Vitamin D receptor and megalin gene polymorphisms and their associations with longitudinal cognitive change in US adults[1–3]

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ABSTRACT

Background: Vitamin D receptor (VDR) and the megalin gene polymorphism's link with longitudinal cognitive change remains unclear.

Objective: The associations of single nucleotide polymorphisms (SNPs) for VDR [rs11568820 (*CdX*-2:T/C), rs1544410 (*Bsm*I:G/A), rs7975232 (*Apa*I:A/C), rs731236 (*Taq*I:G/A)], and Megalin (rs3755166:G/A; rs2075252:C/T; rs4668123:C/T) genes with longitudinal cognitive performance changes were examined.

Design: Data from 702 non-Hispanic white participants in the Baltimore Longitudinal Study of Aging were used. Longitudinal annual rates of cognitive change (LARCCs) between age 50 y and the individual mean follow-up age were predicted with linear mixed models by using all cognitive score time points (prediction I) or time points before dementia onset (prediction II). Latent class, haplotype, and ordinary least squares (OLS) regression analyses were conducted.

Results: Among key findings, in OLS models with SNP latent classes as predictors for LARCCs, $Megalin_2$ [rs3755166(–)/rs2075252(TT)/rs4668123(T−)] compared with $Megalin_1$ [rs3755166(–)/rs2075252(CC)/rs4668123(–)] was associated with greater decline among men for verbal memory (prediction II). Significant sex differences were also found for SNP haplotype (SNPHAP). In women, VDR_1 [*Bsm*I(G−)/*Apa*I(C−)/*Taq*I(A−); baT] was linked to a greater decline in category fluency (prediction I: $\beta = -0.031$, $P = 0.012$). The $Megalin_1$ SNPHAP (GCC) was related to greater decline among women for verbal memory, immediate recall [California Verbal Learning Test (CVLT), List A; prediction II: $\beta = -0.043$, $P = 0.006$) but to slower decline among men for delayed recall (CVLT-DR: $\beta > 0$, $P < 0.0125$; both predictions). In women, the $Megalin_2$ SNPHAP (ACC) was associated with slower decline in category fluency (prediction II: $\beta = +0.026$, $P = 0.005$). Another finding was that Megalin SNP rs3755166:G/A was associated with greater decline in global cognition in both sexes combined and in verbal memory in men.

Conclusion: Sex-specific VDR and Megalin gene variations can modify age-related cognitive decline among US adults. *Am J Clin Nutr* 2012;95:163–78.

INTRODUCTION

Vitamin D's biological effect on the brain function was shown in recent studies, specifically in terms of neuroprotection (mainly mediated by calcium, nerve growth factor, and neurotrophin 3), immunomodulation, and detoxification (1–9). A few receptors and binding proteins mediate vitamin D functions in both animals and humans. VDR[4], a part of the nuclear hormone receptor superfamily, is expressed in many organs. Whereas vitamin D deficiency in animals was associated with changes in brain morphology (10), locomotion (11, 12), learning, and memory (13), a dysfunctional VDR was linked to anxiety-like behavior in mice (14, 15). Among humans, vitamin D deficiency was related to mood disorders and poor cognitive functioning (16, 17). However, only a handful of recent studies have examined VDR gene polymorphisms in relation to cognition among older adults (18, 19), and none so far have examined longitudinal change in cognitive abilities.

Another endocytic vitamin D binding receptor, known as megalin or LRP2, is expressed in many epithelial cells including those of the choroid plexus (ie, blood-brain barrier) and belongs to the LDL receptor family (20, 21). Megalin also binds apoE (22), a protein involved in redistribution of cholesterol for nerve repair (23). In fact, the ApoE genotype was associated with cognitive impairment, decline, and dementia, particularly AD (24, 25), as well as a number of neurobiological factors implicated in dementia: β-amyloid deposition, tangle formation, oxidative stress, lipid homeostasis dysregulation, synaptic plasticity loss, and cholinergic dysfunction (26). Importantly, megalin in choroid plexus directly participates in clearance of β-amyloids (27–30) and is involved in neuroprotection by binding and transcytosis of insulin-like growth factor I (30). The expression of megalin

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[4] Abbreviations used: AD, Alzheimer disease; ApoE, apolipoprotein E; BLSA, Baltimore Longitudinal Study of Aging; BVRT, Benton Visual Retention Test; CVLT, California Verbal Learning Test; CVLT-DR, California Verbal Learning Test, Delayed Recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; DSM-III-R, *Diagnostic and Statistical Manual of Mental Disorders III, revised*; LARCC, longitudinal annual rate of cognitive change; LC, latent class; LCA, latent class analysis; LD, linkage disequilibrium; LRP2, LDL receptor–related protein 2; MMSE, Mini-Mental State Examination; OLS, ordinary least squares; SNP, single nucleotide polymorphism; SNPHAP, single nucleotide polymorphism haplotype; SNPLC, single nucleotide polymorphism latent class; Trails A, Trailmaking Test, part A; Trails B, Trailmaking Test, part B; VDR, vitamin D receptor; VFT-C, Verbal Fluency Test-Categories; VFT-L, Verbal Fluency Test-Letter.

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is regulated by serum vitamin D and vitamin A (31). Vitamin D requires another protein, namely vitamin D binding protein, to bind to megalin and enter cells (32–35). Despite the biologically plausible involvement of megalin in AD pathogenesis, only 2 recent studies thus far have examined the relation between megalin gene polymorphisms and incident AD (20, 36).

To our knowledge, this is the first longitudinal study to test the associations of VDR and megalin SNP, SNP LCs, and SNPHAPs with longitudinal changes in cognitive function with the use of a large long-term study in US adults.

METHODS

Database and study participants

Data from the BLSA were used. The BLSA, initiated in 1958, is an ongoing prospective open-cohort study in community-dwelling, generally highly educated, upper to middle class adults aged 17–97 y at baseline (60.1% men) with a total enrollment of 3005 (37); exclusionary criteria are summarized elsewhere (38). Medical history was determined, and physical, neurological, and neuropsychological examinations were conducted in the BLSA's protocol, which has continued approval from the institutional review board of Medstar Research Institute.

In the present study, eligible participants (n = 2321) had at least one visit at or later than age 50 y and were at risk of dementia; 1917 of whom were non-Hispanic whites. Complete genetic, anthropometric, and other covariate data in eligible participants were available for 702 BLSA participants; data on cognitive function were available for n = 459 for the Trails A and B (megalin gene) and up to n = 616 for the BVRT (VDR gene).

Clinical evaluation of dementia

Annual follow-ups were conducted in all participants, and a consensus conference review was carried out if their Blessed Information Memory Concentration score (39) was $\geq$4, if their informant or subject Clinical Dementia Rating (40) score was $\geq$0.5, or if their Dementia Questionnaire (41) was abnormal. By using DSM-III-R (42) criteria, dementia diagnosis was determined and the age of onset was estimated on the basis of consecutive case conference findings. When participants had either single domain cognitive impairment (usually memory) or cognitive impairment in multiple domains without any significant functional loss in activities of daily living, a diagnosis of mild cognitive impairment was made following the Petersen algorithm (43). In our present analysis, mild cognitive impairment cases were retained. However, 2 sets of analyses were conducted taking into account year of onset of dementia.

Cognitive assessment

A battery of 6 selected cognitive tests was used: the MMSE (44); BVRT (45); CVLT, List A (summation score across 5 learning trials) and delayed free recall score (DR) (46); verbal fluency tests, both letter (VFT-L) (47–49) and category (VFT-C) (50); Trails A and B (51); and DS-F and DS-B (52) (*see* Supplemental Material 1 under "Supplemental data" in the online issue). Linear mixed models with a quadratic age term (to allow for nonlinear age effects) were applied to predict cognitive score values at specific ages, particularly the mean individual age at follow-up, taking all time points until the end of follow-up (prediction I) or time points before the onset of dementia (prediction II), and to predict the slope for annual cognitive change at that particular age. The latter, which is termed LARCC was the main outcome of interest. It can be interpreted as the annual rate of change in the cognitive score between age 50 y and the mean age of follow-up per individual and cognitive test. After this estimation, LARCCs for each cognitive test score were entered into a factor analysis model as measured variables (53) in which a number of common factors were extracted on the basis of common variance, factor loadings estimated, and the residual variance labeled as uniqueness for each LARCC. The common factor model can be summarized as follows:

$$\text{LARCC}_i = \sum_{j=1}^{k} \lambda_{ij} \times Domain_j + \varphi_i \qquad (1)$$

where LARCC_i is the standardized z score for each cognitive test LARCC, λ_{ij} is the factor loading for each LARCC and each factor, domain_j is the standardized z score for each factor j, and φ_i is the residual error, the squared value of which is the uniqueness. The sum of squared factor loadings for each LARCC_i is the communality or the common variance that is accounted for by the extracted factors. An eigenvalue >1 rule was used and the scree plot was observed to determine the adequate number of extracted factors that would produce the best model fit. The factor loadings were then rotated by using varimax orthogonal rotation, and the factors were interpreted and cognitive domains labeled accordingly, with a cutoff of $\geq$0.40 for significant loading. The factor scores (z scores) were predicted and used as markers of LARCC for specific cognitive domains. Domains were labeled on the basis of the combination of significantly high factor loadings and the corresponding measured variables or LARCC_i as follows: domain 1, "Memory and executive function: earlier decline"; domain 2, "Verbal fluency and attention: later decline." With the exception of Trails B, all LARCC_i factor loadings were significant for only 1 of the 2 domains, creating a relatively simple structure that was easy to label and interpret. The labels were determined on the basis of the nature of the cognitive test and the timing that decline in those domains is usually observed during the life course (earlier compared with later). (*See* Supplemental Material 2 under "Supplemental data" in the online issue for results of the factor analysis.)

VDR and megalin SNPs, SNP LCs, and SNPHAPs

Blood samples were collected for DNA extraction, and genomewide genotyping was completed for 1231 subjects by using Illumina 550K. EIGENSTRAT (http://genepath.med.harvard.edu/~reich/Software.htm) analysis using ~10,000 randomly selected SNPs from the 550K SNP panel was used to select the subjects of European descent using HapMap CEU (http://hapmap.ncbi.nlm.nih.gov/) [Utah residents with northern and western European ancestry from the CEPH (Council on Education for Public Health) collection] as the reference population (54). In addition, part of our main analyses was adjusted for the top 2 principal components to control for any residual effects of population structure (54). Moreover, the HapMap CEU sample (build 36) was used as a reference to impute ~2.5 million SNPs using MACH (http://www.sph.umich.edu/csg/abecasis/MACH/tour/imputation.html) (55). Imputed SNPs with an

imputation quality $r^2 < 0.3$ or minor allele frequency of <1% were excluded from the analysis. The selection of SNPs of interest was solely based on those selected in previous confirmatory studies, many of which were identified by genomewide association studies, relating cognitive function, decline, or dementia to VDR (18, 19) and megalin (20, 36) gene polymorphisms. Most of those selected SNPs were available in our database, with few exceptions (eg, VDR SNP rs10735810, *Fok*I: G/A). Note that some VDR SNPs were also studied in relation to other phenotypes, including body composition in old age, obesity, bone mineral density, the metabolic syndrome, type 2 diabetes, and coronary artery disease (56–62). Consequently, 4 VDR SNPs [rs11568820 (*CdX*-2:T/C), rs1544410 (*Bsm*I:G/A), rs7975232 (*Apa*I:A/C), and rs731236 (*Taq*I:G/A)] and 3 megalin SNPs (rs3755166: G/A; rs2075252: C/T; rs4668123: C/T) were chosen as long as they had reliable values. Those SNPs and their locations on each gene and their distributions are shown in **Figure 1**.

