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The effect of genetic variations in the vitamin D receptor gene on the course of depressive symptoms

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Abstract

Purpose: Evidence on the association between single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) and depressive symptoms is inconclusive.

Objective: The primary aim of the study was to investigate the association between SNPs in the *VDR* gene and depressive symptoms.

Methods: In a sample of older adults from the Longitudinal Ageing Study Amsterdam (*n*=922), depressive symptoms were assessed using the Centre for Epidemiological Studies Depression scale (CES-D scale) at baseline and after 3, 6, and 10 years of follow-up. Blood samples for SNP and serum 25-hydroxyvitamin D_3 ($25(OH)D_3$) determination were obtained at baseline. The association between 13 SNPs in the *VDR* gene and the course of depressive symptoms were evaluated using linear mixed models. The interaction between SNPs and serum 25(OH)D3 in relation to depressive symptoms was evaluated using multiple linear regression.

Results: No SNPs were associated with the course of depressive symptoms. Significant interactions between serum 25(OH)D3 and SNPs in the *VDR* gene were found. Stratified analysis revealed that within the GG genotype strata, 10 nmol/L higher serum $25(OH)D_3$ was associated with 0.27 (95% CI -0.50, -0.04) and 0.23 (95% CI -0.48,

0.02) lower scores on the CES-D scale for Cdx-2 and 1b-G-886A respectively. This association was not found in persons having the GA or AA genotype.

Conclusion: No SNPs were associated with the course of depressive symptoms. Stratified analysis showed that the effect of serum 25(OH)D3 levels on depressive symptoms was different among genotypes of Cdx-2 and 1b-G-886A. Future research should elucidate on the function of Cdx-2 and 1b-G-886A to describe their effect.

Lay abstract

The current study aimed at investigating the association between single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) gene and depressive symptoms, as current evidence is inconclusive. Within a sample of older adults from the Longitudinal Ageing Study Amsterdam (n=992), depressive symptoms were assessed at baseline and after 3, 6, and 10 years of follow-up. Serum vitamin D levels and SNPs were determined at baseline. Linear mixed models was used to evaluate the effect of 13 SNPs on the course of depressive symptoms, however no significant effects were found. Multiple linear regression was used to assess the interaction between the 13 SNPs and serum vitamin D levels in relation to depressive symptoms. Stratified analysis revealed that within the GG genotype strata, 10 nmol/L higher serum 25(OH)D_3 was associated with 0.27 (95% CI -0.50, -0.04) and 0.23 (95% CI -0.48, 0.02) lower scores on the CES-D scale for Cdx-2 and 1b-G-886A respectively. This association was not found in persons having the GA or AA genotype. Future research should elucidate on the function of Cdx-2 and 1b-G-886A to describe their effect within the vitamin D metabolism.

Abbreviations:

25(OH)D_3: 25-hydroxy-vitamin D_3

CES-D: Center for Epidemiologic Studies Depression Scale

GDS-15: 15-item geriatric depression scale

HWE: Hardy-Weinberg Equilibrium

LAPAQ: Longitudinal Ageing Study Amsterdam Physical Activity Questionnaire

LASA: Longitudinal Ageing Study Amsterdam

LOESS: locally estimated scatterplot smoothing

MAF: minor allele frequency

SNP: single nucleotide polymorphism

VDR: vitamin D receptor

Keywords: VDR gene, Depressive symptoms, Vitamin D, Older adults, SNPs

Introduction

Depressive symptoms follow a U-shaped relationship with age, increasing again after the age of 60-65 [1]. This is however not solely due to ageing and varies widely between people [2]. A higher medical burden [3], ageing related anxiety [4], and non-health related events [5] have been associated with depressive symptoms in older adults. The prevalence of depressive symptoms in older adults is estimated at 17% [6], but they are often overlooked [7] due to amongst others the overlap with ageing symptoms [6,8]. The burden will continue to rise due to the ageing population and increasing life expectancy [9], thereby increasing years lived with disease [10] and societal costs [11].

Different factors (e.g., biological, social, and psychological) play a role in the onset and course of depressive symptoms [12–15]. Vitamin D – a modifiable risk factor of which the status decreases with age [16] – has been associated with the course of depressive symptoms. In a previous study of the Longitudinal Ageing Study Amsterdam, an association between low vitamin D status and an increase in depressive symptoms among women was observed [17]. Two recent meta-analyses found a similar association in both men and women [18,19]. Genetic factors, such as single nucleotide polymorphisms (SNPs) in vitamin D related genes may influence the response to, and therefore the requirement of, vitamin D [20]. The Vitamin D Receptor gene (*VDR* gene) encodes the vitamin D receptor (VDR), the transporter of active vitamin D ($1,25(OH)_2D$), and thereby influences the availability of $1,25(OH)_2D$ [21].

