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State and Trait-Dependent Associations of Vitamin-D with Brain Function During Aging

Alexandra M. Kueider[1], **Toshiko Tanaka**[2], **Yang An**[3], **Melissa H. Kitner-Triolo**[3], **Elango Palchamy**[2], **Luigi Ferrucci**[4], and **Madhav Thambisetty**[1]

[1]Unit of Clinical and Translational Neuroscience, Laboratory of Behavioral Neuroscience, National Institute on Aging, Baltimore, MD, USA

[2]Translational Gerontology Branch, National Institute on Aging, Baltimore, MD, USA

[3]Laboratory of Behavioral Neuroscience, National Institute on Aging, Baltimore, MD, USA

[4]Longitudinal Studies Section, National Institute on Aging, Baltimore, MD, USA

Abstract

We investigated whether: 1) serum levels of 25-hydroxyvitamin D [25(OH)D]; and 2) single nucleotide polymorphisms (SNPs) in the group-specific component (GC) gene regulating serum 25(OH)D levels are associated with cognition in older individuals; and 3) whether causal relationships exist between 25(OH)D and cognition during aging.

Data from 1207 participants in the Baltimore Longitudinal Study of Aging were analyzed (mean follow-up 10.4 years) to test associations between serum 25(OH)D and cognition. Two GC SNPs were used to derive a composite genetic risk score associated with lower 25(OH)D concentrations.

Lower serum 25(OH)D and higher GC composite scores were associated with lower executive function at baseline. Mendelian randomization analyses suggested a causal relationship between lower serum 25(OH)D and poorer executive function and psychomotor speed. The SNP score was also associated with lower performance on measures of visuospatial abilities at baseline but with attenuated declines over time in visuospatial abilities and executive function.

Widespread associations between vitamin-D regulatory SNPs and cognition suggest a mechanistic basis for the relationship between serum 25(OH)D levels and cognition during aging.

Keywords

Vitamin D; cognitive performance; Mendelian randomization

Corresponding author: Madhav Thambisetty, M.D., Ph.D., Clinical and Translational Neuroscience Unit, Laboratory of Behavioral Neuroscience, National Institute on Aging, Baltimore, MD, USA, 251 Bayview Blvd, Room 4B311, National Institute on Aging NIA, National Institutes of Health NIH, Baltimore, MD 21224, thambisettym/at/mail.nih.gov, Telephone: 410-558-8572, Fax: 410-558-8674.

Disclosure statement

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1. Introduction

Both state (i.e. serum/plasma concentrations) and trait (i.e., polymorphic variation in genes) markers of vitamin-D are associated with several adverse health outcomes including hypertension, common cancers, and autoimmune diseases (Holick and Chen, 2008) as well as lower cognitive performance and an increased risk of cognitive decline (Balion et al., 2012, van der Schaft et al., 2013). Epidemiological and observational studies report associations between low serum levels of vitamin-D and age-related cognitive decline and dementia risk (Annweiler et al., 2011, Balion et al., 2012, Lee et al., 2009, Littlejohns et al., 2014, Llewellyn et al., 2010, Oudshoorn et al., 2008, Slinin et al., 2012, van der Schaft et al., 2013). However, these findings have been inconsistent (Breitling et al., 2012, McGrath et al., 2007, Slinin et al., 2010, Tolppanen et al., 2011). Furthermore, evidence from randomized controlled trials evaluating the effects of vitamin-D supplementation on cognition and dementia risk is scarce. Reverse causation or uncontrolled confounding may be important considerations in observational studies of vitamin-D. For example, given the role sunlight plays in the synthesis of vitamin-D, lower levels of serum vitamin-D could be the result of less exposure to sunlight due to an underlying disease rather than its cause (Berry et al., 2012).

The most widely accepted biomarker for vitamin-D is 25-hydroxyvitamin D (25(OH)D) (Ahn et al., 2010). Approximately 25% of variability in serum 25(OH)D concentrations can be explained by such factors as diet, exposure to sunlight, and dietary supplements (Snellman et al., 2009). Genetic factors also contribute significantly to variability in vitamin-D with heritability estimates ranging from 23% to 80% (Karohl et al., 2010, Shea et al., 2009). A number of studies have identified genetic polymorphisms associated with vitamin-D concentrations, including common variants in the group-specific component (GC) gene (i.e., Vitamin-D binding protein) (Ahn et al., 2010, Berry et al., 2012, Nissen et al., 2014, Wang et al., 2010) which play an important role in 25(OH)D distribution (Speeckaert et al., 2006).

GC is a key 25(OH)D carrier protein and determines bioavailability of vitamin-D metabolites to key target cells. Variants in GC may define genetic susceptibility to vitamin-D deficiency in certain individuals (Speeckaert et al., 2006). Protein-bound vitamin-D metabolites have a longer half-life in circulation which suggests that an important function of the vitamin-D binding protein (VDBP) is the stabilization and maintenance of 25(OH)D concentrations (Zella et al., 2008). There are three common isoforms of VDBP based on the combination of alleles from GC SNPs rs7041 and rs4588 (Fu et al., 2009; Speeckaert et al., 2006). In samples of European ancestry, the minor alleles of these SNPs are consistently associated with lower 25(OH)D levels (Ahn et al., 2010, Fu et al., 2009; Wang et al., 2010). Furthermore, healthy individuals with different VDBP genotypes have varying responses to the same vitamin-D dose (Fu et al., 2009) which may have important implications for randomized controlled trials of vitamin-D supplementation. It must be noted however, that although several genome-wide association studies in diverse populations have consistently demonstrated a relationship between polymorphisms in the GC and 25(OH)D

concentrations, the precise interplay between GC SNPs, 25(OH)D concentrations, and binding/bioavailability of 25(OH)D are unclear (Ahn et al., 2010).