VDR and megalin SNP LCs were obtained by using LC analysis (PROC LCA in SAS version 9.1; SAS Institute) (63, 64), in which sex and first-visit age were introduced as potential covariates and each selected SNP per gene was entered into that model (one gene per model) as a 3-level categorical variable. Model fit was determined on the basis of Akaike Information Criterion and Bayesian Information Criterion, which led to deciding the appropriate number of LCs. Posterior probabilities were estimated by using the Bayes theorem, and those were the same for all individuals with a specific SNP pattern per gene. On the basis of those posterior probabilities, each individual was labeled as belonging to a specific LC when the posterior probability for this class was >0.50, and the higher this probability the more the certainty of belonging to this class. In most cases, it is expected that this posterior probability is >0.90 (63). These SNPLCs, in terms of SNP combinations, are shown in more detail in Figure 1.

SNPHAPs were also considered as main predictors in our analysis for each of the 2 genes. For the VDR gene, the *Bsm*I:G/A, *Apa*I:G/A, and *Taq*I:G/A SNPs were combined in that order to form SNPHAP, and their proportions in the population were found to be similar to those found in at least one previous study (18). As a result, 3 SNPHAPs were found in this population with one of the following SNP combinations for 1 or 2 alleles: VDR_1, GCA (baT); VDR_2, AAG (BAt); or VDR_3, GAA (bAT). Participants were coded as 0 = having no VDR_x haplotype, 1 = having one allele carrying the VDR_x haplotype, 2 = having 2 alleles carrying the VDR_x haplotype. This approach was also applied to the 3 megalin SNPs, and 8 haplotypes were found. However, only 3 of them were considered in the main analysis because their proportion in the population (with 1 or 2 copies) was >10% (*see* Figure 1 for more details).

Covariates

Three sets of covariates were considered as potential confounders in the main associations of interest: *1*) sociodemographic factors, namely individual age at first visit and mean ages at follow-up (per individual and cognitive test), sex, educational attainment (years of schooling), and one lifestyle-related factor, namely smoking status (never, former or current smoker); *2*) self-reported history of type 2 diabetes, hypertension, cardiovascular disease (stroke, congestive heart failure, nonfatal myocardial infarction, or atrial fibrillation), and dyslipidemia at first visit; and *3*) measured first-visit BMI (in kg/m^2). Moreover, first-visit blood pressure (systolic and diastolic in mm Hg), plasma total and HDL cholesterol, and fasting blood glucose (in mg/dL) were analyzed only in relation to availability of genetic data for descriptive purposes, given their higher proportion with missing data compared with the self-reported conditions.

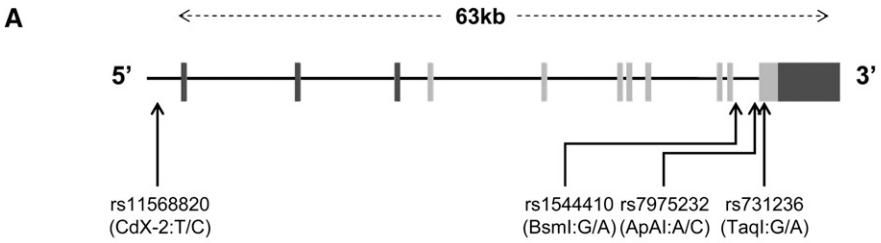
Statistical analysis

For each gene SNP that was included in our analyses, Hardy-Weinberg equilibrium was examined by using an exact test, and pairwise LD was calculated by using the Haploview version 4.2 package (65, 66). The LD map for all available SNPs of VDR and megalin genes are presented in Supplemental Material 3 under "Supplemental data" in the online issue. To describe the study participant characteristics and to compare them by genetic data availability, 1-factor ANOVA, *t* test, and chi-square test were used.

Furthermore, multiple OLS linear regression analysis was carried out to examine the association of VDR and megalin SNPs, SNPLCs, and SNPHAPs with predicted LARCCs for each cognitive test or domain and from each predictive model, after potential confounding variables including first-visit age, mean age of follow-up, sex, education, first-visit smoking status, self-reported comorbid conditions, and BMI were controlled for. SNPs (wild-type with variant v) were examined both in terms of genotype, comparing the 2 variant genotypes (wv, wild type-variant; vv, variant-variant) with the wild-type genotype (ww), and in terms of dosage of the variant allele (v). In the latter case, a *P* value for trend was computed. *P* values for trend were also computed when testing the association between each haplotype dosage (0, 1, and 2 copies) and the cognitive outcomes of interest.

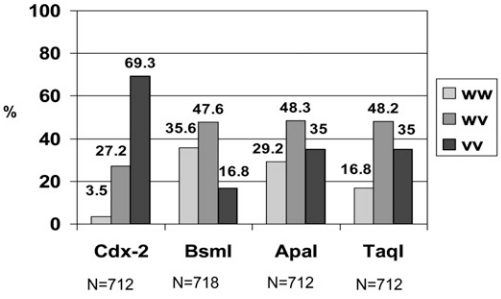
To account for potential selection bias in OLS models (due to the nonrandom selection of participants with genetic data from the target study population), a 2-stage Heckman selection model was constructed (67) by using a probit model to obtain an inverse mills ratio at the first stage (derived from the predicted probability of being selected, conditional on the covariates in the probit model), as was done in an earlier study (24). The inverse mills ratio was then included into the main OLS models at a second stage to adjust for this selection bias. Stratification was made, and effect modification was tested (by adding interaction terms) by sex for the analysis when SNPs, SNPLCs, and SNPHAPs were the main predictors, particularly when the megalin SNP was included in the analysis. In fact, sex differences in the association between the megalin gene polymorphism and cognitive outcomes were hypothesized a priori, as discussed later (68–70).

A type I error of 0.05 was considered for all analyses, and *P* values between 0.05 and 0.10 were considered to be borderline significant for main effects, whereas a $P < 0.10$ was considered significant for interaction terms (71), before correction for multiple testing. Correction for multiple testing was performed by using a family-wise Bonferroni procedure whereby a family was defined by a cognitive test or a cognitive domain, with the assumption that the family was independent in content though not necessarily in its degree of correlation (72). Within each cognitive test, there were generally 2 test scores and 2 predictions to take into account for correction. This was the case for the CVLT-DR and CVLT-List A, Trails A and B, DS-F and DS-B, and VFT-C and VFT-L. For these cognitive tests, the significance criterion for *P* and *P*-trend was reduced to $P = 0.05/$



VDR SNP		Dprime			
		rs11568820	rs1544410	rs7975232	rs731236
R^2	rs11568820	1	0.168	0.216	0.177
	rs1544410	0.009	1	1	1
	rs7975232	0.009	0.571	1	1
	rs731236	0.01	0.985	0.579	1

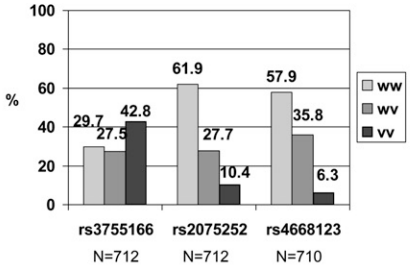
SNPHAP (any allele, three SNP) BsmI/ApaI/TaqI	% (n=702)	SNPLC (two alleles) BsmI/TaqI	% (n=719)
VDR_1: GCA: baT	45.6%	VDR_1: AG/GA	47.8%
VDR_2: AAG: BAt	40.5%	VDR_2: AA/GG	17.0%
VDR_3: GAA: bAT	13.5%	VDR_3: GG/AA	35.2%



SNPHAP (allelic copies of the haplotype) BsmI/ApaI/TaqI	No copy (0)	One copy (1)	Two copies (2)
VDR_1: GCA: baT	29.2%	50.4%	20.4%
VDR_2: AAG: BAt	35.6%	47.7%	16.7%
VDR_3: GAA: bAT	74.4%	24.2%	1.4%

LRP2		Dprime		
		rs3755166	rs4668123	rs2075252
R^2	rs3755166	1	0.183	0.191
	rs4668123	0.009	1	0.697
	rs2075252	0.008	0.427	1

SNPHAP (any allele, three SNP) (rs3755166 /rs4668123 /rs2075252)	% (n=702)	SNPLC (two alleles) rs4668123 (n=719)	% (n=719)
$Megalin_1$:GCC	37.4%	$Megalin_1$:CC	63.8%
$Megalin_2$:ACC	33.3%	$Megalin_2$:TT	8.2%
$Megalin_3$:GTT	11.0%	$Megalin_3$:CT	28.0%
$Megalin_4$:ATT	5.9%		
$Megalin_5$:GTC	4.3%		
$Megalin_6$:ATC	3.0%		
$Megalin_7$:GCT	3.0%		
$Megalin_8$:ACT	2.1%		



SNPHAP (allelic copies of the haplotype) (rs3755166/rs4668123/rs2075252)	No copy (0)	One copy (1)	Two copies (2)
$Megalin_1$:GCC	47.3%	38.9%	13.8%
$Megalin_2$:ACC	35.5%	53.4%	11.1%
$Megalin_3$:GTT	74.1%	24.2%	1.7%

FIGURE 1. A: Gene structure of the VDR gene. The SNP and gene coordinates are based on NCBI build 36 (hg18, March 2006) using RefSeq gene prediction. The VDR gene on chromosome 12 is composed of ≤11 exons spanning ~63 kb. Note: More than 99% of eligible participants had well-defined SNPLCs that could be summarized by *Bsm*I and *Taq*I SNP combinations. SNPHAPs were defined on the basis of 3 VDR SNP combinations (*Bsm*I, *Apa*I, and *Taq*I) and were expressed as dosage (0 = none, 1 = one copy, 2 = 2 copies) in the main analysis. B: Gene structure of the megalin gene. The SNP and gene coordinates are based on NCBI build 36 (hg18, March 2006) using RefSeq gene prediction. The megalin (*LRP2*) gene on chromosome 2 has 79 exons and is ~235 kb in size. Note: More than 96% of eligible participants had well-defined SNPLCs that could be summarized by the genotype of rs4668123. SNPHAPs were defined on the basis of all 3 megalin SNP combinations (rs3755166, rs4668123, and rs2075252) and were expressed as dosage (0 = none, 1 = one copy, 2 = 2 copies) in the main analysis. hg, human genome; LRP2, LDL receptor-related protein 2; NCBI, National Center for Biotechnology Information; RefSeq, reference sequence; SNP, single nucleotide polymorphism; SNPLC, single nucleotide polymorphism latent class; SNPHAP, single nucleotide polymorphism haplotype; VDR, vitamin D receptor gene; vv, variant-variant; wv, wild-type–variant; ww, wild-type–wild-type.

4 = 0.0125 (marginal significance: $P = 0.10/4 = 0.025$). In the case of the MMSE (a measure of global cognition), BVRT, and cognitive domains (domains 1 and 2, produced by factor analysis of LARCCs with orthogonal rotation), only prediction was taken into account. In fact, they were deemed to be independent in content of other tests and of each other, while having only one score each, and thus the significance criterion was reduced to only $P = 0.05/2 = 0.025$ (marginal significance to $P = 0.10/2 = 0.05$). After correction for multiple testing, and due to their lower statistical power compared with main effects (71), interaction terms had their critical P values reduced to 0.05. All analyses (except for LCA) were performed by using Stata version 11.0 (73).