To date, three studies investigated this topic. In the Leiden 85-plus study, a Dutch cohort study (*n*=563), it was found that the alternative allele on Apa1 (A>C) was associated with a decreased score on the 15-item geriatric depression scale (GDS-15) after 4.2 years of follow-up, while other SNPs (Cdx-2, Fok1, Bsm1 and Taq1) were not associated with these scores [22]. In the B-Vitamins for the Prevention Of Osteoporotic Fractures (B-PROOF) study, another Dutch cohort study (n=2839), no associations were found between SNPs in the *VDR* gene (Cdx-2, Fok1, Bsm1 and Taq1) nor where interactions with serum 25-hydroxy-vitamin D_3 ($25(OH)D_3$) in relation to depressive symptoms identified [23]. In a third study based on a sample of German older adults (*n*=101), solely Fok1 was investigated in relation to depression. The researchers found the alternative allele (G>A) to be associated with a lower prevalence of depression [24].

Further research is needed to elucidate the effect of SNPs in the *VDR* gene as previous studies have been inconsistent and results were not replicated. Besides this, evidence on the effect of SNPs in the *VDR* gene on the course of depressive symptoms is underreported, which is important as depressive symptoms change

over time. Therefore, the primary aim of the study was to investigate whether 13 SNPs (Cdx-2, GATA, 1b-C-2481A, 1b-G-886A, 1b-C-673T, 1b-C25A, Fok1, Bsm1, Apa1, Taq1, 291M, 444K and 282W) within the *VDR* gene were associated with the course of depressive symptoms. A secondary aim was to investigate whether there was an interaction effect of serum 25(OH)D_3 in the association between these SNPs and depressive symptoms.

Methods

Study design and population

Data from a representative sample of Dutch older adults, obtained from an ongoing population-based cohort study, the Longitudinal Ageing Study Amsterdam (LASA), was used. Details of the study are described elsewhere and summarised below [25,26]. The LASA study is conducted in line with the Declaration of Helsinki and received approval by the medical ethics committee of the VU University Medical Centre (IRB numbers: 92/138, 2002/141, 2012/361 and 2016.301). All included participants signed an informed consent. In 1992, participants were recruited from municipal registries in three different regions in the Netherlands. LASA focusses on physical, cognitive, emotional, and social functioning and has follow-up measurements every three years, including a main interview, a medical interview with clinical measurements, and a self-administered questionnaire. For this study data from 1995/1996 were used as baseline measurement (*n=2546*); the reason being that blood (i.e., needed for serum 25 (OH)D_3 determinations) was not collected in all three regions in 1992/1993. Follow-up measurements were performed in 1998/1999, 2001/2002 and 2005/2006. Participants with a non-Dutch ethnicity (*n=26*), and persons not participating in the medical interview and the consecutive blood-collection (*n=1598)* were excluded, leaving n=922 in the study sample. For the longitudinal analyses, participants with missing information on the exposure for three or more timepoints were excluded (*n=195*), resulting in n=727 in the sample for analysis. For the cross-sectional interaction analyses, participants with missing information on baseline depressive symptoms (*n=41*) and serum 25(OH)D_3 concentration (*n=2*) were excluded, resulting in n=879 in the sample for analysis. Visual representation of the study sample is presented in **Figure 1**.

Data collection

Depressive symptoms were assessed by self-report with help of the Center for Epidemiologic Studies Depression Scale (CES-D scale) based on a 20-question questionnaire with answers ranging from never (*0*) to always (*3*) [27]. This leads to continuous score between 0 to 60 points, with higher scores indicating more depressive symptoms.

Measurements were taken at baseline and after three-, six- and ten-years of follow-up. The CES-D scale has been validated and is a reliable measure of depressive symptoms in older adults [28,29].

Morning blood samples obtained at the healthcare centre in 1995/1996 were centrifuged and frozen at -80℃ until determination of the *VDR* gene in 2004/2005, and at -20 ℃ until determination of the serum 25(OH)D_3 concentrations (nmol/L) in 1997/1998. Genotyping was performed in Rotterdam in cooperation with the endocrinology laboratory of the VUmc using the Taqman allelic discrimination assay. Samples with pipetting errors, sex mismatch, inconsistencies, and too low DNA were excluded. During this project 13 SNPs in the *VDR* gene were investigated [30]: Cdx-2, GATA, 1b-C-2481A, 1b-G-886A, 1b-C-673T, 1b-C25A, Fok1, Bsm1, Apa1, Taq1, 291M, 444K and 282W. The analysis of 25(OH)D_3 was performed at the Endocrine Laboratory of the VUmc by competitive protein-binding assay (Nichols Diagnostic Capistrano, CA, USA). All measures of serum 25(OH)D_3 concentrations were standardised in 2015 [31] using the Vitamin D Standardisation Program protocol [32]. Seasonality in serum 25(OH)D_3 concentrations was removed by performing locally estimated scatterplot smoothing (LOESS) (**Supplementary Figure 1**)[33]

