs385564	-0.04 (0.17)	.80	-0.05 (0.05)	.41	-0.68 (0.27)	.01	0.03 (0.10)	.82
rs575536	0.03 (0.17)	.84	0.08 (0.05)	.16	0.44 (0.28)	.12	-0.04 (0.10)	.67
rs576404	-0.06 (0.15)	.70	0.01 (0.05)	0.98	0.22 (0.24)	.37	0.01 (0.09)	.91
rs2283368	0.35 (0.24)	.14	-0.19 (0.07)	.008	0.03 (0.38)	.94	0.03 (0.14)	.81
rs9526984	0.24 (0.27)	.38	-0.14 (0.09)	.10	-0.01 (0.43)	.99	-0.38 (0.16)	.02
rs1207362	-0.02 (0.17)	.91	0.08 (0.05)	.13	-0.08 (0.27)	.78	0.10 (0.10)	.30
rs2320762	0.08 (0.15)	.61	0.03 (0.05)	.57	-0.02 (0.25)	.94	-0.09 (0.09)	.31
rs1888057	0.30 (0.20)	.13	-0.09 (0.07)	.18	0.24 (0.32)	.46	0.08 (0.12)	.53
rs657049	0.28 (0.17)	.11	-0.10 (0.06)	.09	0.13 (0.28)	.65	-0.05 (0.10)	.61
rs687045	-0.22 (0.15)	.15	0.03 (0.05)	.52	-0.11 (0.25)	.65	0.08 (0.10)	.39
rs9536314†	-0.01 (0.21)	.99	-0.11 (0.07)	.10	-0.62 (0.34)	.07	-0.12 (0.13)	.33
rs9527026	-0.03 (0.21)	.90	-0.10 (0.07)	.13	-0.51 (0.34)	.13	-0.15 (0.13)	.23
rs522796	0.16 (0.15)	.29	0.04 (0.05)	.39	0.35 (0.25)	.16	-0.02 (0.09)	.87
rs564481	-0.19 (0.15)	.22	0.04 (0.05)	.45	-0.03 (0.25)	.90	0.09 (0.09)	.34
rs648202	0.41 (0.22)	.06	-0.05 (0.08)	.48	0.73 (0.35)	.04	0.04 (0.13)	.79

Notes: CCS = cognitive composite score; MMSE = Mini Mental State Examination; SE = standard error; SNP = single-nucleotide polymorphism. Analyses were adjusted for sex and time from intake. Significant results are in italic (*p* < .05).

*Upstream of the KL gene.

†KL-VS variant.

because the AG haplotype, which is composed of the major alleles of the two variants, was robustly associated with a poorer cognitive level across both cognitive measures and cohort strata and even independent of KL-VS. As the effect was even stronger than that of KL-VS, we suggest that this haplotype is a risk factor for cognitive deterioration and function in the oldest old group. The notion that multiple genetic variations in KL are important is supported by other genetic variants, C1818T and G395A, which were previously associated with various phenotypes.

There are several advantages to this study. First of all, we used tagging SNPs to cover the major part of the genetic variation in KL, and thus, we were able to detect novel associations with SNPs other than the most obvious candidate SNPs, C1818T, G395A, and KL-VS. Secondly, the span of cognitive function varies more at extreme old ages compared with at younger ages, and thus, there is likely a gain of power by studying oldest old participants. Thirdly, it is possible that some gene variants associate more strongly with cognition in selected populations, such as the oldest old group, compared to less

age-selected populations. If these scenarios indeed are the reasons why we observed associations between novel *KL* variants and cognition, then our study setup might be ideal for identifying other novel gene variants in relation to cognitive functioning.

Our study also included a longitudinal analysis enabling us to examine the genetic effect of cognitive decline at the very old ages. We did not find a consistent impact of any of the *KL* variants on the slope of cognitive decline, but a tendency towards an association between a higher cognitive level and a more rapid decline was observed for the rs398655 C allele. This is similar to an observation found for variants in the *CLU* gene, and it probably indicates a regression towards the mean. We previously suggested that this trend could be explained by individuals with a high initial cognitive level having more room for declining compared to those with a lower initial cognitive level (38). In contrast, Deary and coworkers stressed that their longitudinal cohort study showed that the *KL-VS* gene variant associated with intelligence similarly in early and old age, thus suggesting that the *KL-VS* gene variant may impact on cognition in a large span of life (27). However, their study participants were younger than participants in the present study, and their findings may therefore not necessarily apply to the oldest old group.

There are also several drawbacks to our study that should be mentioned. First of all, the study only includes oldest old Danes; thus, our novel findings may not necessarily be generalized to younger cohorts. Also, our results may only apply to participants who are within the normal range cognitively and cannot necessarily be extrapolated to clinically relevant functional decline. Secondly, there may be population differences that could influence the effects of the association between *KL* variants and cognition when generalized to other populations. One such explanation could be that the *KL-VS* variant is common in European populations and almost nonexistent in East Asian populations.

In conclusion, we suggest that several *KL* variants are important for normal-range cognitive function in the oldest old group. Also, we present evidence that suggests the genetic effect of *KL-VS* carriers on cognitive function is dependent on the ages of the elderly individuals. Thus, our results suggest that age is a crucial factor both when exploring novel associations between gene variants and age-related phenotypes and when exploring known gene candidates in associations with new phenotypes.

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