RESULTS

Study sample characteristics

Study sample characteristics are presented in **Table 1,** and the eligible group with genetic data available was compared with those without available genetic data. Generally, participants with complete genetic data were younger (mean age: 52.3 compared with 60.8 y), had higher proportion of women (47.8% compared with 26.9%), had higher educational attainment (mean education: 16.8 compared with 16.6 y), were less likely to be current smokers (18.5% compared with 25.3%), and were healthier in terms of continuous BMI, systolic and diastolic blood pressure, total cholesterol, fasting glucose concentration, and some comorbid conditions (type 2 diabetes, hypertension, and cardiovascular disease) ($P < 0.05$ on the basis of t test or chi-square test). By the end of follow-up, 50 participants developed dementia in the group with available genetic data (7.1%) as compared with 17.4% ($n = 212$) in the group with unavailable genetic data. Moreover, LARCCs were indicative of larger cognitive declines among participants with no genetic data available compared with those with genetic data for most cognitive tests (Table 1).

All of the SNPs examined were in Hardy-Weinberg equilibrium ($P > 0.05$). Within the VDR gene, 3 SNPs (*Bsm*I, *Apa*I, *Taq*I) were in LD ($r^2 > 0.5$), whereas the *CdX*-2 SNP was independent. In the megalin gene, rs4668123 and rs2075252 were in moderate LD ($r^2 = 0.42$), whereas rs3755166 was independent (Figure 1). Genotypic frequencies indicated that, for each SNP, one genotype had a relative frequency >40% and thus was dominant compared with the other genotypes. The percentage distributions of VDR and megalin SNP LC as determined by LCA and SNPHAP (1 or 2 copies) are presented in Figure 1. Note that the SNPHAP distribution is presented in non–mutually exclusive fashion because it reflects allelic combinations for each individual. When each SNPHAP was cross-tabulated with the SNP LC per gene, they were found to be significantly associated ($P < 0.001$ on the basis of chi-square test). In particular, participants with 2 copies of an SNPHAP belonged exclusively to a single SNP LC.

VDR SNPs and LARCCs

The association between VDR SNPs (entered alternatively, models A–D) and LARCCs (predictions I and II), with the use of multiple OLS models, is shown in Supplemental Material 4 under "Supplemental data" in the online issue. After correction for multiple testing, none of the associations (main effects in the total population) remained significant. When effect modification by sex was tested in the association between VDR SNP dosage and LARCC, sex differences ($P < 0.05$ for null hypothesis sex × SNP interaction term = 0) emerged in many of those associations, indicating in some cases that there were significant associations in women only (*Bsm*I, *Apa*I, and *Taq*I in relation to VFT-C LARCC, both predictions; *Apa*I and *Taq*I in relation to DS-F LARCC, both predictions).

Megalin SNPs and LARCCs: sex-stratified findings

Similarly, in OLS models that included only the megalin gene SNP (**Table 2**), significant associations were found between the rs3755166: G/A megalin SNP and LARCC on MMSE, whereby an increasing dose of the "A" nucleotide was associated with faster decline (prediction I) in both sexes combined ($\beta = -0.011$, $P = 0.033$), an association deemed only marginally significant after correction of main effects for multiple testing ($P < 0.05$). An examination of prediction I of LARCC in verbal memory resulted in a significant association between the rs3755166: G/A megalin SNP and faster decline on tests scores in men (CVLT-List A: $\beta = -0.038$, $P = 0.008$; CVLT-DR: $\beta = -0.011$, $P = 0.003$) but a slower decline in women (CVLT-List A: $\beta = +0.038$, $P = 0.016$; CVLT-DR: $\beta = +0.006$, $P = 0.082$), with a significant interaction with sex ($P < 0.05$). Those associations remained significant only in men after correction for multiple testing ($P < 0.0125$). The finding of a sex interaction ($P < 0.05$) was replicated for most of those associations in prediction II. Similarly, decline in cognitive domain 1 was faster in men but not in women among those with a higher dose of the rs3755166:G/A megalin SNP (ie, the "A" nucleotide), with a significant interaction by sex for both predictions. In particular, for prediction I, men declined in this domain by −0.16 SD faster with each "A" nucleotide ($P = 0.009$), an association deemed significant even after correction for multiple testing ($P < 0.025$).

When examining the association between rs2075252: C/T and cognitive outcomes, faster decline among men only was found on VFT-L (prediction I: $\beta = -0.029$, $P = 0.008$; prediction II: $\beta = -0.021$, $P = 0.006$), without significant sex differences in this main association.

In contrast, slower decline in VFT-L, deemed marginally significant after correction for multiple testing, was found among men with increasing dosage of the "T" nucleotide for the third megalin SNP (rs4668123: C/T) for prediction I ($\beta = +0.028$, $P = 0.019$). In this case, sex differences were also nonsignificant.

VDR and Megalin SNP LC associations with LARCC: sex-stratified findings

As shown in **Table 3**, OLS regression models were conducted for SNP LCs as exposures and LARCCs as outcomes, stratifying by sex. After correction for multiple testing, $Megalin_2$ (compared with $Megalin_1$) was linked to a greater rate of decline with the CVLT-DR in men only (prediction II: $\beta = -0.025$, $P = 0.011$; $P < 0.05$ for sex × SNP LC interaction). A similar pattern was noted whereby the $Megalin_2$ SNP LC (compared with $Megalin_1$) was associated with a greater rate of decline with the CVLT-List A (prediction II: $\beta = -0.108$, $P = 0.008$) and cognitive domain 1 (prediction II: $\beta = -0.529$, $P = 0.012$) in men only, although without any significant sex differences ($P > 0.05$ for sex × SNP LC interaction). None of the other sex-specific associations retained their significance after correction for multiple testing.

TABLE 1
Study sample characteristics by availability of gene SNP data: BLSA[1]

	Eligible study sample with visit at age ≥50 y			Genetic data available			Genetic data not available			
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*P* value[2]
Female (%)	1917	32.7		702	47.85		1215	26.91		<0.001
Age at first visit (%)	1901	57.7	16.37	702	52.34	16.7	1199	60.81	15.34	<0.001
≤20 y	1	0.05		1	0.14		0	0		
21–29 y	93	4.89		62	8.83		31	2.59		
30–39 y	239	12.57		130	18.52		109	9.09		
40–49 y	336	17.67		160	22.79		176	14.68		
50–59 y	334	17.62		113	16.10		222	18.52		
60–69 y	350	18.41		94	13.39		256	21.35		
70–79 y	378	19.88		92	13.11		286	23.85		
≥80 y	169	8.89		50	7.12		119	9.92		
Education at first visit (y)	1854	16.65	2.90	674	16.85	2.53	1180	16.55	3.09	0.0333
Smoking status at first visit (%)	1823			644			1179			0.004
Never	689	37.79		254	39.44		435	36.9		
Former	717	39.33		271	42.08		446	37.83		
Current	417	22.87		119	18.48		298	25.28		
Type 2 diabetes at first visit (%)	1901	2.89		702	1.28		1199	3.84		0.001
Hypertension at first visit (%)	1882	37.25		689	27.00		1193	43.17		<0.001
Cardiovascular disease at first visit (%)[3]	1917	7.25		702	3.85		1215	9.22		<0.001
Dyslipidemia at first visit (%)	1901	5.84		702	6.70		1199	5.34		0.223
BMI at first visit (kg/m^2)	1892	24.95	3.40	698	24.75	3.42	1194	25.07	3.39	0.0453
Underweight [BMI (in kg/m^2) ≤18.5] (%)	25	1.32		10	1.43		15	1.26		0.192
Normal weight (18.5 < BMI ≤ 24.9) (%)	1015	53.65		396	56.73		619	51.84		
Overweight (25.0 < BMI ≤ 29.9) (%)	718	37.95		244	34.96		474	39.7		
Obese (BMI ≥ 30) (%)	134	7.08		48	6.88		86	7.2		
Systolic blood pressure (mm Hg)	1881	130.57	20.72	689	124.74	17.97	1192	133.94	21.45	<0.001
Diastolic blood pressure (mm Hg)	1880	79.91	10.98	689	78.36	10.16	1191	80.8	11.34	<0.001
Total cholesterol concentration (mg/dL)	1443	221.67	41.54	616	213.59	39.14	827	227.68	42.28	<0.001
HDL cholesterol (mg/dL)	581	49.46	13.03	323	49.68	12.65	258	49.18	13.52	0.6465
Fasting plasma glucose (mg/dL)	951	98.34	12.75	459	96.84	12.62	492	99.74	12.73	0.0004
Dementia (%)	1916	13.67		701	7.13		1215	17.45		<0.001
Predicted annual rate of cognitive change between age 5 y and mean age at follow-up[4]										
MMSE										
All time points: prediction I	944	0.017	0.117	500	0.039	0.095	444	−0.008	0.134	<0.001
Time points before dementia onset: prediction II	888	−0.007	0.055	492	0.001	0.052	396	−0.017	0.058	<0.001
BVRT										
All time points: prediction I	1394	0.126	0.076	630	0.109	0.077	764	0.140	0.073	<0.001
Time points before dementia onset: prediction II	1319	0.121	0.065	622	0.107	0.066	697	0.135	0.061	<0.001
CVLT-List A										
All time points: prediction I	920	−0.306	0.242	620	−0.274	0.239	300	−0.371	0.236	<0.001
Time points before dementia onset: prediction II	870	−0.271	0.192	601	−0.251	0.196	269	−0.315	0.175	<0.001
CVLT-DR										
All time points: prediction I	920	−0.087	0.061	620	−0.080	0.059	300	−0.103	0.062	<0.001
Time points before dementia onset: prediction II	870	−0.075	0.048	601	−0.071	0.048	269	−0.085	0.048	<0.001
VFT-C										
All time points: prediction I	1025	−0.040	0.137	519	−0.011	0.132	506	−0.071	0.135	<0.001
Time points before dementia onset: prediction II	961	−0.055	0.089	511	−0.040	0.091	450	−0.071	0.083	<0.001
VFT-L										
All time points: prediction I	1023	−0.002	0.115	519	0.020	0.116	504	−0.024	0.110	<0.001
Time points before dementia onset: prediction II	939	−0.008	0.079	508	0.001	0.084	431	−0.020	0.072	<0.001
Trails A										
All time points: prediction I	960	0.391	1.114	484	0.080	0.927	476	0.708	1.198	<0.001
Time points before dementia onset: prediction II	882	0.309	0.641	473	0.132	0.558	409	0.513	0.670	<0.001
Trails B										
All time points: prediction I	955	0.466	1.990	484	−0.134	1.693	471	1.083	2.082	<0.001
Time points before dementia onset: prediction II	879	0.446	1.668	472	0.014	1.455	407	0.947	1.759	<0.001
DS-F										

(Continued)

TABLE 1 (*Continued*)

	Eligible study sample with visit at age ≥50 y			Genetic data available			Genetic data not available			
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*P* value[2]
All time points: prediction I	959	−0.031	0.010	623	−0.030	0.011	338	−0.032	0.009	0.009
Time points before dementia onset: prediction II	904	−0.029	0.010	607	−0.029	0.010	297	−0.030	0.008	0.316
DS-B										
All time points: prediction I	961	−0.046	0.016	623	−0.046	0.017	338	−0.046	0.013	0.673
Time points before dementia onset: prediction II	906	−0.045	0.016	607	−0.045	0.017	299	−0.044	0.013	0.101
Cognitive domain 1										
All time points: prediction I	707	−0.16	0.97	475	0.00	0.94	232	−0.50	0.94	<0.001
Time points before dementia onset: prediction II	649	−0.13	0.94	453	0.00	0.93	196	−0.43	0.89	<0.001
Cognitive domain 2										
All time points: prediction I	707	−0.07	0.86	475	0.00	0.86	232	−0.22	0.84	0.001
Time points before dementia onset: prediction II	649	−0.05	0.83	649	0.00	0.84	196	−0.16	0.80	0.025

[1] BLSA, Baltimore Longitudinal Study of Aging; BVRT, Benton Visual Retention Test; CVLT-List A, California Verbal Learning Test, List A; CVLT-DR, California Verbal Learning Test, Delayed Recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; MMSE, Mini-Mental State Examination; SNP, single nucleotide polymorphism; Trails A, Trailmaking Test, part A; Trails B, Trailmaking Test, part B; VFT-C, Verbal Fluency Test-Categorical; VFT-L, Verbal Fluency Test-Letter.