Covariables and known confounders of serum 25(OH)D_3 concentrations included were: sex, age, physical activity, body mass index (BMI), educational status, smoking status, alcohol consumption and chronic diseases [18]. All variables were measured at baseline in 1995/1996, except sex and educational status, which were measured in 1992/1993 as part of the main interview. Sex was included as a binary variable (male or female). Educational status was split in three categories; low (elementary school or less), middle (lower vocational, intermediate vocational, general intermediate or general secondary school), or high (higher vocational, college or university). Age at date of main interview was included as baseline age. Information on physical activity was measured with the LASA Physical Activity Questionnaire (LAPAQ) [34]. The LAPAQ covers activities such as walking, bicycling, gardening, light to heavy household activities, and sport activities. Physical activity was included as a continuous variable of minutes of physical activity per week. Self-report of chronic diseases was obtained during the main interview and included among others arthritis, cardiac disease, cancer, chronic non-specific lung disease, diabetes, peripheral arterial disease, and stroke; incontinence and head trauma were not included. Chronic diseases were included in three categories; participants having 0 chronic diseases, 1 chronic diseases or ≥2 chronic diseases. Anthropometric measures for calculating the BMI (kg/m^2), smoking status, alcohol consumption and anti-depressant use were obtained during the medical interview. Smoking status was defined as former, current, and never. Self-reported alcohol consumption was categorised

with the alcohol consumption index for LASA, adapted from Garretsen [35,36], leading to the following categories: no alcohol use, light, moderate, excessive, and very excessive. Anti-depressants were identified with the Anatomic Therapeutic Chemical (ATC) code based on the World Health Organisation Collaboration Centre for Drug Statistics Methodology [37]. All participants using medication included under de anti-depressant code N06A were categorised as users of anti-depressants. Information on vitamin D supplementation was not available. Descriptive statistics will be presented as number and percentages (n, %), mean and standard deviation (SD) or median and inter quartile range (IQR).

Statistical analysis

Descriptive statistics was used to describe the baseline characteristics of the study population. In a non-response analysis, a T-test, Wilcoxon signed-rank-test and a two proportions z-test were used to evaluate whether the respective mean, median or proportion differed between responders (*n*=922) and non-responders (*n*=1598). Genetic characteristics of the SNPs were described as MAF and Hardy-Weinberg Equilibrium (HWE). Seasonality in serum 25(OH)D_3 concentrations was removed by performing locally estimated scatterplot smoothing (LOESS) [38]. SNPs in the *VDR* gene were included as dichotomised variables, having no (=0) or at least 1 (=1) alternative allele. In the main analysis variables were dichotomised as the sample sizes for heterogeneous alternative allele subgroups for all the SNPs were too small (*n*=1 to *n*=24) to include it as a separate category (**Supplementary Table 1**). All analyses were complete case analysis. The results of this study will be compared with previously conducted studies, which did not use correction for multiple testing. Therefore, unadjusted p-values will be presented in the results. However, when multiple tests are conducted, controlling for the False Discovery Rate (FDR) is important. Therefore, the Benjamini-Hochberg corrected p-values were presented as well, to investigate whether the found effect measures were still significant after controlling for multiple testing [39].

Linear mixed models was used to examine the association between SNPs in the *VDR* gene and the course of depressive symptoms. Participants were added as a random effect, and all other variables (i.e., SNPs, years of follow-up, age and sex) as fixed effects, resulting in a random intercept model. Depressive symptoms from baseline, three-, six- and ten years of follow-up were used. Time was included as a continuous variable of years after baseline. To test whether the slope for depressive symptoms differed across genotype of the SNP, an interaction term (time*SNP) was added to the model. Exact p-values were presented, with a p-value of $p<0.10$ deemed statistically significant for the interaction term. If no interaction was present, an additive model (time +

SNP) was used. Exact p-values were presented with a p-value of $p<0.05$ deemed statistically significant. Age and sex adjusted regression coefficients and 95% confidence intervals (95% CI) were calculated.

For the interaction analysis, multiple linear regression was used to examine the interaction between SNPs in the *VDR* gene and serum 25(OH)D_3 concentrations, in relation to depressive symptoms at baseline. Levene's Test was used to test for equal variance among the genotypes. The serum 25(OH)D_3 concentration was divided by ten, so a one-step increase in serum 25(OH)D_3 was equivalent to 10 nmol/L. The interaction of the SNP with serum 25(OH)D_3 concentration was tested by adding an interaction term to the model (SNP*25(OH)D_3). Exact p-values were presented with a p-value of $p<0.10$ deemed statistically significant. In case of a significant interaction, stratified analysis was performed. Three models were used: Model 1; adjusted for age and sex, Model 2; additionally adjusted for lifestyle factors (BMI, physical activity, smoking, and alcohol use) Model 3; additionally adjusted for educational status and comorbidities. Exact p-values were presented, with a p-value $p<0.05$ deemed statistically significant.

Sensitivity analysis was performed for the linear mixed and multiple linear regression model to investigate whether analysing the associations among three genotypes (reference allele homozygous, heterozygous, and alternative allele homozygous) would have resulted in different effect estimates. All statistical analysis were performed in R, version 4.3.1. Packages lme4, genetics and effects were used.