In the current study, we used data from the Baltimore Longitudinal Study of Aging (BLSA) to explore whether: 1) serum levels of 25(OH)D are associated with cognition during aging; 2) polymorphic variation in the GC gene regulating serum 25(OH)D levels are associated with cognition during aging; and 3) causal relationships exist between 25(OH)D levels and cognitive performance during aging.

2. Methods

2.1. Subjects

Written informed consent was obtained from participants at each visit, and the study was approved by local Institutional Review Board and the National Institute on Aging.

2.1.1. Baltimore Longitudinal Study of Aging—The BLSA is a prospective cohort study of community-dwelling adult volunteer participants in Baltimore, MD which began in 1958 (Figure 1) (Shock et al., 1984). Data from BLSA participants who did not develop dementia or mild cognitive impairment at any point during follow-up were used in the current analyses (n=1207, mean follow-up interval 10.4 years). All participants in this study sample were Caucasian. Participants received detailed examinations, including neurological, neuropsychological, and laboratory assessments every two years. Starting in 2003, participants aged 80 and older were seen annually.

2.2. Measurement of serum 25(OH)D

Concentration of 25(OH)D was measured in serum samples from BLSA participants collected annually over a 7 year period (2007–2014; n= 2411). Samples were stored at −80°C until analysis. Concentrations of 25(OH)D were assessed by liquid chromatography-mass spectrometry at Mayo Clinic laboratories (Rochester, MN, USA). The inter-assay CV was 10% while the lower limit of detection was 4 ng/mL.

2.3. Cognitive performance

Neuropsychological testing was performed by an experienced tester using a standardized protocol and battery. Mental status was measured with the Mini-Mental State Examination (Folstein et al., 1975). Memory was assessed using the California Verbal Learning Test (Delis et al., 1987). The Trail Making Test Parts A and B assessed attention and executive function respectively (Reitan, 1958), and the Clock Drawing to command (Rouleau et al., 1992) measured executive functioning. Letter and category fluency measured phonetic and semantic fluency (Benton, 1968). The Boston Naming Test (BNT) assessed confrontation naming. Two subtests from the Wechsler Adult Intelligence Scale-Revised (WAIS-R), Digit Span backwards and Similarities, assessed working memory and verbal concept formation and reasoning. The Wide Range Achievement Test Letter and Word Reading sub-test (WRAT) (Wilkinson, 1993) measured verbal abilities. The Purdue Pegboard assessed psychomotor speed (Tiffin and Asher, 1948), while the Rey-Osterrieth Complex Figure (Rey-O) (Rey, 1941) measured visuospatial abilities and figural memory.

2.4. SNP selection and genotyping

Blood samples were collected for DNA extraction, and genome-wide genotyping was completed for 1231 subjects using the Illumina 550K platform. The analysis was restricted to Caucasian participants and each analysis was further adjusted for the top two principal components derived from an EIGENSTRAT analysis utilizing ~10,000 randomly selected SNPs from the 550K SNP panel (Price et al., 2006). Genotyping was completed for 848 participants of European ancestry using a call rate of >98.5% without sex discrepancy based on X-chromosome homozygosity rates (Purcell et al., 2007). 501,704 autosomal SNPs that passed quality control (completeness ≥99%, MAF ≥1%, HWE $\geq 10^{-4}$) were used for imputation (Tanaka et al., 2009). In the BLSA, ~2.5 million HapMap SNPs were imputed using CEU sample (Phase II, release 22, build 36) as a reference using MACH (Li et al., 2010).

SNPs selected for analyses in the current report were identified from two large genome-wide association studies (GWAS) of circulating vitamin-D levels performed in participants of European ancestry (Ahn et al., 2010, Wang et al., 2010). SNPs significantly associated with circulating vitamin-D levels in these prior studies included those in genes encoding vitamin-D binding protein; DBP (i.e. group-specific component gene GC), CYP2R1, encoding the enzyme catalyzing C-25 hydroxylation of vitamin D3 and DHCR7, encoding the enzyme 7-dehydrocholesterol (7-DHC) reductase, which converts 7-DHC, a precursor of 25-hydroxyvitamin D3 to cholesterol (Ahn et al., 2010, Wang et al., 2010). Eighteen SNPs (see Table A.1) were identified based on these previous GWAS of circulating vitamin-D levels (Ahn et al., 2010, Wang et al., 2010). Figure 2 summarizes the exclusion criteria we applied to these 18 SNPs to further identify the most suitable SNPs in our current analyses. Briefly, we excluded one SNP that did not meet our call rate inclusion criteria and eight SNPs that were not significantly associated with serum vitamin-D levels in our study sample. Of the remaining nine SNPs, three were excluded because they were judged to be weak instrumental variables for Mendelian Randomization analyses, as estimated by an F-statistic <10.0 (Burgess et al., 2013) (please see Section 2.6). Of the six SNPs that remained, four (rs17467825, rs3755967, rs4588, and rs2298850) were in significant linkage disequilibrium and were not further considered (Broad Institute SNP Annotation and Proxy Search; SNAP, https://www.broadinstitute.org/mpg/snap/ldsearch.php; HapMap-3, Release-2, CEU sample). The two remaining SNPs (rs2282679 and rs7041) were judged to be appropriate for our current analyses as they were not in linkage disequilibrium ($r^2<0.3$) and were within the GC gene. The F-statistic for the composite SNP was >10.0 (F-statistic = 10.6) after adjustment for age and sex.