[2] *P* value for null hypothesis of no difference between those with and those without genetic data.

[3] Reported any of the following conditions at first visit: stroke, congestive heart failure, nonfatal myocardial infarction, or atrial fibrillation.

[4] Cognitive scores were predicted at mean age at follow-up before onset of dementia or for all time points by using a linear mixed model with control for sex, race-ethnicity, education (y), and smoking status, with age added among the fixed-effects variables to allow for quadratic nonlinear change. The slope or annual rate of change was predicted from these models at the mean age at follow-up (ie, between age 50 and the individual mean age of follow-up for each cognitive test). By using factor analysis, 2-factor scores were estimated and were labeled as Longitudinal Annual Rate of Cognitive Change in the following domains: domain 1, "Memory and executive function: earlier decline"; domain 2, "Verbal fluency and attention: later decline" (*see* Supplemental Material 2 under "Supplemental data" in the online issue for more details).

VDR and Megalin SNPHAP associations with LARCC: sex-stratified findings

VDR SNPHAPs, consisting of [rs1544410 (*Bsm*I:G/A), rs7975232 (*Apa*I:A/C), and rs731236 (*Taq*I:G/A)] SNP combinations, and megalin SNPHAPs, consisting of [rs3755166:G/A; rs2075252:C/T; and rs4668123:C/T] combinations were examined in terms of haplotype dosage in relation to LARCCs for each sex and each prediction (**Table 4**).

Among women only, VDR_1 (GCA) was associated with greater decline with the VFT-C (prediction I: $\beta = -0.031$, $P = 0.012$; $P < 0.05$ for sex × SNPHAP interaction). VDR_2 (AAG), however, was associated with a marginally significant slower decline in women only (prediction I) with the MMSE ($\beta = +0.017$, $P = 0.042$). The third VDR SNPHAP (VDR_3: GAA) was associated with marginally significant slower decline in men only with the MMSE (prediction II: $\beta = +0.014$, $P = 0.047$; $P < 0.05$ for sex × SNPHAP interaction), the CVLT-List A (prediction I: $\beta = +0.059$, $P = 0.025$; prediction II: $\beta = +0.052$, $P = 0.019$; $P > 0.05$ for sex × SNPHAP interaction), and the CVLT-DR (prediction II: $\beta = +0.013$, $P = 0.018$; $P < 0.05$ for sex × SNPHAP interaction).

When megalin SNPHAPs were examined in relation to LARCCs, several key findings emerged. $Megalin_1$ (GCC) was associated with a significantly faster decline with the CVLT-List A among women (prediction II: $\beta = -0.043$, $P = 0.006$). For the CVLT-DR, however, a slower decline was found among men (prediction I: $\beta = +0.011$, $P = 0.009$; prediction II: $\beta = +0.010$, $P = 0.007$), although the associations were not significant among women. However, for both cognitive test scores and predictions, sex differences were significant ($P < 0.05$ for sex × SNPHAP interaction). $Megalin_1$ (GCC) was also associated with slower decline for cognitive domain 1 among men only (prediction II: $\beta = +0.17$, $P = 0.021$; $P < 0.05$ for sex × SNPHAP interaction). To assess confounding effects of covariates included in the latter model (prediction II, cognitive domain 1), a change-in-estimate analysis was conducted with backward elimination of covariates. This analysis indicated that the strongest confounding effect was found for education (years) among men, whereas among women baseline age, smoking status, education, and sample selectivity were found to affect the estimate in an appreciable manner (>7% change in estimate; data not shown).

The $Megalin_2$ (ACC) SNPHAP was associated with slower cognitive decline among women with the VFT-C (prediction II: $\beta = +0.026$, $P = 0.005$), without significant sex differences. There was no significant link between $Megalin_3$ SNPHAP (GTT) and decline on any of the cognitive test scores. A sensitivity analysis was conducted in which the 2 principal components analysis factor scores were added to each model to address the issue of the residual effects of population structure within the sample. This adjustment did not alter any of our key findings, particularly the strong positive association between the $Megalin_1$ SNPHAP (GCC) and LARCC in cognitive domain 1 (reflecting less decline) among men.

DISCUSSION

We examined associations of SNPs for VDR [rs11568820 (*CdX*-2:T/C), rs1544410 (*Bsm*I:G/A), rs7975232 (*Apa*I:A/C), rs731236 (*Taq*I:G/A)] and Megalin [rs3755166:G/A; rs2075252:C/T; rs4668123:C/T] genes with longitudinal cognitive performance

TABLE 2
Megalin gene SNP associations with predicted annual rate of cognitive change between age 50 y and mean age of follow-up: multiple OLS regression analysis, BLSA[1]

	Predicted annual rate of cognitive change between age 50 y and mean age of follow-up[2]							
	All time points: prediction I				Time points before dementia onset: prediction II			
	n	β[3]	SEE	*P*-trend	*n*	β[3]	SEE	*P*-trend
MMSE								
Megalin: rs3755166: G/A	484	−0.011	0.005	0.033[4]	477	−0.004	0.003	0.126
Men	312	−0.014	0.007	0.038[4]	306	−0.007	0.004	0.076
Women	172	−0.008	0.007	0.282	171	−0.001	0.003	0.856
Megalin: rs2075252: C/T	484	−0.008	0.007	0.279	477	−0.005	0.004	0.208
Men	312	−0.009	0.010	0.384	306	−0.006	0.006	0.264
Women	172	−0.009	0.011	0.407	171	−0.004	0.005	0.373
Megalin: rs4668123: C/T	484	+0.005	0.008	0.540	477	+0.006	0.004	0.191
Men	312	+0.016	0.011	0.170	306	+0.011	0.006	0.088
Women	172	−0.009	0.012	0.456	171	−0.001	0.005	0.875
BVRT								
Megalin: rs3755166: G/A	608	+0.002	0.003	0.600	601	+0.000	0.003	0.898
Men	364	+0.003	0.004	0.390	357	+0.002	0.004	0.647
Women	244	−0.002	0.005	0.608	244	−0.003	0.004	0.524
Megalin: rs2075252: C/T	608	+0.003	0.005	0.580	601	+0.005	0.004	0.229
Men	364	+0.001	0.006	0.838	357	+0.005	0.005	0.327
Women	244	+0.006	0.008	0.467	244	+0.006	0.006	0.364
Megalin: rs4668123: C/T	608	+0.000	0.005	0.929	601	−0.001	0.005	0.803
Men	364	+0.006	0.007	0.326	357	+0.003	0.006	0.587
Women	244	−0.010	0.009	0.256	244	−0.010	0.007	0.190
CVLT-List A								
Megalin: rs3755166: G/A	598	−0.006	0.011	0.562[5]	580	−0.000	0.009	0.959[5]
Men	356	−0.038	0.014	0.008[6]	343	−0.025	0.012	0.045
Women	242	+0.038	0.016	0.016[4]	237	+0.033	0.013	0.013[4]
Megalin: rs2075252: C/T	598	−0.030	0.016	0.069	580	−0.028	0.014	0.047
Men	356	−0.042	0.022	0.053	343	−0.042	0.019	0.026
Women	242	−0.028	0.024	0.245	237	−0.021	0.020	0.307
Megalin: rs4668123: C/T	598	+0.026	0.018	0.157	580	+0.020	0.015	0.190
Men	356	+0.016	0.024	0.511	343	+0.010	0.020	0.633
Women	242	+0.054	0.028	0.053	237	+0.045	0.023	0.052
CVLT-DR								
Megalin: rs3755166: G/A	598	−0.003	0.003	0.174[5]	580	−0.002	0.002	0.298[5]
Men	356	−0.011	0.003	0.003[6]	343	−0.008	0.003	0.009[6]
Women	242	+0.006	0.004	0.082	237	+0.005	0.003	0.075
Megalin: rs2075252: C/T	598	−0.008	0.004	0.051	580	−0.007	0.003	0.038
Men	356	−0.011	0.005	0.045	343	−0.010	0.005	0.026
Women	242	−0.006	0.006	0.321	237	−0.004	0.005	0.417
Megalin: rs4668123: C/T	598	+0.007	0.004	0.135	580	+0.005	0.004	0.166
Men	356	+0.004	0.006	0.537	343	+0.002	0.005	0.634
Women	242	+0.012	0.006	0.076	237	0.010	0.005	0.078
VFT-C								
Megalin: rs3755166: G/A	504	0.003	0.006	0.655[5]	497	+0.006	0.004	0.139
Men	319	−0.008	0.008	0.310	313	−0.000	0.005	0.953
Women	185	+0.017	0.010	0.104	184	+0.015	0.007	0.037
Megalin: rs2075252: C/T	504	−0.011	0.009	0.228	497	−0.004	0.006	0.513
Men	319	−0.010	0.012	0.381	313	−0.002	0.008	0.832
Women	185	−0.018	0.015	0.228	184	−0.010	0.010	0.335
Megalin: rs4668123: C/T	504	+0.014	0.010	0.167	497	+0.007	0.007	0.313
Men	319	+0.019	0.013	0.155	313	+0.007	0.008	0.384
Women	185	+0.012	0.017	0.501	184	+0.008	0.011	0.465
VFT-L								
Megalin: rs3755166: G/A	507	−0.007	0.006	0.206	494	−0.004	0.004	0.298
Men	319	−0.010	0.007	0.176	311	−0.006	0.005	0.212
Women	185	−0.006	0.009	0.524	183	−0.002	0.007	0.728
Megalin: rs2075252: C/T	507	−0.015	0.008	0.072	494	−0.010	0.006	0.099
Men	319	−0.029	0.011	0.008[6]	311	−0.021	0.008	0.006[6]
Women	185	+0.007	0.013	0.602	183	+0.008	0.010	0.374

(Continued)

TABLE 2 (*Continued*)