Results

The study sample consisted of 922 participants who were on average 75.6 (SD 6.6) years old, were 51% women, and had a median CES-D score of 13.6 (IQR, 11.6-15.6). The prevalence of depressive symptom [40] and anti-depressant use at baseline was 24% and 2.5%, respectively. Mean serum 25(OH)D_3 concentration was 46.6 nmol/L (SD 17.7), mean BMI was 26.6 kg/m^2 (SD 5.2) and median physical activity in minutes per week was 128.6 (IQR 67.7 – 189.7). The basic characteristics are summarized in **Table 1**. Compared to non-responders, participants in the study sample were older and more often women, never smokers, moderate to excessive alcohol users and more often had two or more comorbidities (all $p < 0.05$; **Supplementary Table 2**). The Minor Allele Frequency (MAF) was smallest for 282W (n=63/919; 3%), and no SNPs departed from HWE (**Supplementary Table 1**).

The effect of SNPs on the course of depressive symptoms

The prevalence of depressive symptoms was 24% at baseline, and 26% after 10 years of follow-up (**Supplementary Table 3**). To test whether the SNPs influenced depressive symptoms, first the interaction with time was tested to investigate if the slope for the genotypes differed. The interaction between years of follow-up (time) and the SNP was only significant for GATA (p = 0.07) (**Supplementary Table 4**). All other models were simplified to an additive model with SNP and time as the independent variables and adjusted for age and sex (**Table 2**). Time was before Benjamini-Hochberg correction significantly associated with 0.05 points increase on the CES-D scale per year, within the additive models of all different SNPs. However, after Benjamini-Hochberg correction time was no longer significantly associated with an increase of depressive symptoms. No SNPs were associated before and after Benjamini-Hochberg correction with the course of depressive symptoms ($p > 0.05$). The significant interaction before Benjamini-Hochberg correction of GATA and time suggested a different course of depressive symptoms over time. The increase on the CES-D scale was 0.11 (95% CI, 0.03, 0.19) points per year for AA genotype, but 0.09 (95% CI, -0.18, 0.00) points lower per year for the AG+GG genotype. However, this interaction was not significant after Benjamini-Hochberg correction.

The interaction between SNPs and serum 25(OH)D_3 in relation to depressive symptoms

An increase of 10 nmol/L serum 25(OH)D_3 was significantly ($p < 0.001$) associated with a decrease of -0.40 (95% CI -0.54, -0.21) points on the CES-D scale. To test whether there was an interaction effect of SNPs and serum 25(OH)D_3 in the association with depressive symptoms multiple linear regression was used. The interaction was significant for Cdx-2 (p = 0.008) and 1b-886A (p=0.03) before Benjamini-Hochberg correction for the fully adjusted model, Model 3 (**Supplementary Table 5**). After Benjamini-Hochberg correction the interaction in the fully adjusted model (i.e., Model 3) for Cdx-2 remained significant (p=0.10), however the interaction for 1b-G-886A did not (p=0.20). Stratified analysis was performed for both SNPs, and for Cdx-2 it showed that within the stratum of the homozygous reference allele genotype (GG), an increase of 10 nmol/L in the model was significantly associated with lower scores of 0.32 (95% CI, -0.54, -0.09), 0.28 (95% CI, -0.51, 0.05) and 0.27 (95% CI, -0.50, -0.04) on the CES-D scale, in Models 1, 2 and 3, respectively (**Table 3**). This association was not found in the stratum of the GA+AA genotype. For 1b-G-886A it showed that in the stratum of homozygous reference allele genotype (GG), an increase of 10 nmol/L in the model was significantly associated with lower scores of 0.26 (95% CI, -0.51, -0.01) in Model 1 and borderline associated with lower scores of 0.23 (95% CI, -0.48, 0.02)

in Model 2 and 3 on the CES-D scale (**Table 3**). This association was not found in the stratum of the GA+AA genotype.

Sensitivity analysis

As a sensitivity analysis, the prior analysis were repeated using an additive model, so three genotypes of each SNP (reference allele homozygous, heterozygous, and alternative allele homozygous). Sensitivity analysis for the linear mixed model yielded no different results (**Supplementary Table 6**). The stratified analysis showed that for Cdx-2, as well as within the stratum of homozygous reference allele genotype (GG) as in the stratum of homozygous alternative allele genotype (AA), higher serum 25(OH)D_3 concentrations were significantly associated with a lower CES-D score (**Supplementary Table 7**).