2.5. Linear mixed models analyses

Linear mixed models were used to determine the association between serum 25(OH)D and cognition as well as to examine the association of the composite GC SNP score with cognition (both at baseline and rates of change over time).

Age, sex (male=1, female=0), years of education, significant depressive symptoms (1=yes, 0=no), body mass index (BMI), and *APOE* ε4 status were included as covariates in all analyses. Because obesity has been associated with decreased bioavailability of vitamin-D

(Wortsman et al., 2000), BMI was included as a covariate in all analyses. A cut-off score of ≥16 on the Center for Epidemiological Studies Depression Scale (CESD) (Radloff and Teri, 1986) was used to determine significant depressive symptomatology (Lewinsohn et al., 1997). Season of serum vitamin-D collection was included as a covariate in all analyses with serum 25(OH)D. Time was treated as a continuous variable in all models. For all analyses, outcome variables were standardized (Mean=0, SD=1).

2.6. Mendelian Randomization analysis

To examine the causal relationship between vitamin-D and cognition, a Mendelian randomization (MR) analysis was used. First, we regressed serum 25(OH)D on the GC SNP score, adjusting for the previously mentioned covariates. The strength of the association was assessed using the F-statistic, with values <10 considered weak instrumental variables not suitable for MR analyses (Burgess et al., 2013). Next, we regressed the GC SNP score on each cognitive outcome adjusting for the previously mentioned covariates.

A MR analysis was used by adopting a two-stage least-squares (2SLS) (Leong et al., 2014) estimator that regressed each outcome against predicted values of 25(OH)D level per composite GC SNP score using the command “ivreg2” in the Stata SE13.1 software package. This method allows for the estimation of the unconfounded association of genetically predicted concentrations of 25(OH)D with cognition. The Durbin-Wu-Hausman chi-square test for endogeneity in a regression estimated using instrumental variables was computed using the “ivendog” command in which the null hypothesis is that an ordinary least squares estimator of the same equation would yield consistent estimators (Baum et al., 2003). The MR approach thus controls for unmeasured confounders and reverse causality that may distort the directly assessed association between outcome and the exposure of interest (i.e., serum vitamin-D).

To determine whether the relationship between the GC SNPs and 25(OH)D was caused by other factors or genetic confounding, we first included several other available biomarkers (HbA1c, triglycerides, low density lipoproteins, high density lipoproteins, total cholesterol, systolic and diastolic blood pressure, D-dimer, CRP, IL-6, and IGF1) as covariates in a linear regression model examining the associations between GC SNPs and 25(OH)D (Berry et al., 2012).

Next, we tested for pleiotropy by examining the associations between GC SNPs and the biomarkers listed above after adjustment for 25(OH)D. If pleiotropy is present, the association between the GC SNPs and biomarkers should be strong and should not be affected by 25(OH)D adjustment. Finally, interactions between 25(OH)D and GC SNPs with other biomarkers mentioned above were also explored. A Bonferroni corrected p-value was used for the analyses (0.05/11 = <0.004; where the denominator is the number of biomarker tests for each SNP; Table A.2).

2.7. GC SNP composite score

Minor allele frequencies of GC SNPs in the BLSA were 0.25 for rs17467825, 0.25 for rs2282679, 0.25 for rs3755967, 0.28 for rs4588, 0.44 for rs7041, and 0.25 for rs2298850 which are consistent with previous studies (Wang et al., 2010). A composite GC SNP score

was created using the two GC SNPs that passed MR requirements (rs2282679, rs7041) by summing the minor alleles in each SNP (Figure 1; sample 2).

3. Results

3.1. Demographic characteristics

Table 1 shows the demographic characteristics. In the BLSA sample examining associations between serum 25(OH)D and cognition, participants were 52.6 years of age (SD=16.0) and attained 17.1 (SD=2.6 years) years of education.

3.2. Aim 1: Serum 25(OH)D and cognition

On average, serum 25(OH)D concentrations were 32.9 ng/mL (SD=12.3, range 6.4–201) at the initial measurement (Figure 1; sample 1). At baseline, higher concentrations of 25(OH)D were associated with better performance on the clock drawing test (clock 3:25: β=0.02, 95% Confidence Interval (CI): 0.01,0.04, p=.007 and clock 11:10:β=0.02 95% CI: −0.001, 0.03, p=.056). Serum 25(OH)D was not significantly associated with any other cognitive measures (Table 2).

3.3. Aim 2: Composite GC SNP score and cognition

A one unit increase in the GC composite SNP score was associated with a reduction in serum Vitamin-D levels of approximately 0.61 ng/mL (95% CI −0.93, −0.40; p<.0001) after adjustment for age and sex.

There was no clear evidence for pleiotropic effects of the selected SNPs since they were not significantly associated with any of the variables examined at baseline (Table S2). This illustrates that the GC SNP score can be used largely as an unconfounded instrument to assess causality between serum 25(OH)D and cognition. Prior studies have used these GC SNPs as instrumental variables in MR analyses (Berry et al., 2012, Vimaleswaran et al., 2013).

At baseline, higher composite GC SNP score (i.e., more risk alleles) was associated with lower performance on the Clock Drawing tests [3:25 (β=−0.47; 95% CI: −0.81, −0.13; p=.007); 11:10 (β=−0.58; 95% CI: −0.89, −0.26; p=.003; Figure 3)]; however a higher GC composite SNP score was associated with attenuated declines over time on the Clock drawing test [3:25 (β=0.03; 95% CI: 0.01, 0.05; p=.0001)]. The composite GC score was also associated with lower scores on the WRAT (β=−0.23; 95% CI −0.46, −0.002; p=.04) and the Rey-O total copy (β=−0.46; 95% CI −0.87, −0.03; p=.03) at baseline, but with attenuated declines on the Rey-O total copy over time (β=0.03; 95% CI 0.001, 0.06; p=.04).