	Predicted annual rate of cognitive change between age 50 y and mean age of follow-up[2]							
	All time points: prediction I				Time points before dementia onset: prediction II			
	n	β[3]	SEE	*P*-trend	*n*	β[3]	SEE	*P*-trend
Megalin: rs4668123: C/T	507	+0.010	0.011	0.271	494	+0.006	0.006	0.354
Men	319	+0.028	0.012	0.019[4]	311	+0.017	0.008	0.037
Women	185	−0.020	0.015	0.180	183	−0.014	0.011	0.211
Trails A								
Megalin: rs3755166: G/A	469	−0.002	0.052	0.971	459	+0.008	0.031	0.803
Men	302	+0.049	0.076	0.523	294	+0.041	0.042	0.330
Women	167	−0.062	0.053	0.241	165	−0.039	0.041	0.337
Megalin: rs2075252: C/T	469	+0.018	0.080	0.816	459	+0.028	0.047	0.548
Men	302	+0.062	0.115	0.589	294	+0.074	0.064	0.250
Women	167	+0.006	0.082	0.942	165	−0.008	0.063	0.902
Megalin: rs4668123: C/T	469	+0.076	0.086	0.379	459	+0.053	0.051	0.292
Men	302	+0.058	0.125	0.641	294	+0.061	0.069	0.377
Women	167	+0.062	0.092	0.502	165	+0.022	0.070	0.749
Trails B								
Megalin: rs3755166: G/A	469	+0.082	0.086	0.337	458	+0.072	0.075	0.337
Men	302	+0.151	0.111	0.180	294	+0.122	0.095	0.199
Women	167	0.000	0.134	0.998	164	+0.022	0.124	0.858
Megalin: rs2075252: C/T	469	+0.046	0.130	0.720	458	+0.063	0.113	0.579
Men	302	+0.102	0.169	0.548	294	+0.145	0.144	0.315
Women	167	0.071	0.207	0.730	164	+0.017	0.190	0.928
Megalin: rs4668123: C/T	469	+0.010	0.142	0.946	458	+0.001	0.123	0.990
Men	302	+0.065	0.184	0.726	294	+0.091	0.155	0.559
Women	167	−0.096	0.231	0.677	164	−0.135	0.212	0.526
DS-F								
Megalin: rs3755166: G/A	600	+0.000	0.000	0.886	585	+0.000	0.001	0.840
Men	360	+0.001	0.000	0.214	348	+0.001	0.000	0.153
Women	240	−0.001	0.001	0.104	237	−0.001	0.000	0.080
Megalin: rs2075252: C/T	600	+0.000	0.000	0.373	585	+0.001	0.001	0.202
Men	360	+0.001	0.001	0.152	348	+0.001	0.001	0.126
Women	240	−0.000	0.001	0.828	237	+0.000	0.001	0.760
Megalin: rs4668123: C/T	600	+0.000	0.001	0.515	585	+0.000	0.001	0.860
Men	360	+0.001	0.001	0.376	348	+0.000	0.001	0.533
Women	240	+0.000	0.001	0.968	237	−0.000	0.001	0.654
DS-B								
Megalin: rs3755166: G/A	600	−0.000	0.001	0.678	585	−0.000	0.001	0.607
Men	359	+0.000	0.001	0.727	347	+0.000	0.001	0.771
Women	241	−0.001	0.001	0.150	238	−0.001	0.001	0.150
Megalin: rs2075252: C/T	600	+0.000	0.001	0.645	585	+0.001	0.001	0.443
Men	359	+0.001	0.001	0.459	347	+0.001	0.001	0.338
Women	241	+0.000	0.001	0.912	238	+0.001	0.002	0.704
Megalin: rs4668123: C/T	600	+0.000	0.001	0.785	585	+0.000	0.001	0.953
Men	359	+0.001	0.001	0.376	347	+0.001	+0.001	0.487
Women	241	−0.001	0.002	0.422	238	−0.002	0.002	0.344
Cognitive domain 1								
Megalin: rs3755166: G/A	460	−0.05	0.05	0.260[5]	439	−0.03	0.05	0.559[5]
Men	295	−0.16	0.06	0.009[6]	280	−0.12	0.06	0.048
Women	165	+0.12	0.08	0.114	159	+0.13	0.08	0.105
Megalin: rs2075252: C/T	460	−0.08	0.07	0.270	439	−0.10	0.07	0.189[5]
Men	295	−0.11	0.09	0.251	280	−0.16	0.09	0.090
Women	165	−0.11	0.12	0.366	159	−0.07	0.12	0.541
Megalin: rs4668123: C/T	460	+0.04	0.08	0.596	439	+0.03	0.08	0.698[5]
Men	295	−0.04	0.10	0.670	280	−0.07	0.10	0.486
Women	165	+0.22	0.13	0.104	159	+0.22	0.13	0.101
Cognitive domain 2								
Megalin: rs3755166: G/A	460	−0.00	0.03	0.965	439	+0.00	0.03	0.922
Men	295	−0.00	0.05	0.928	280	+0.01	0.04	0.819
Women	165	−0.03	0.06	0.608	159	−0.03	0.06	0.597

(Continued)

TABLE 2 (*Continued*)

	Predicted annual rate of cognitive change between age 50 y and mean age of follow-up[2]							
	All time points: prediction I				Time points before dementia onset: prediction II			
	n	β[3]	SEE	*P*-trend	*n*	β[3]	SEE	*P*-trend
Megalin: rs2075252: C/T	460	−0.01	0.05	0.804	439	−0.00	0.05	0.964
Men	295	−0.00	0.07	0.998	280	−0.00	0.07	0.945
Women	165	−0.05	0.08	0.594	159	+0.00	0.09	0.995
Megalin: rs4668123: C/T	460	+0.04	0.06	0.473	439	+0.04	0.06	0.513
Men	295	+0.09	0.07	0.239	280	+0.07	0.07	0.355
Women	165	−0.02	0.10	0.838	159	−0.02	0.10	0.841

[1] Note that each SNP is denoted by an rs number followed by the polymorphism in which one nucleotide is replaced by another (eg, C/T or G/A). BLSA, Baltimore Longitudinal Study of Aging; BVRT, Benton Visual Retention Test; CVLT-List A, California Verbal Learning Test, List A; CVLT-DR, California Verbal Learning Test, Delayed Recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; MMSE, Mini-Mental State Examination; OLS, ordinary least squares; SNP, single nucleotide polymorphism; Trails A, Trailmaking Test, part A; Trails B, Trailmaking Test, part B; VFT-C, Verbal Fluency Test-Categorical; VFT-L, Verbal Fluency Test-Letter.

[2] Cognitive scores were predicted at the mean age at follow-up before onset of dementia or for all time points by using a linear mixed model controlled for sex, race-ethnicity, education (y), and smoking status, with age added among the fixed-effects variables to allow for quadratic nonlinear change. The slope or annual rate of change was predicted from these models at the mean age at follow-up (ie, between age 50 and the individual mean age of follow-up for each cognitive test). By using factor analysis, 2-factor scores were estimated and were labeled as Longitudinal Annual Rate of Cognitive Change in the following domains: domain 1, "Memory and executive function: earlier decline"; domain 2, "Verbal fluency and attention: later decline" (*see* Supplemental Material 2 under "Supplemental data" in the online issue for more details).

[3] On the basis of multiple OLS regression models with outcome being cognitive annual rate of change and main exposures being the 3 megalin SNPs. The model controlled for first-visit age, mean age at follow-up, education, first-visit smoking status, first-visit self-reported type 2 diabetes, hypertension, cardiovascular disease, and BMI.

[4] Marginally significant main effects after family-wise Bonferroni correction: $P < 0.05$ for MMSE or BVRT or cognitive domains and $P < 0.025$ for other cognitive tests.

[5] $P < 0.05$ for the null hypothesis that sex × SNP interaction term = 0 in a model in which the main effect of sex was added.

[6] Significant main effects after family-wise Bonferroni correction: $P < 0.025$ for MMSE and cognitive domains and $P < 0.0125$ for other cognitive tests.

changes. Data from 702 non-Hispanic white BLSA participants were used. LARCCs between age 50 y and the age at individual mean follow-up were predicted with linear mixed models by using all cognitive score time points (prediction I) or time points before dementia onset (prediction II). LC, haplotype, and OLS regression analyses were conducted. Among many key findings, in OLS models with SNP LCs as predictors for LARCC, Megalin_2 [rs3755166(–)/rs2075252(TT)/rs4668123(T−)] compared with Megalin_1 [rs3755166(–)/rs2075252(CC)/rs4668123(–)] was associated with greater decline among men in verbal memory (prediction II), with significant sex differences ($P < 0.05$). When examining SNPHAPs, in women VDR_1 [*Bsm*I (G−)/*Apa*I(C−)/*Taq*I(A−); baT] was linked to greater decline in category fluency (prediction I: $\beta = -0.031$, $P = 0.012$). The Megalin_1 SNPHAP (GCC) was related to greater decline among women in verbal memory, immediate recall (CVLT-List A; prediction II: $\beta = -0.043$, $P = 0.006$), but slower decline among men in delayed recall (CVLT-DR: $\beta > 0$, $P < 0.0125$; both predictions). In women, the Megalin_2 SNPHAP (ACC) was associated with slower decline in category fluency (VFT-C; prediction II: $\beta = +0.026$, $P = 0.005$). Another finding was that Megalin SNP rs3755166:G/A was associated with greater decline in global cognition in both sexes combined and in verbal memory in men.

Four recent studies have examined Megalin (20, 36) and VDR (18, 19) genetic polymorphisms as potential risk markers for cognitive impairment or, more specifically, AD. Overall, cognitive impairment risk appears to be associated with various genetic SNPs and haplotypes pertaining to megalin and VDR. In a case-control study (1158 patients with sporadic AD compared with 1025 healthy controls), out of 3 megalin SNPs (rs3755166, rs2075252, rs4668123), only one (rs3755166:G/A) was found to be associated with apparent increased AD risk. Note that the rs3755166 "A" variant had 20% less transcriptional activity than did the "G" variant (20).

This finding was replicated by a recent case-control study in Chinese middle-aged and older adults ($n = 361$), in which cases were found to be 38% more likely than controls to have the "A" variant of that SNP (rs3755166 G/A: OR: 1.38; 95% CI: 1.02, 1.87; $P = 0.039$) (36). Similarly, in our study, we found a marginally significant inverse relation between rs3755166 G→A and MMSE LARCC, indicative of greater cognitive decline for the "A" variant SNP. Moreover, this SNP variant was associated with significantly greater decline in verbal memory among men only, even after correction for multiple testing. Whereas the previously described study (20) did not find a significant relation between rs2075252 or rs4668123 and AD, our present study found that the rs2075252 SNP LC (TT compared with CC) may be associated with greater decline in verbal memory (CVLT-DR), particularly before the onset of dementia and more so among men.