Discussion

The objective of this study was to investigate whether 13 SNPs in the *VDR* gene were associated with the course of depressive symptoms among older adults. In this study, no SNPs were associated with the course of depressive symptoms. We found no significant interactions between the SNPs and time in relation to depressive symptoms, suggesting that the course of depressive symptoms was the same for genetic predisposition of the SNPs. We also investigated the interaction of SNPs in the *VDR* gene and serum 25(OH)D_3 in relation to depressive symptoms. An interaction was found for Cdx-2 and 1b-G-886A, although no longer statistically significant after Benjamini-Hochberg correction for 1b-G-886A. Stratified analyses showed that for both Cdx-2 and 1b-G-886A, within the stratum of homozygous reference allele genotype a higher concentration of serum 25(OH)D_3 was associated with a lower score of depressive symptoms on the CES-D scale, whilst this association was not found within the stratum of genotype with at least one alternative allele.

Even though we found no significant effects of SNPs in the VDR gene and the course of depressive symptoms, we found some associations that are comparable with prior research. The Leiden 85-plus study found that the heterozygous genotype for Apa1 was significantly associated with a 0.56 lower mean score and the homozygous alternative allele genotype was associated with a 0.72 lower mean score based on the GDS-15 scale during a follow-up of 4.2 years without correction for multiple testing [22]. Although the GDS-D scale is another scale to measure depressive symptoms than the CES-D scale and therefore results cannot be directly compared, we found non-significant effect estimates in the same direction with 0.32 lower scores per year for the genotype with at least one alternative allele. One reason for the lack of significance could be that in our study the strata

of heterozygous and homozygous alternative allele were taken together, due to the small sample size in the latter strata. Another study, based on a sample of the German older adult population, did a cross-sectional analysis of Fok1 and depression and found that the genotype with at least one alternative allele was associated with a lower prevalence of depression [24]. We did not focus on depression in our study; therefore, results cannot be directly compared. In our study, no significant effect estimates for Fok1 on depressive symptoms were observed. To our knowledge, no study has found an interaction between SNPs in the *VDR* gene and time in relation to depressive symptoms. We found an interaction for the GATA SNP, however after Benjamini-Hochberg correction the interaction did not remain significant. The results suggested that the increase of points on the CES-D scale over time was lower when having a genotype with at least one alternative allele. The 0.09 points per year found in our results is a very small difference, and therefore the effect of this SNP on its own does not have clinical implications, as the clinical relevance threshold is estimated at about 6.5 points on the CES-D scale [41]. Further research should elucidate whether a combination of SNPs in the VDR gene or other vitamin D related genes have a clinically relevant impact on the course of one's depressive symptoms [42].

In our study we found interactions between serum 25(OH)D_3 concentrations, Cdx-2 and 1b-G-886A before Benjamini-Hochberg correction, which is not in line with prior research[23]. The interaction of serum 25(OH)D_3 and Cdx-2 remained significant after Benjamini-Hochberg correction. The B-PROOF study investigated the interaction between seven SNPs, including Cdx-2 and serum 25(OH)D_3 concentrations without adjusting for multiple testing. This study did not observe the interaction between Cdx-2 and serum 25(OH)D_3 concentrations, we observed within our study [23]. To our knowledge 1b-G-886A has not been studied in relation to serum 25(OH)D_3 and depressive symptoms priorly. Results of our study suggested that the genotype with at least one alternative allele for Cdx-2 and 1b-G-886A alters the beneficial effect of serum 25(OH)D_3 concentrations on depressive symptoms. The sensitivity analysis showed conflicting results within the homozygous alternative allele genotype stratum for Cdx-2, where higher levels of serum 25(OH)D_3 concentrations were associated with lower depressive symptoms on the CES-D scale. However, this stratum consisted of 32 participants, and had a wide CI and therefore most likely does not reflect the true association. The other results of our study are in line with the B-PROOF study, finding no interaction between Fok1, Bsm1 and Taq1 and serum 25(OH)D_3 concentrations in relation to depressive symptoms [23].

The effect of vitamin D is mediated through the VDR [43], so therefore the SNPs in the *VDR* gene might influence the impact of vitamin D. The exact relationship of vitamin D and depressive symptoms is not known;

however, there are different hypothesis. Firstly, SNPs could alter the impact of vitamin D on depressive symptoms by influencing inflammation, calcium homeostasis or mono-amine neurotransmission in the brain [43–46]. GATA, Cdx-2 and 1b-G-886A lie within the promoter region of the *VDR* gene [47–49]. SNPs in this region may alter the *VDR* gene expression or translation capacity, impacting vitamin D signalling efficiency or the levels of the VDR present in different areas of the brain [50–52]. Prior research mentioned that the alternative allele in GATA increases the *VDR* expression [53]; possibly leading to more $1,25(OH)_2D_3$ in the brain [54]. Higher concentrations of $1,25(OH)_2D_3$ might be the reason we observed a slower increase of depressive symptoms among the genotype with at least one alternative allele for GATA. It can also be hypothesised that the SNPs Cdx-2 and 1b-G-886A could affect the VDR levels among different genotypes, so that higher vitamin D levels would not be associated with less depressive symptoms due to less opportunity to transport vitamin D. The SNPs that were found to have an effect could also be in linkage disequilibrium with another SNP which influences the course of depressive symptoms or serum $25(OH)D_3$ concentrations [55]. Secondly, vitamin D might not play a role in the course of depressive symptoms but might be a marker of depressive symptoms among older adults. Older adults could be less active and go outside less often, for example due to the presence of chronic diseases, which could influence the vitamin D status. Results of the effect of vitamin D on depressive symptoms is inconclusive. Results from a recent study based on longitudinal data from 139,128 participants from the UK biobank found that vitamin D status predated depressive symptoms in older adults [56]. More studies found positive findings [57] where others found no association [58–60]. A causal role for vitamin D as assessed in a randomized controlled trial has not been established yet; longitudinal studies with long follow-up time including multiple measures of vitamin D and depressive symptoms over time might elucidate on this relationship.