3.4. Aim 3: Evaluation of causal association between serum Vitamin-D and cognition using MR

In MR analyses to examine a causal association between serum 25(OH)D concentrations and cognition (Figure 1, samples 1 and 2) we observed a causal association between 25(OH)D and the Clock Drawing task (3:25 β=0.05; 95% CI 0.01, 0.08; p=.002; test for endogeneity p=.001; 11:10 β=0.03; 95% CI 0.006, 0.06; p=.02; test for endogeneity p=.03;

Table 2) as well as the Trail Making Test–Part B (β=0.04; 95% CI 0.01, 0.08; *p*=.006; test for endogeneity *p*=.001). The MR results also showed a significant causal association between 25(OH)D and psychomotor speed (pegboard dominant hand β=0.02; 95% CI 0.006, 0.05; test for endogeneity *p*=.003; pegboard nondominant hand β=0.04; 95% CI 0.01, 0.06; test for endogeneity *p*=.01).

3.5. Sensitivity analyses

We performed several sensitivity analyses to explore potential explanations for attenuated declines noted in longitudinal analyses of the GC SNP score and cognitive performance. We first tested whether extreme values of serum vitamin-D, GC SNP scores, or age influenced these results. In none of the cases did extreme values affect significant findings. We also tested whether age, sex, *APOE*, or BMI affected the relationship between vitamin-D (serum and GC SNP score) and the outcome by adding an interaction term as an additional covariate (e.g., BMI×serum 25(OH)D, BMI×GC SNP score). In none of the cases did the interaction term add significantly to the amount of variance explained. To determine if results were due to regression to the mean, a linear mixed effects model was used with intercept and time as both fixed and random effects using an unstructured correlation matrix. If the correlation between the random intercept and random slope is positive, higher baseline values are associated with slower rates of decline. We did not observe a significant positive correlation between the random intercept (baseline cognitive values) and random slope in the mixed effects models.

4. Discussion

The aim of the current study was to examine the influence of both state and trait markers of vitamin-D on cognition during aging in non-demented older individuals. We asked whether: 1) serum 25(OH)D concentrations are associated with cognition; 2) a composite GC SNP risk score related to lower serum 25(OH)D concentrations was associated with cognition; and 3) causal relationships exist between serum 25(OH)D and cognition.

We find that low levels of serum vitamin-D are associated with lower performance on measures of executive function (i.e. clock drawing to command). SNPs within the GC gene that are associated with lower serum concentrations of vitamin-D are also associated with lower performance in executive function, visuospatial and verbal abilities. Furthermore, using Mendelian randomization, we are able to demonstrate that lower concentrations of serum vitamin-D may causally mediate lower performance in executive function and psychomotor speed during aging. We did not find any associations with serum vitamin-D or the GC composite SNP score on memory performance.

Our results indicate domain-specific effects of vitamin-D on cognition during aging suggesting that executive function, visuospatial and verbal abilities, and psychomotor speed may be especially susceptible to perturbations in vitamin-D physiology. The effects of genetic variation in vitamin-D related SNPs on specific cognitive domains appear to be more extensive compared to the effects of serum vitamin-D levels alone. While we observed a significant detrimental effect of GC SNPs on cognition at baseline, suggesting that genetic predisposition to lower serum vitamin-D levels is associated with lower cognitive

performance, these individuals show attenuated declines in cognition during follow-up. It is worth noting that the mean age of our sample at baseline was 52 years with participants having achieved an average of 17 years of education. Given these analyses were performed in a high functioning, middle-aged, and well educated sample, it is plausible that greater cognitive reserve in these individuals may attenuate longitudinal detrimental effects of both genetic and environmental risk factors for cognitive decline. It is plausible that longer follow-up periods may capture differential rates of cognitive decline as our sample entered the highest risk periods for cognitive impairment and dementia. We also suggest that in this sample of healthy older individuals who maintain cognitive health throughout follow-up, these findings may point to compensatory mechanisms recruited in those 'at-risk' to preserve cognitive function over time. These may include as yet unidentified gene×environment interactions that overcome the effects of genetic vulnerability to lower serum vitamin-D levels during aging. The eventual failure of such compensatory mechanisms in older individuals at genetic risk for lower vitamin-D levels may predispose them to accelerated cognitive decline during aging. While the precise mechanisms responsible for the attenuated declines in longitudinal cognitive performance associated with genetic risk for lower vitamin-D levels in our sample are unclear, our sensitivity analyses suggest that neither extreme values in serum vitamin-D concentrations nor age were driving these findings. Furthermore, regression to the mean was ruled out as there was not a significant positive correlation between the random intercept (baseline cognitive values) and random slope in the mixed effects models.

Despite accumulating evidence linking vitamin-D deficiency with various diseases, a recent report from the Institute of Medicine (IOM, 2011) on 'dietary reference intakes for calcium and vitamin D' concluded that the evidence about the potential benefits of vitamin-D supplementation is unreliable. Similarly, while several previous observational studies have consistently shown an association between low serum vitamin-D levels and lower cognitive performance (Annweiler et al., 2011, Balion et al., 2012, Lee et al., 2009, Littlejohns et al., 2014, Llewellyn et al., 2010, Oudshoorn et al., 2008, Slinin et al., 2012, van der Schaft et al., 2013), they do not establish a causal relationship between peripheral vitamin-D concentrations and cognition. Equally importantly, such observational studies may be confounded by reverse causality wherein it is impossible to determine whether lifestyle factors such as a poor diet or lower exposure to sunlight in cognitively impaired subjects, relative to healthy individuals may account for their lower serum vitamin-D concentration. The power of Mendelian randomization relies upon the use of genetic variants associated with serum vitamin-D levels as unconfounded instrumental variables to establish a causal relationship between peripheral vitamin-D concentrations and cognitive performance. For this reason, the MR approach has been referred to as nature's randomized clinical trial in the post-genome era (Thanassoulis et al., 2009).