When testing VDR SNP associations with AD, a recent case-control study (104 patients with late-onset AD compared with 109 age-matched controls) found that heterozygous *Apa*I genotype (AC) was linked to an increased risk of AD, compared with homozygous AA genotype (19). In our study, we found only a marginally significant *P*-trend ($P < 0.10$), indicating that AA may be protective against cognitive decline compared with AC and CC, particularly for changes in global cognitive performance

TABLE 3
VDR and Megalin SNP LC associations with predicted annual rate of cognitive change between age 50 y and mean age of follow-up: multiple OLS regression analysis, BLSA[1]

	Predicted annual rate of cognitive change between age 50 y and mean age of follow-up[2]															
	All time points: prediction I								Time points before dementia onset: prediction II							
	Men				Women				Men				Women			
	n	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*
MMSE	315				179				309				178			
VDR_2 vs VDR_1		+0.019	0.016	0.241		+0.024	0.016	0.125		+0.014	0.009	0.118		+0.006	0.007	0.435
VDR_3 vs VDR_1		+0.004	0.013	0.742		−0.009	0.013	0.504		+0.006	0.007	0.381		−0.003	0.006	0.543
$Megalin_2$ vs $Megalin_1$		−0.000	0.023	0.985		−0.015	0.021	0.493		−0.003	0.013	0.833		−0.011	0.010	0.244
$Megalin_3$ vs $Megalin_1$		+0.010	0.013	0.432		−0.025	0.013	0.060		+0.004	0.007	0.566		−0.003	0.006	0.641
BVRT	368				254				361				254			
VDR_2 vs VDR_1		−0.007	0.009	0.441		+0.011	0.011	0.316		−0.002	0.008	0.799		+0.007	0.010	0.485
VDR_3 vs VDR_1		−0.008	0.007	0.280		+0.009	0.009	0.320		−0.007	0.007	0.305		+0.007	0.008	0.383
$Megalin_2$ vs $Megalin_1$		+0.011	0.013	0.408		−0.002	0.014	0.874		+0.014	0.012	0.233		+0.000	0.012	0.969
$Megalin_3$ vs $Megalin_1$		+0.007	0.007	0.327		+0.003	0.009	0.738		+0.008	0.007	0.221		−0.000	0.008	0.949
CVLT-List A	360				252				347				247			
VDR_2 vs VDR_1		+0.049	0.034	0.148		−0.029	0.036	0.421		+0.039	0.029	0.176		−0.017	0.031	0.586
VDR_3 vs VDR_1		+0.035	0.027	0.199		−0.017	0.029	0.567		+0.026	0.023	0.258		−0.004	0.025	0.880
$Megalin_2$ vs $Megalin_1$		−0.102	0.048	0.034		+0.003	0.046	0.954		−0.108	0.040	0.008[4]		−0.008	0.038	0.829
$Megalin_3$ vs $Megalin_1$		+0.002	0.027	0.930		+0.007	0.030	0.827		−0.002	0.002	0.915		+0.025	0.026	0.325
CVLT-DR	360				252				347				247			
VDR_2 vs VDR_1		+0.010	0.008	0.218		+0.000	0.008	0.992		+0.007	0.007	0.348		+0.004	0.007	0.581
VDR_3 vs VDR_1		+0.015	0.007	0.019[5]		−0.001	0.007	0.915		+0.012	0.006	0.029		+0.002	0.006	0.643
$Megalin_2$ vs $Megalin_1$		−0.026	0.012	0.026		+0.001	0.011	0.922		−0.025	0.010	0.011[4,6]		−0.002	0.009	0.853
$Megalin_3$ vs $Megalin_1$		−0.002	0.006	0.709		+0.009	0.007	0.184		−0.003	0.006	0.542[6]		+0.014	0.006	0.023[5]
VFT-C	322				192				316				191			
VDR_2 vs VDR_1		+0.006	0.018	0.758		+0.033	0.023	0.155		+0.004	0.012	0.729		+0.022	0.016	0.165
VDR_3 vs VDR_1		+0.022	0.015	0.161		−0.021	0.019	0.284		+0.012	0.010	0.192		−0.012	0.013	0.340
$Megalin_2$ vs $Megalin_1$		−0.002	0.026	0.929		+0.010	0.030	0.729		−0.007	0.017	0.684		+0.005	0.020	0.812
$Megalin_3$ vs $Megalin_1$		+0.019	0.015	0.205		−0.010	0.030	0.435		+0.013	0.017	0.169		+0.000	0.013	0.978
VFT-L	322				192				314				190			
VDR_2 vs VDR_1		−0.015	0.017	0.383		−0.006	0.021	0.757		−0.011	0.012	0.358		−0.005	0.015	0.720
VDR_3 vs VDR_1		−0.005	0.014	0.712		−0.004	0.017	0.799		−0.005	0.009	0.582		+0.000	0.012	0.952
$Megalin_2$ vs $Megalin_1$		−0.001	0.024	0.978		−0.006	0.027	0.829		−0.009	0.017	0.590		−0.008	0.019	0.668
$Megalin_3$ vs $Megalin_1$		−0.007	0.014	0.607		+0.006	0.018	0.806		−0.007	0.010	0.461		+0.021	0.012	0.085
Trails A	304				172				296				170			
VDR_2 vs VDR_1		−0.342	0.174	0.050		+0.036	0.121	0.768		−0.174	0.100	0.076		+0.025	0.093	0.786
VDR_3 vs VDR_1		−0.221	0.138	0.112		+0.039	0.100	0.701		−0.093	0.077	0.228		+0.040	0.075	0.598
$Megalin_2$ vs $Megalin_1$		+0.122	0.249	0.623		+0.078	0.162	0.633		+0.169	0.141	0.233		−0.006	0.122	0.963
$Megalin_3$ vs $Megalin_1$		+0.107	0.141	0.450		+0.040	0.102	0.693		+0.093	0.078	0.234		−0.038	0.077	0.622
Trails B	304				172				296				169			
VDR_2 vs VDR_1		−0.504	0.258	0.052		−0.101	0.309	0.744		−0.485	0.218	0.027		−0.177	0.286	0.537
VDR_3 vs VDR_1		−0.230	0.205	0.264		+0.023	0.255	0.929		−0.249	0.173	0.151		−0.007	0.228	0.974
$Megalin_2$ vs $Megalin_1$		+0.209	0.369	0.572		−0.185	0.414	0.655		+0.417	0.317	0.189		−0.203	0.369	0.584
$Megalin_3$ vs $Megalin_1$		+0.072	0.209	0.935		+0.272	0.260	0.297		+0.135	0.175	0.441		−0.042	0.236	0.857
DS-F	364				250				352				247			
VDR_2 vs VDR_1		+0.001	0.001	0.869[6]		+0.000	0.001	0.739		+0.000	0.001	0.769[6]		−0.000	0.001	0.920
VDR_3 vs VDR_1		+0.002	0.001	0.145[6]		−0.002	0.001	0.062		+0.001	0.001	0.103[6]		−0.002	0.001	0.043
$Megalin_2$ vs $Megalin_1$		+0.003	0.001	0.041		+0.001	0.001	0.518		+0.003	0.001	0.041		+0.001	0.001	0.676
$Megalin_3$ vs $Megalin_1$		+0.001	0.001	0.115		−0.001	0.001	0.199		+0.001	0.001	0.146		−0.001	0.001	0.190
DS-B	363				251				351				248			
VDR_2 vs VDR_1		−0.001	0.002	0.735		+0.001	0.002	0.499		−0.000	0.002	0.829		+0.001	0.002	0.774
VDR_3 vs VDR_1		+0.000	0.002	0.907		−0.003	0.002	0.085		+0.000	0.002	0.923		−0.003	0.002	0.064
$Megalin_2$ vs $Megalin_1$		+0.004	0.002	0.137		+0.002	0.003	0.481		+0.005	0.002	0.096		+0.002	0.003	0.508
$Megalin_3$ vs $Megalin_1$		+0.001	0.002	0.639		−0.003	0.002	0.079		+0.001	0.002	0.667		−0.003	0.002	0.092
Cognitive domain 1	297				170				282				164			
VDR_2 vs VDR_1		+0.194	0.144	0.178		−0.077	0.178	0.666		+0.167	0.144	0.249		+0.056	0.184	0.759
VDR_3 vs VDR_1		+0.177	0.113	0.119		−0.101	0.145	0.485		+0.186	0.114	0.107		−0.022	0.146	0.879
$Megalin_2$ vs $Megalin_1$		−0.366	0.212	0.084		+0.099	0.147	0.937		−0.529	0.210	0.012[4]		+0.047	0.233	0.841
$Megalin_3$ vs $Megalin_1$		−0.008	0.115	0.947		+0.11	0.147	0.937		−0.07	0.116	0.558		+0.246	0.153	0.109

(Continued)

TABLE 3 (*Continued*)

	Predicted annual rate of cognitive change between age 50 y and mean age of follow-up[2]															
	All time points: prediction I								Time points before dementia onset: prediction II							
	Men				Women				Men				Women			
	n	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*
Cognitive domain 2	297				170				282				164			
VDR_2 vs VDR_1		+0.072	0.107	0.501		+0.153	0.128	0.235		+0.090	0.105	0.390		+0.094	0.133	0.481
VDR_3 vs VDR_1		+0.066	0.084	0.433		−0.038	0.105	0.718		+0.073	0.083	0.380		−0.051	0.106	0.630
$Megalin_2$ vs $Megalin_1$		+0.172	0.157	0.274		+0.049	0.169	0.774		+0.143	0.152	0.348		+0.052	0.168	0.757
$Megalin_3$ vs $Megalin_1$		+0.040	0.086	0.641		−0.102	0.106	0.338		+0.024	0.084	0.773		−0.007	0.110	0.946

[1] Note that VDR_1, VDR_2, and VDR_3 denote VDR SNP LCs, whereas $Megalin_1$, $Megalin_2$, and $Megalin_3$ denote Megalin SNP LCs. BLSA, Baltimore Longitudinal Study of Aging; BVRT, Benton Visual Retention Test; CVLT-List A, California Verbal Learning Test, List A; CVLT-DR, California Verbal Learning Test, Delayed Recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; LC, latent class; MMSE, Mini-Mental State Examination; OLS, ordinary least squares; SNP, single nucleotide polymorphism; Trails A, Trailmaking Test, part A; Trails B, Trailmaking Test, part B; VDR, vitamin D receptor gene; VFT-C, Verbal Fluency Test-Categorical; VFT-L, Verbal Fluency Test-Letter.

[2] Cognitive scores were predicted at mean age at follow-up before onset of dementia or for all time points by using a linear mixed model controlled for sex, race-ethnicity, education (y), and smoking status, with age added among the fixed-effects variables to allow for quadratic nonlinear change. The slope or annual rate of change was predicted from these models at the mean age at follow-up (ie, between age 50 y and the individual mean age of follow-up for each cognitive test). By using factor analysis, 2-factor scores were estimated and were labeled as Longitudinal Annual Rate of Cognitive Change in the following domains: domain 1, "Memory and executive function: earlier decline"; domain 2, "Verbal fluency and attention: later decline" (see Supplemental Material 2 under "Supplemental data" in the online issue for more details).

[3] On the basis of multiple OLS regression models with outcome being cognitive annual rate of change and main exposures being the 3 megalin and VDR SNP LCs. *See* Figure 1 for more details on definition of the LCs. The model controlled for first-visit age, mean age at follow-up, education, first-visit smoking status, first-visit self-reported type 2 diabetes, hypertension, cardiovascular disease, and BMI.

[4] Significant main effects after family-wise Bonferroni correction: $P < 0.025$ for MMSE and cognitive domains and $P < 0.0125$ for other cognitive tests.

[5] Marginally significant main effects after family-wise Bonferroni correction: $P < 0.05$ for MMSE or BVRT or cognitive domains and $P < 0.025$ for other cognitive tests.