One strong aspect of this study was the possibility to investigate depressive symptoms longitudinally, as CES-D scores were available for multiple timepoints. Another strong aspect was the inclusion of unknown SNPs which broadens the evidence on less known and studied single SNPs. Also, we included correction for multiple testing to control for the False Discovery Rate, which is a common practice when multiple hypotheses are tested. A few limitations should be mentioned which give opportunity for future research. The first limitation is that the sample size was relatively small for a genetic study, making it difficult to detect a gene-environment interaction. Besides this, even though the LASA cohort is a representative sample of the Dutch population, participants in study sample, in comparison with the non-response population, were on average older, had a higher prevalence of former smokers, a higher prevalence of moderate and excessive alcohol users and had more often two or

more comorbidities, therefore representing an unhealthier sample of Dutch older adults. This likely does not have any implications for the genetic analyses, however, it could have impacted the analyses including serum 25(OH)D_3 as lifestyle does impact vitamin D status [61]. Also, results are not generalisable to other ethnicities, as solely Dutch participants were included in the study. Secondly, vitamin D supplementation was not considered, therefore participants could have had a higher total of vitamin D (i.e., 25(OH)D_3 + 25(OH)D_2) status, possibly weakening, or strengthening the found interaction effects. Vitamin D3 supplementation is directly linked to serum 25(OH)D_3 levels [62]; meaning that 25(OH)D_3 status is a result from supplementation, sunlight and diet. Vitamin D2 supplementation could have decreased the levels of 25(OH)D_3 and increased the levels of 25(OH)D_2 [63], thereby possibly leading to an underestimation of the real effect. However, as vitamin D supplementation often consists vitamin D3, compared to vitamin D2, and the Dutch nutrition guidelines also recommend taking vitamin D3 [64], it is not likely that potential vitamin D supplementation would have changed the outcomes of this study much. Future research should include a more representative study sample representing the general Dutch older adult population, to improve study results and being able to generalise the results. Furthermore, the previously found U-shaped relationship of depressive symptoms with age [1,65] was not evaluated during this study as we did not include enough time-points to test for non-linearity [66]. The association we found between time and depressive symptoms might become stronger, as the non-linear prediction curve would fit the data better. To be able to test for non-linearity, future research should focus on increasing the sample size or time-point of the exposure variable. Lastly, other vitamin D related genes such as *CYP27A1*, *CYP27B1* and *RXR* could be investigated [67]. To elucidate the underlying biological mechanisms of the SNPs, functional research is needed to test whether *VDR* gene activity or VDR levels are different among genotypes.

Conclusion

To conclude, no SNPs in the *VDR* gene were associated with the course of depressive symptoms. Two SNPs in the *VDR* gene interacted with serum 25(OH)D_3 concentrations in relation to depressive symptoms. Stratified analysis revealed that within the GG genotype strata for the SNPs 1b-G-886A and Cdx-2, higher serum 25(OH)D3 was associated with lower scores on the CES-D scale. This association was not found in stratum of the GA+AA genotype. Future research should focus on increasing the sample size, taking effect estimates of SNPs in the *VDR* gene together in a GRS and should include functional research to elucidate the underlying biological mechanisms.

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536

537 **Table 1** Baseline characteristics of the study population

	Study sample (n=922)[a]
CES-D score [b] (median (IQR))	13.6 (11.6 – 15.6)
Depressive symptoms [c] (n (%))	214 (24%)
Missing (n (%))	41 (4%)
Age (mean, SD)	75.6 (6.6)
Sex -male (%)	454 (49%)
Body Mass Index, kg/m^2 (mean (SD))	26.6 (24.0 – 29.2)
Missing (n (%))	8 (1%)
Educational status [d] (%)	
Low	337 (41%)
Middle	432 (47%)
High	112 (12%)
Missing (n (%))	2 (< 1%)
Minutes physical activity /week (median (IQR))	128.6 (67.6 – 189.7)
Missing (n (%))	31 (3%)
Smoking (%)	
Never	306 (33%)
Current	188 (20%)
Former	429 (47%)
Missing	0 (0%)
Alcohol [e] (%)	
No drinking	214 (23%)
Light	453 (49%)
Moderate	191 (21%)