The molecular mechanisms mediating a protective role of vitamin-D on cognition are diverse and may include neurotrophic effects mediated through nerve growth factor and glial cell line derived neurotrophic factor (Wrzosek et al., 2013). Moreover, vitamin-D has been shown to protect against both age-related inflammatory changes within key memory circuits as well as excitotoxic neuronal damage (Wrzosek et al., 2013).

The strengths of our study include a well characterized and longitudinally followed cohort of older individuals with both serial cognitive and neuroimaging assessments. Furthermore, the availability of genetic data on GC SNPs, together with biochemical measures of analytes related to potentially confounding biological pathways, allowed us to apply a robust MR strategy to examine causal relationships between vitamin-D levels and cognition. Some limitations and methodological considerations must be acknowledged. As serum measurements of vitamin-D levels were only available over a 7 year period, our sample size was limited to participants who had cognitive assessments performed during this interval. Another potential limitation of our study is that our analyses only used participants of European ancestry that were highly educated, which may limit the generalizability of our results.

5. Conclusion

In conclusion, we demonstrated that vitamin-D exerts both state and trait-dependent effects on brain function during aging. The widespread associations between vitamin-D regulatory SNPs and cognition as well as results from the Mendelian randomization analyses, suggest a mechanistic basis for the relationship between vitamin-D and cognition during aging.

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Appendix

Table A.1

Single nucleotide polymorphisms identified in previous genome-wide association studies[16, 20] and their association with serum Vitamin D in the Baltimore Longitudinal Study of Aging

Nearest gene	Role in Vitamin D	SNP	MAF	Association with serum Vitamin D‡ (β; *p*-value)
GC	Metabolism	rs17467825	0.22	−3.44; p<.0001
		rs3755967	0.22	−3.44; p<.0001
		rs2298850	0.20	−3.48; p<.0001
		rs7041	0.39	−2.72; p<.0001
		rs4588	0.22	−3.41; p<.0001
		rs842999	0.36	−2.51; p<.0001
		rs2282679	0.22	−3.44; p<.0001
		rs12512631	0.30	−1.48; p<.0001
		rs16846876	0.26	−2.34; p<.0001
CYP24A1	Metabolism	rs73913757	0.18	*
		rs6013897	0.23	0.72; p>.05
CYP27B1	Metabolism	rs10877012	0.35	0.17; p>.05
CYP2R1	Metabolism	rs2060793	0.36	−1.11; p>.05
		rs1993116	0.34	−1.06; p>.05
		rs12794714	0.48	−1.32; p>.05
		rs10741567	0.33	0.55; p>.05
		rs2060793	0.36	−1.11; p>.05

Nearest gene	Role in Vitamin D	SNP	MAF	Association with serum Vitamin D‡ (β; *p*-value)
DHCR7	Synthesis	rs12785878	0.47	0.77; p>.05

*Poor imputation quality;

‡Models adjusted for age and sex; MAF: minor allele frequency

Table A.2

Association between GC SNPs and other biomarkers adjusted for 25(OH)D and sex

Biomarker	SNP	Coefficient (95% CI)	p-value	Interaction p-value†
Lipid markers				
Triglycerides	rs7041	0.02 (−0.05,0.08)	0.58	0.25
	rs2282679	0.02 (−0.05,0.08)	0.55	0.25
LDL	rs7041	−0.02 (−0.06,0.03)	0.43	0.36
	rs2282679	−0.02 (−0.06,0.03)	0.43	0.38
HDL	rs7041	0.00 (−0.04,0.04)	0.84	0.33
	rs2282679	0.00 (−0.04,0.04)	0.92	0.33
Cholesterol	rs7041	0.00 (−0.03,0.03)	0.95	0.10
	rs2282679	0.00 (−0.03,0.03)	0.89	0.10
Cardiovascular disease related markers				
Diastolic BP	rs7041	0.00 (−0.01,0.02)	0.78	0.33
	rs2282679	0.00 (−0.02,0.02)	0.75	0.33
Systolic BP	rs7041	0.01 (−0.01,0.02)	0.34	0.79
	rs2282679	0.01 (−0.01,0.02)	0.34	0.79
HbA1c	rs7041	0.00 (−0.01,0.02)	0.76	0.92
	rs2282679	0.00 (−0.01,0.02)	0.76	0.92
IGF-1	rs7041	0.01 (−0.05,0.07)	0.77	0.90
	rs2282679	0.01 (−0.06,0.07)	0.86	0.89
Inflammatory markers				
IL-6	rs7041	−0.34 (−0.67,−0.00)	0.05	0.03
	rs2282679	−0.34 (−0.67,−0.00)	0.05	0.03
CRP	rs7041	−0.43 (−1.08,0.22)	0.19	0.39
	rs2282679	−0.43 (−1.08,0.22)	0.19	0.39
Coagulation markers				
D-dimer	rs7041	−0.02 (−0.14,0.10)	0.71	0.56
	rs2282679	−0.02 (−0.13,0.10)	0.78	0.57
Fibrogen	rs7041	−0.16 (−0.39, 0.06)	0.16	0.02
	rs2282679	−0.16 (−0.39, 0.06)	0.16	0.02

†Bonferroni corrected p-value 0.05/11 = 0.004;

- Where the biomarkers have been log transformed to achieve a normal distribution

Highlights

- Lower serum 25(OH)D is associated with lower executive function during aging.
- GC gene variants are associated with lower serum 25(OH)D concentrations.
- GC risk allele carriers show lower executive function, visuospatial and verbal abilities.
- Mendelian randomization tested causality between serum 25(OH)D and cognition.
- Low serum 25(OH)D may be causally linked to poorer executive function and psychomotor speed during aging.