[6] $P < 0.05$ for the null hypothesis that sex × SNP LC interaction term = 0 in a model in which the main effect of sex was added.

and verbal memory, when all time points were considered (ie, MMSE and CVLT-List A; prediction I). However, this association was deemed nonsignificant after correction for multiple testing. In a recent prospective population-based cohort study (Leiden 85-plus Study; $n = 563$) that examined 5 VDR SNPs in relation to cognitive functioning at follow-up, a number of key findings were reported (18). Three of 5 SNPs were associated with cognitive function, namely *Bsm*I, *Apa*I, and *Taq*I. In contrast to the study by Gezen-Ak et al (19), *Apa*I (A/C) variant allele (ie, CC or AC compared with AA) was associated with better cognitive function at follow-up, particularly in immediate recall (18). In our present study, SNPHAPs combining *Bsm*I, *Apa*I, and *Taq*I were associated with cognitive change. Specifically, after correction for multiple testing, the VDR_1 SNPHAP (GCA or baT) was associated with greater decline with the VFT-C among women but not among men, a finding that was not replicated by Kuningas et al (18), who found that worse performance was, in fact, ascribed to the VDR_2 SNPHAP (AAG), which they labeled as BAt.

Sex differences in some of the observed associations of Megalin SNP LCs with cognitive change may be related to the interaction of Megalin with both estrogen, established to affect cognitive function (74), and with vitamin D, also known to affect cognitive performance (16, 17, 75). Notably, Megalin's role may be explained by some of the recent experimental evidence indicating that the binding of vitamin D and estrogen to their shared plasma membrane receptor megalin (33, 68, 76) may be competitive, because megalin is also now an established nonrecycled endocytosis receptor of both 25-hydroxyvitamin D bound to vitamin D binding protein and estrogen bound to sex hormone–binding globulin (68), a key mediator of androgen and estrogen dose and biological response. Indeed, an emerging body of evidence indicates that sex hormone–binding globulin bound estrogen and testosterone become biologically active via receptor-mediated endocytosis (68–70), mediated primarily via the Megalin receptor (68). In fact, megalin gene knockout has been shown to induce both estrogen deficiency and vitamin D deficiency (33, 68), and the cross-effect modification of estrogen and vitamin D interventions was observed for colorectal cancer incidence in the Women's Health Initiative trial (77). Taken together, multiple lines of evidence indicate the interplay of estrogen and vitamin D via their shared receptor, Megalin.

Our study has several strengths, including frequency of follow-up (the median frequency ranged between 13 and 15 depending on the outcome) and use of advanced statistical techniques by combining linear mixed models with OLS multiple linear regression analyses to examine associations between gene SNP, SNP LC (defined by using LCA), SNPHAP (defined by using haplotype analysis), and annual rates of change in cognitive function.

Despite its strengths, our present study has a number of limitations. First, the BLSA is a sample of convenience; the cohort was not fixed, and recruitment and dropout were continuous throughout the follow-up. We used a number of statistical techniques to diminish resulting biases, including a 2-stage Heckman selection model (67). Second, even though observation frequency for cognitive function was high, first-visit age and duration between visits varied across participants, making the

TABLE 4
VDR and Megalin SNPHAP associations with predicted annual rate of cognitive change between age 50 y and mean age of follow-up: multiple OLS regression analysis, BLSA[1]

	Predicted annual rate of cognitive change between age 50 y and mean age of follow-up[2]															
	All time points: prediction I								Time points before dementia onset: prediction II							
	Men				Women				Men				Women			
	n	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*
MMSE: models A–F																
VDR$_1$: GCA (0, 1, 2)	312	−0.009	0.008	0.260	172	−0.010	0.009	0.246	306	−0.006	0.004	0.184	171	−0.001	0.004	0.846
VDR$_2$:AAG (0, 1, 2)	312	+0.005	0.009	0.527	172	+0.017	0.008	0.042[4]	306	+0.002	0.004	0.649	171	+0.005	0.004	0.173
VDR$_3$: GAA (0, 1, 2)	312	+0.017	0.012	0.163	172	−0.017	0.012	0.169	306	+0.014	0.007	0.047[4,5]	171	−0.010	0.006	0.078
Megalin$_1$: GCC (0, 1, 2)	312	+0.010	0.008	0.207	172	+0.012	0.008	0.161	306	+0.004	0.004	0.419	171	0.000	0.004	1.000
Megalin$_2$: ACC (0, 1, 2)	312	−0.017	0.009	0.056	172	+0.004	0.010	0.684	306	−0.006	0.005	0.197	171	+0.007	0.004	0.107
Megalin$_3$: GTT (0, 1, 2)	312	+0.011	0.013	0.408	172	−0.008	0.012	0.513	306	+0.008	0.007	0.255	171	−0.001	0.005	0.817
BVRT: models A–F																
VDR$_1$: GCA (0, 1, 2)	364	−0.004	0.005	0.356	243	−0.001	0.006	0.837	357	−0.005	0.004	0.273	243	−0.001	0.005	0.810
VDR$_2$:AAG (0, 1, 2)	364	+0.001	0.005	0.797	243	−0.001	0.006	0.914	357	+0.003	0.004	0.550	243	−0.001	0.005	0.772
VDR$_3$: GAA (0, 1, 2)	364	+0.003	0.007	0.660	243	+0.004	0.009	0.652	357	+0.001	0.006	0.863	243	+0.006	0.007	0.422
Megalin$_1$: GCC (0, 1, 2)	364	−0.006	0.005	0.219	243	+0.001	0.006	0.919	357	−0.005	0.004	0.227	243	+0.002	0.005	0.691
Megalin$_2$: ACC (0, 1, 2)	364	+0.001	0.005	0.899	243	+0.003	0.006	0.676	357	−0.001	0.005	0.896	243	+0.000	0.005	0.972
Megalin$_3$: GTT (0, 1, 2)	364	+0.006	0.007	0.418	243	−0.005	0.008	0.561	357	+0.007	0.007	0.312	243	−0.004	0.007	0.544
CVLT-List A: models A–F																
VDR$_1$: GCA (0, 1, 2)	356	−0.019	0.018	0.270	241	−0.006	0.019	0.735	343	−0.019	0.015	0.217	236	−0.001	0.016	0.934
VDR$_2$:AAG (0, 1, 2)	356	−0.004	0.017	0.797	241	+0.004	0.018	0.819	343	−0.003	0.015	0.859	236	+0.003	0.016	0.838
VDR$_3$: GAA (0, 1, 2)	356	+0.059	0.026	0.025[4]	241	+0.008	0.028	0.779	343	+0.052	0.022	0.019[4]	236	−0.001	0.023	0.953
Megalin$_1$: GCC (0, 1, 2)	356	+0.039	0.018	0.028[5]	241	−0.043	0.019	0.022[4]	343	+0.033	0.015	0.027[4,5]	236	−0.043	0.016	0.006[6]
Megalin$_2$: ACC (0, 1, 2)	356	−0.023	0.019	0.207[5]	241	+0.037	0.020	0.072	343	−0.011	0.016	0.468	236	+0.036	0.017	0.039
Megalin$_3$: GTT (0, 1, 2)	356	−0.018	0.027	0.519	241	−0.018	0.027	0.511	343	−0.021	0.023	0.366	236	+0.023	0.022	0.292
CVLT-DR: models A–F																
VDR$_1$: GCA (0, 1, 2)	356	−0.000	0.004	0.990	241	−0.002	0.004	0.717	343	−0.000	0.004	0.930	236	−0.000	0.004	0.958
VDR$_2$:AAG (0, 1, 2)	356	−0.005	0.004	0.216	241	+0.002	0.004	0.619	343	−0.005	0.004	0.193	236	+0.002	0.004	0.533
VDR$_3$: GAA (0, 1, 2)	356	+0.014	0.006	0.032	241	−0.000	0.007	0.958	343	+0.013	0.005	0.018[4,5]	236	−0.004	0.005	0.500
Megalin$_1$: GCC (0, 1, 2)	356	+0.011	0.004	0.009[5,6]	241	−0.007	0.004	0.112	343	+0.010	0.004	0.007[5,6]	236	−0.007	0.004	0.052
Megalin$_2$: ACC (0, 1, 2)	356	−0.007	0.005	0.132	241	+0.005	0.005	0.322	343	−0.005	0.004	0.207	236	+0.004	0.004	0.276
Megalin$_3$: GTT (0, 1, 2)	356	−0.003	0.07	0.657	241	+0.009	0.006	0.145	343	−0.004	0.006	0.478	236	+0.007	0.005	0.192
VFT-C: models A–F																
VDR$_1$: GCA (0, 1, 2)	319	+0.011	0.009	0.258[5]	184	−0.031	0.012	0.012[6]	313	+0.005	0.006	0.419[5]	183	−0.019	0.009	0.024[4]
VDR$_2$:AAG (0, 1, 2)	319	−0.012	0.009	0.207[5]	184	+0.024	0.012	0.043	313	−0.007	0.006	0.249[5]	183	+0.015	0.008	0.067
VDR$_3$: GAA (0, 1, 2)	319	+0.002	0.014	0.895	184	+0.010	0.018	0.572	313	+0.004	0.009	0.696	183	+0.006	0.012	0.603
Megalin$_1$: GCC (0, 1, 2)	319	−0.003	0.010	0.734	184	−0.014	0.012	0.241	313	−0.008	0.006	0.205	183	−0.016	0.008	0.042
Megalin$_2$: ACC (0, 1, 2)	319	−0.004	0.010	0.695	184	+0.031	0.014	0.030	313	+0.005	0.007	0.440	183	+0.026	0.009	0.005[6]
Megalin$_3$: GTT (0, 1, 2)	319	+0.009	0.015	0.542	184	−0.001	0.017	0.945	313	+0.006	0.010	0.525	183	−0.001	0.011	0.926
VFT-L: models A–F																
VDR$_1$: GCA (0, 1, 2)	319	+0.006	0.009	0.465	184	−0.003	0.011	0.801	313	+0.003	0.006	0.659	182	−0.001	0.009	0.937
VDR$_2$:AAG (0, 1, 2)	319	−0.003	0.009	0.698	184	−0.001	0.011	0.872	311	+0.005	0.009	0.562	182	−0.004	0.008	0.567
VDR$_3$: GAA (0, 1, 2)	319	−0.008	0.013	0.558	184	+0.010	0.016	0.542	311	−0.003	0.009	0.743	182	+0.011	0.011	0.333
Megalin$_1$: GCC (0, 1, 2)	319	+0.010	0.009	0.944	184	+0.005	0.011	0.672	311	+0.001	0.006	0.856	182	−0.002	0.008	0.720
Megalin$_2$: ACC (0, 1, 2)	319	−0.003	0.010	0.742	184	+0.003	0.012	0.834	311	+0.000	0.007	0.996	182	+0.007	0.009	0.461
Megalin$_3$: GTT (0, 1, 2)	319	+0.000	0.014	0.978	184	−0.002	0.015	0.870	311	−0.000	0.009	0.987	182	+0.007	0.011	0.533
Trails A: models A–F																
VDR$_1$: GCA (0, 1, 2)	302	+0.000	0.091	0.997	167	+0.019	0.064	0.766	294	+0.066	0.051	0.198	165	+0.031	0.049	0.525
VDR$_2$:AAG (0, 1, 2)	302	−0.018	0.090	0.845	167	+0.001	0.061	0.991	294	−0.022	0.051	0.659	165	−0.001	0.047	0.973
VDR$_3$: GAA (0, 1, 2)	302	+0.034	0.135	0.797	167	−0.043	0.09	0.648	294	−0.100	0.076	0.189	165	−0.063	0.072	0.379
Megalin$_1$: GCC (0, 1, 2)	302	−0.096	0.091	0.294	167	−0.037	0.062	0.558	294	−0.104	0.051	0.041	165	−0.006	0.048	0.898
Megalin$_2$: ACC (0, 1, 2)	302	+0.031	0.099	0.750	167	−0.046	0.071	0.518	294	+0.008	0.055	0.883	165	−0.027	0.054	0.622
Megalin$_3$: GTT (0, 1, 2)	302	+0.187	0.141	0.183	167	+0.083	0.086	0.336	294	+0.130	0.078	0.097	165	+0.004	0.067	0.853
Trails B: models A–F																
VDR$_1$: GCA (0, 1, 2)	302	+0.136	0.134	0.310	167	+0.016	0.016	0.920	294	+0.154	0.114	0.177	164	+0.015	0.149	0.919
VDR$_2$:AAG (0, 1, 2)	302	−0.067	0.133	0.618	167	−0.047	0.153	0.757	294	−0.054	0.114	0.635	164	−0.053	0.144	0.713
VDR$_3$: GAA (0, 1, 2)	302	−0.144	0.200	0.470	167	−0.136	0.191	0.474	294	−0.211	0.169	0.214	164	+0.088	0.216	0.686
Megalin$_1$: GCC (0, 1, 2)	302	−0.172	0.135	0.203	167	−0.172	0.129	0.181	294	−0.158	0.114	0.169	164	+0.055	0.144	0.704