Excessive	19 (5%)
Very excessive	15 (2%)
Missing (n (%))	1 (< 1%)
Anti-depressant use (n/%)	23 (2.5%)
Chronic diseases (%)	
0	163 (18%)
1	267 (29%)
≥2	492 (53%)
Missing (n (%))	1 (< 1%)
Vitamin D concentration nmol/L (mean (SD)) [f]	46.6 (17.6)

[a] For the variables age, sex, smoking and anti-depressant use there were no missing values

[b] Depressive symptom assessed with CES-D scale (0-60) at baseline

[c] Prevalence of depressive symptoms is classified based on ≥16 points on the CES-D scale [40]

[d] Educational status is categorised as low (elementary school or less), middle (lower vocational, intermediate vocational, general intermediate or general secondary school), high (higher vocational, college or university)

[e] Classification based on alcohol consumption index for LASA adapted from Garretsen [35,36]

[f] Serum vitamin D ($25(OH)D_3$) concentration was seasonally adjusted with locally estimated scatterplot smoothing (LOESS)

Table 2 Longitudinal analysis with linear mixed models of the association between SNPs in de VDR gene and the course of depressive symptoms among older adults

CES-D score [a]	n	Coefficient (95% CI) [b]	p-value[c*]	p_{BH}[d*]
Cdx-2				
Cdx-2	GG = 485, GA + AA = 236	-0.01 (-0.54, 0.51)	0.96	1.00
Time		0.05 (0.01, 0.09)	0.02*	0.13
282W				
282W	AA = 677, AT + TT= 48	0.55 (-0.43, 1.53)	0.27	1.00
Time		0.05 (0.01, 0.09)	0.02*	0.13
291M				
291M	AA = 211, AC + CC = 513	-0.28 (-0.82, 0.26)	0.31	1.00
Time		0.05 (0.01, 0.09)	0.02*	0.13
GATA				
GATA	AA = 213, AG + GG = 513	0.22 (-0.41, 0.86)	0.44	0.95

Time		0.11 (0.03, 0.19)	0.006*	0.078
GATA * Time		-0.09 (-0.18, 0.004)	0.07*	0.91
444K				
444K	CC = 591, CA + AA = 134	-0.23 (-0.86, 0.40)	0.47	0.87
Time		0.05 (0.01, 0.09)	0.02*	0.13
1b-C-2481A				
1b-C-2481A	CC = 609, CA + AA = 116	0.24 (-0.42, 0.91)	0.47	0.87
Time		0.05 (0.01, 0.09)	0.02*	0.13
1b-G-886A				
1b-G-886A	GG = 421, GA + AA = 304	-0.30 (-0.80, 0.20)	0.24	1.00
Time		0.05 (0.01, 0.09)	0.02*	0.13
1b-C-673T				
1b-C-673T	CC = 591, CT + TT = 134	0.10 (-0.53, 0.73)	0.76	1.00
Time		0.05 (0.01, 0.09)	0.03*	0.13
Fok1				
Fok1	CC = 335 CT + TT = 448	0.07 (-0.44, 0.58)	0.79	1.00
Time		0.05 (0.01, 0.09)	0.02*	0.13
1b-C25A				
1b-C25A	CC = 291, CA + AA = 434	0.09 (-0.41, 0.60)	0.73	1.00
Time		0.05 (0.01, 0.09)	0.02*	0.13
Bsm1				
Bsm1	GG = 222, GA + AA = 468	0.32 (-0.23, 0.86)	0.26	1.00
Time		0.05 (0.01, 0.10)	0.02*	0.13
Apa1				
Apa1	TT = 201, TG + GG = 489	-0.32 (-0.88, 0.24)	0.26	1.00
Time		0.05 (0.01, 0.10)	0.02*	0.13
Taq1				
Taq1	TT = 230, TC + CC = 460	0.23 (-0.31, 0.77)	0.41	1.00
Time		0.05 (0.01, 0.10)	0.02*	0.13

[a] Centre for Epidemiologic Studies - Depression Scale (CES-D scale) with continuous score ranging from 0 – 60

[b] Associations are adjusted for age and sex

[c] P-values not adjusted for multiple testing with Benjamini-Hochberg

[d] P-values adjusted for multiple correction with Benjamini-Hochberg

[*] p-values p <0.05 were deemed statistically significant for the main effects, and p <0.10 were deemed statistically significant for the interaction effects

Table 3 Stratified cross-sectional analysis of the association between serum 25(OH)D_3 concentrations and depressive symptoms in strata of genotype for Cdx-2 and 1b-G-886A