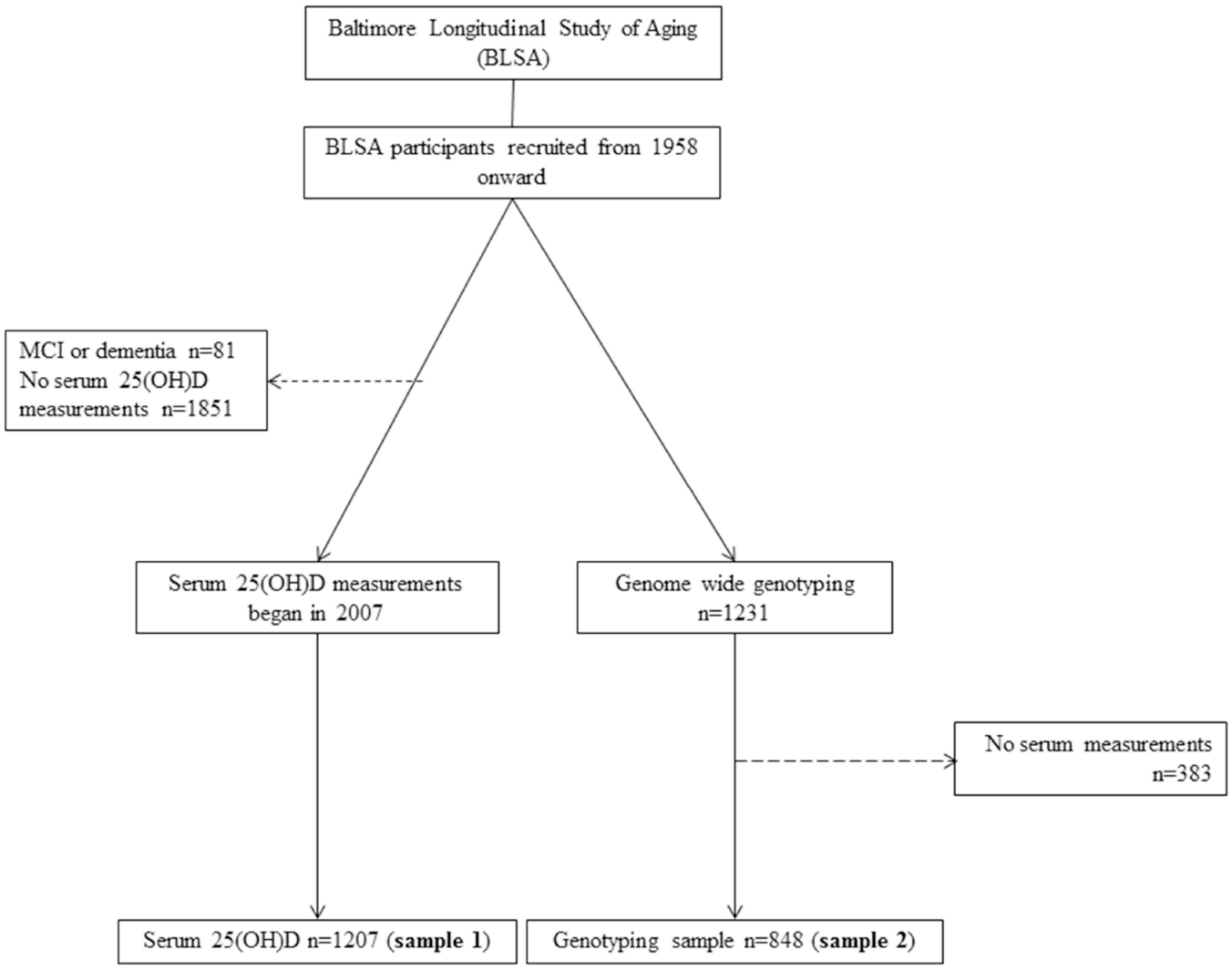

Figure 1.

Schematic summary of the samples used in the present analyses from the Baltimore Longitudinal Study of Aging. Dashed lines indicate excluded participants.