(Continued)

TABLE 4 (*Continued*)

	Predicted annual rate of cognitive change between age 50 y and mean age of follow-up[2]															
	All time points: prediction I								Time points before dementia onset: prediction II							
	Men				Women				Men				Women			
	n	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*	*n*	β^3	SEE	*P*
Megalin$_2$*: ACC* (0, 1, 2)	302	+0.071	0.146	0.625	167	−0.027	0.178	0.878	294	+0.005	0.124	0.967	164	+0.023	0.165	0.889
Megalin$_3$*: GTT* (0, 1, 2)	302	+0.091	0.208	0.664	167	−0.014	0.217	0.946	294	+0.168	0.175	0.338	164	+0.109	0.201	0.588
DS-F: models A–F																
VDR$_1$*: GCA* (0, 1, 2)	360	+0.001	0.000	0.259[5]	240	−0.001	0.000	0.045	348	+0.001	0.001	0.283[5]	237	−0.001	0.000	0.064
VDR$_2$*:AAG* (0, 1, 2)	360	−0.000	0.000	0.267[5]	240	+0.001	0.001	0.099	348	−0.001	0.000	0.231	237	+0.001	0.001	0.156
VDR$_3$*: GAA* (0, 1, 2)	360	+0.000	0.001	0.838	240	+0.000	0.001	0.848	348	+0.000	0.001	0.685	237	+0.000	0.001	0.769
Megalin$_1$*: GCC* (0, 1, 2)	360	−0.001	0.000	0.014[4]	240	+0.000	0.001	0.711	348	−0.001	0.000	0.020[4]	237	+0.000	0.001	0.766
Megalin$_2$*: ACC* (0, 1, 2)	360	+0.000	0.001	0.634	240	−0.000	0.001	0.413	348	+0.000	0.001	0.582	237	−0.001	0.001	0.416
Megalin$_3$*: GTT* (0, 1, 2)	360	+0.001	0.001	0.065	240	−0.000	0.001	0.583	348	+0.001	0.001	0.094	237	−0.001	0.001	0.525
DS-B: models A–F																
VDR$_1$*: GCA* (0, 1, 2)	359	+0.001	0.001	0.464[5]	241	−0.002	0.001	0.035	347	+0.001	0.001	0.532	238	−0.002	0.001	0.468
VDR$_2$*:AAG* (0, 1, 2)	359	−0.000	0.001	0.640	241	+0.002	0.001	0.054	347	−0.000	0.001	0.687	238	+0.002	0.001	0.091
VDR$_3$*: GAA* (0, 1, 2)	359	−0.000	0.002	0.837	241	−0.000	0.002	0.959	347	−0.000	0.002	0.878	238	+0.000	0.002	0.969
Megalin$_1$*: GCC* (0, 1, 2)	359	−0.020	0.001	0.064	241	+0.001	0.001	0.278	347	−0.002	0.001	0.085	238	+0.001	0.001	0.336
Megalin$_2$*: ACC* (0, 1, 2)	359	+0.000	0.001	0.919	241	−0.001	0.001	0.538	347	−0.000	0.001	0.980	238	−0.001	0.001	0.516
Megalin$_3$*: GTT* (0, 1, 2)	359	+0.002	0.002	0.221	241	−0.001	0.002	0.436	347	+0.002	0.002	0.215	238	−0.001	0.002	0.507
Cognitive domain 1: models A–F																
VDR$_1$*: GCA* (0, 1, 2)	295	−0.02	0.07	0.742	165	−0.07	0.09	0.461	280	−0.05	0.08	0.504	159	−0.04	0.10	0.693
VDR$_2$*:AAG* (0, 1, 2)	295	−0.03	0.07	0.603	165	+0.03	0.09	0.763	280	−0.05	0.07	0.486	159	+0.03	0.10	0.728
VDR$_3$*: GAA* (0, 1, 2)	295	−0.16	0.08	0.061	165	+0.08	0.14	0.538	280	+0.22	0.11	0.052	159	+0.01	0.14	0.966
Megalin$_1$*: GCC* (0, 1, 2)	295	+0.16	0.74	0.028[4,5]	165	−0.14	0.09	0.129	280	+0.17	0.07	0.021[5,6]	159	−0.18	0.09	0.052
Megalin$_2$*: ACC* (0, 1, 2)	295	−0.10	0.10	0.239	165	+0.11	0.10	0.309	280	−0.03	0.08	0.724	159	+0.13	0.10	0.228
Megalin$_3$*: GTT* (0, 1, 2)	295	−0.09	0.11	0.438[5]	165	+0.15	0.13	0.221	280	−0.13	0.11	0.233[5]	159	+0.20	0.13	0.131
Cognitive domain 2: models A–F																
VDR$_1$*: GCA* (0, 1, 2)	295	+0.03	0.06	0.630	165	−0.090	0.067	0.181	280	+0.00	0.06	0.985	159	−0.07	0.07	0.326
VDR$_2$*:AAG* (0, 1, 2)	295	−0.01	0.05	0.876	165	+0.10	0.06	0.126	280	−0.00	0.05	0.941	159	+0.07	0.07	0.292
VDR$_3$*: GAA* (0, 1, 2)	295	−0.04	0.08	0.619	165	−0.04	0.10	0.712	280	+0.02	0.08	0.795	159	−0.02	0.10	0.868
Megalin$_1$*: GCC* (0, 1, 2)	295	−0.07	0.06	0.193	165	+0.00	0.06	0.959	280	−0.07	0.05	0.223	159	−0.03	0.07	0.619
Megalin$_2$*: ACC* (0, 1, 2)	295	−0.00	0.06	0.966	165	+0.04	0.07	0.587	280	+0.02	0.06	0.738	159	+0.04	0.07	0.553
Megalin$_3$*: GTT* (0, 1, 2)	295	+0.06	0.08	0.505	165	−0.06	0.09	0.495	280	+0.05	0.08	0.556	159	−0.01	0.09	0.948

[1] Note that VDR_1, VDR_2, VDR_3 denote VDR SNPHAPs, whereas $Megalin_1$, $Megalin_2$, and $Megalin_3$ denote Megalin SNPHAPs. (0, 1, 2) refers to ordinal coding with 0, 1, and 2 copies of each haplotype. Three VDR SNPs were combined to form the haplotypes, namely *Bsm*I, *Apa*I, and *Taq*I. Only haplotypes 1 through 3 were selected for megalin because their overall prevalence was >10%. BLSA, Baltimore Longitudinal Study of Aging; BVRT, Benton Visual Retention Test; CVLT-List A, California Verbal Learning Test, List A; CVLT-DR, California Verbal Learning Test, Delayed Recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; MMSE, Mini-Mental State Examination; OLS, ordinary least squares; SNP, single nucleotide polymorphism; SNPHAP, single nucleotide polymorphism haplotype; Trails A, Trailmaking Test, part A; Trails B, Trailmaking Test, part B; VDR, vitamin D receptor gene; VFT-C, Verbal Fluency Test-Categorical; VFT-L, Verbal Fluency Test-Letter.

[2] Cognitive scores were predicted at mean age at follow-up before the onset of dementia or for all time points by using a linear mixed model controlled for sex, race-ethnicity, education (y), and smoking status, with age added among the fixed-effects variables to allow for quadratic nonlinear change. The slope or annual rate of change was predicted from these models at the mean age at follow-up (ie, between age 50 y and the individual mean age of follow-up for each cognitive test). By using factor analysis, 2-factor scores were estimated and were labeled as Longitudinal Annual Rate of Cognitive Change in the following domains: domain 1, "Memory and executive function: earlier decline"; domain 2, "Verbal fluency and attention: later decline" (*see* Supplemental Material 2 under "Supplemental data" in the online issue for more details). *See* Figure 1 for more details on definition of the SNPHAPs.

[3] On the basis of multiple OLS regression models with outcome being cognitive annual rate of change and main exposures being the 3 megalin and VDR SNP haplotypes. Each haplotype was entered separately in each of the six models per outcome (models A-F). The model controlled for first-visit age, mean age at follow-up, education, first-visit smoking status, first-visit self-reported type 2 diabetes, hypertension, cardiovascular disease, and BMI.

[4] Marginally significant main effects after family-wise Bonferroni correction: $P < 0.05$ for MMSE or BVRT or cognitive domains and $P < 0.025$ for other cognitive tests.

[5] $P < 0.05$ for the null hypothesis that sex × SNPHAP interaction term = 0 in a model in which the main effect of sex was added.

[6] Significant main effects after family-wise Bonferroni correction: $P < 0.025$ for MMSE and cognitive domains and $P < 0.0125$ for other cognitive tests.

data structure unbalanced in terms of follow-up. To this end, we used mixed models to predict cognitive scores and annual rates of change at specific ages where a large proportion of the data were available (mean age at follow-up for each subject; ie, LARCC). We also controlled for the mean age at follow-up as well as first-visit age in the statistical models we conducted. Third, there were no data on serum vitamin D concentrations, and no adequate information on lifestyle factors including dietary intakes of calcium and vitamin D, as well as other potentially confounding factors such as physical activity or alcohol or drug use. Fourth,

although diagnosis of dementia was available, the number of incident dementia cases in our present study was limited ($n = 50$), which precluded analysis of time to onset of dementia in relation to SNPs, SNP LCs, or SNPHAPs. However, this information was used to predict cognitive change by using time points before onset of dementia as well as all time points of follow-up. Finally, several of our positive findings may have been due to chance, residual confounding, or selection bias, whereas other negative findings may have been caused by lack of adequate power. Thus, until those findings are replicated elsewhere, they should be interpreted with caution.

In summary, variance in VDR and megalin SNPs, SNP LCs, and SNPHAPs were shown to affect longitudinal changes in cognitive function in our study population in a sex-specific fashion. Future studies should attempt to examine associations of those SNPs, SNP LCs, and SNPHAPs with incident dementia, AD, and mild cognitive impairment in comparable populations.

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