Depressive symptoms [a]		Model 1 [b]			Model 2 [c]			Model 3 [d]		
	n	β (95%CI)	p	n	β (95%CI)	p	n	β (95%CI)	p [b]
Cdx-2									
GG	*584*	-0.32 (-0.54, -0.09)	<0.01*	*571*	-0.28 (-0.51, - 0.05)	0.02*	*570*	-0.27 (-0.50, -0.04)	0.02*
GA + AA	*288*	0.05 (-0.25, 0.36)	0.73	*285*	0.08 (-0.23, 0.40)	0.61	*285*	0.07 (-0.25, 0.38)	0.68
1b-G-886A									
GG	*509*	-0.26 (-0.51, -0.01)	0.04*	*497*	-0.23 (-0.48, 0.02)	0.07	*496*	-0.23 (-0.48, 0.02)	0.07
GA + AA	*366*	-0.10 (-0.38, 0.17)	0.45	*362*	-0.05 (-0.33, 0.23)	0.73	*362*	-0.06 (-0.34, 0.22)	0.68

[a] Centre for Epidemiologic Studies - Depression Scale (CES-D scale) with continuous score ranging from 0 – 60

[b] Model 1 adjusted for age and sex

[c] Model 2 adjusted for age, sex, BMI, physical activity, smoking and alcohol use

[d] Model 3 adjusted for age, sex, BMI, physical activity, smoking alcohol use, educational status and comorbidities

[*] p-value <0.05 were deemed statistically significant

Figure 1 Flowchart of study participants

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethics declarations

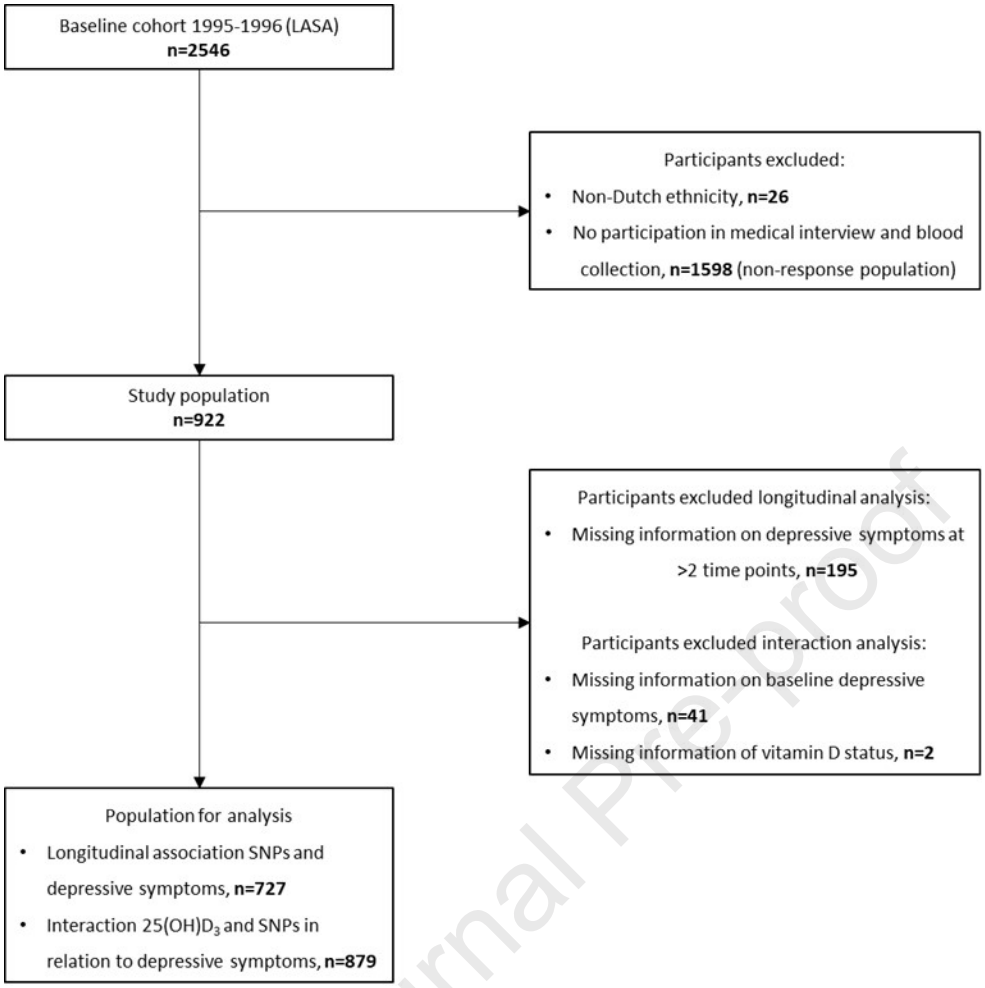
The LASA study is conducted in line with the Declaration of Helsinki and received approval by the medical ethics committee of the VU University Medical Centre (IRB numbers: 92/138, 2002/141, 2012/361 and 2016.301). All included participants signed an informed consent.

Data accessibility

Data from the Longitudinal Ageing Study Amsterdam (LASA) are available after submission of a research proposal for a specific research question to the LASA Steering Group. A standard analysis proposal form can be obtained from the LASA website: www.lasa-vu.nl. The LASA Steering Group will ensure that proposals do not violate privacy regulations and are in keeping with informed consent that is provided by all participants.

Supplementary information

Below is the link to the SI Online Resources.



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