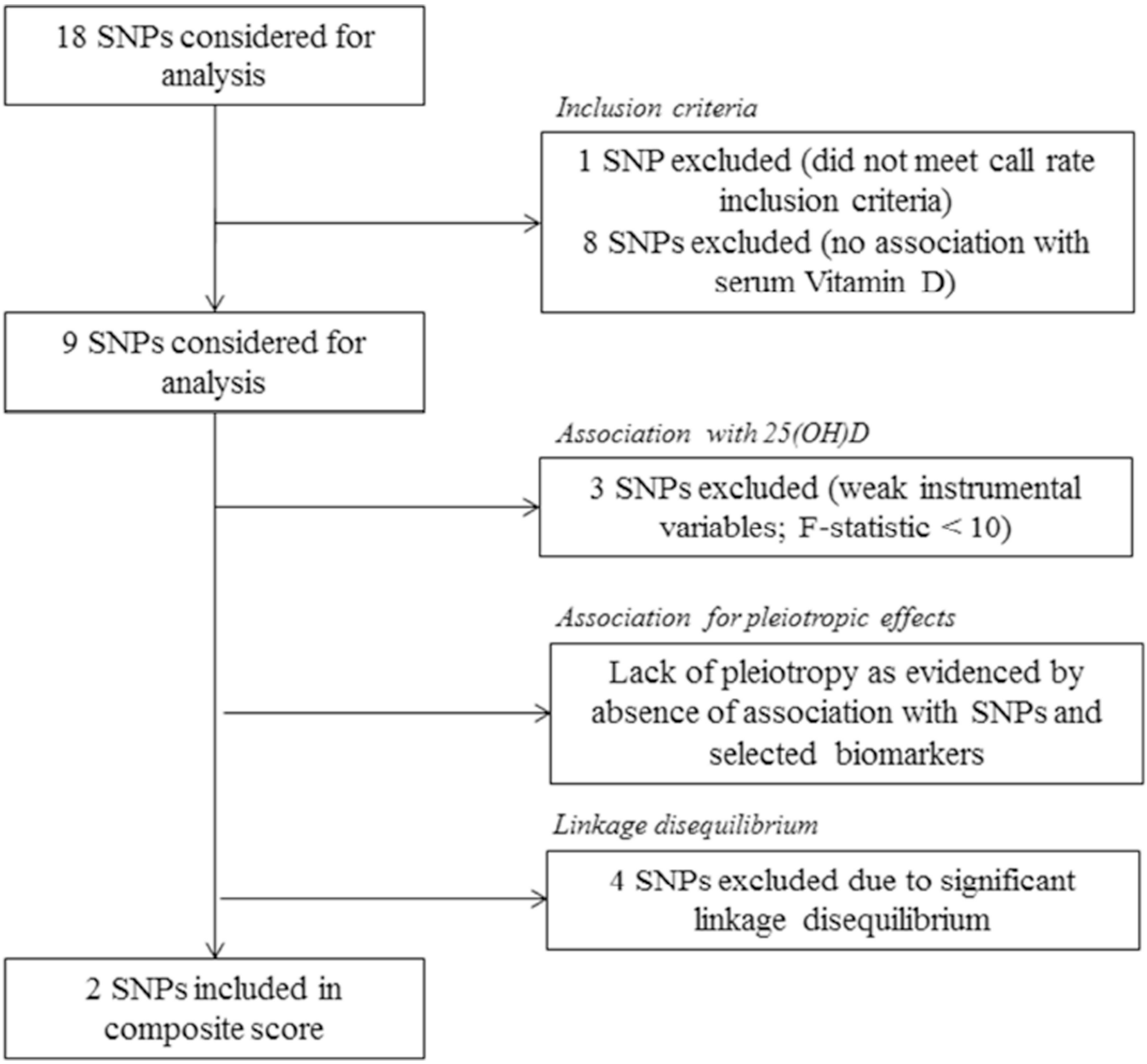

Figure 2.
Selection of 25(OH)D single nucleotide polymorphisms for Mendelian randomization analysis

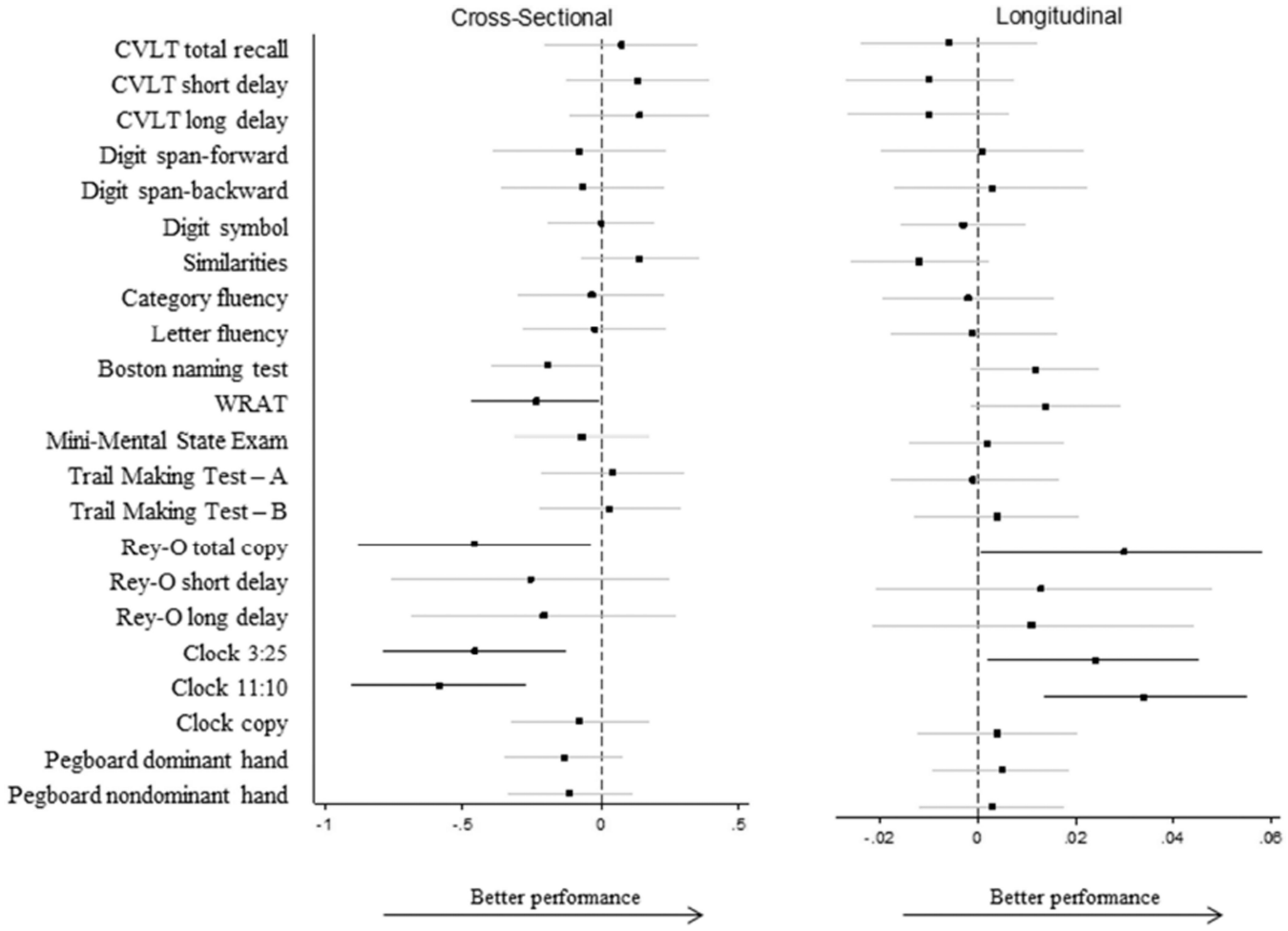

Figure 3. Association of composite GC SNP score with cognitive performance

Effect estimates are adjusted for age, sex, education, APOEε4, body mass index, and depressive symptoms. Bold lines are significant at p<.05. All cognitive outcomes have been standardized with M=0, SD=1. Abbreviations: CVLT – California Verbal Learning Test; Rey-O: Rey-Osterrieth Complex Figure Test; WRAT – Wide Range Achievement Test

Table 1

Demographic characteristics of BLSA sample

	n=1,207
Age at baseline, years M±SD, range	52.6±16.0, 18–91
Male, %	49.8
Education in years, M±SD, range	17.1±2.6, 7–30
25(OH)D, M±SD, range	32.9±12.3, 6.4–201.0
CESD score ≥16, %	5.9
APOE ε4 status, %	21.6
Body mass index, M±SD, range	26.9±4.9, 16.8–53.5

Abbreviations: CESD: Center for Epidemiological Studies Depression Scale

Table 2

Observational and Mendelian randomization analyses for the causal association of the composite GC SNP score with 25-hydroxy-Vitamin D and cognition

	Observational Regression Analysis		Mendelian randomization analysis		
Outcome	**Effect estimate* (95% CI)**	**p-value**	**Effect estimate* (95% CI)**	**p-value**	**Endogeneity p-value**
CVLT total recall	0.00 (−0.01,0.01)	.80	0.004 (−0.03, 0.03)	.82	.98
CVLT short delay	0.00 (−0.01,0.01)	.85	−0.001 (−0.03, 0.03)	.96	.71
CVLT long delay	0.00 (−0.01,0.01)	.83	−0.005 (−0.03, 0.02)	.73	.62
Digit span forward	−0.01 (−0.02,0.01)	.22	0.02 (−0.007, 0.05)	.16	.22
Digit span backward	−0.01 (−0.02,0.01)	.34	0.007 (−0.02, 0.03)	.67	.81
Digit symbol substitution	0.01 (−0.0003,0.02)	.06	0.02 (−0.001, 0.04)	.07	.12
Similarities	−0.01 (−0.02,0.00)	.06	0.01 (−0.01, 0.03)	.35	.68
WRAT	0.00 (−0.01,0.01)	.92	0.006 (−0.006, 0.05)	.15	.44
Mini-Mental State Exam	0.00 (−0.01,0.01)	.87	0.01 (−0.01, 0.06)	.41	.18
Trail Making Test - A	0.00 (−0.01,0.02)	.38	0.007 (−0.01, 0.02)	.51	.19
Trail Making Test - B	0.01 (0.00,0.02)	.14	0.04 (0.01, 0.08)	.006	.001
Category fluency	0.00 (−0.01,0.01)	.72	0.02 (−0.005, 0.03)	.14	.08
Letter fluency	0.00 (−0.01,0.01)	.78	−0.01 (−0.04, 0.01)	.25	.45
Boston naming test	0.00 (−0.01,0.01)	.50	−0.04 (−0.07, −0.01)	.004	.56
Rey-O copy	0.01 (−0.01,0.03)	.45	0.01 (−0.01, 0.04)	.35	.62
Rey-O short delay	0.00 (−0.02,0.02)	.87	0.02 (−0.01, 0.06)	.17	.29
Rey-O long delay	−0.01 (−0.03,0.02)	.60	0.02 (−0.02, 0.08)	.30	.42
Clock 3:25	0.02 (0.01,0.04)	.007	0.05 (0.01, 0.08)	.002	.001
Clock 11:10	0.02 (−0.001,0.03)	.06	0.03 (0.006, 0.06)	.02	.03
Clock copy	0.00 (−0.01,0.02)	.49	0.008 (−0.01, 0.03)	.46	.67
Pegboard dominant hand	0.00 (−0.01,0.01)	.99	0.02 (0.006, 0.05)	.01	.003
Pegboard nondominant hand	0.00 (−0.01,0.01)	.54	0.04 (0.01, 0.06)	.003	.009

Adjusted for age, sex, education, APOEε4, depressive symptoms, body mass index, and season of 25(OH)D collection.

Abbreviations: CVLT - California Verbal Learning Test; Rey-O: Rey-Osterrieth Complex Figure Test; WRAT - Wide Range Achievement Test