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A.N. Wenzler, B. van de Loo, N. van der Velde, N.M. Schoor



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1 The effect of genetic variations in the vitamin 2 D receptor gene on the course of depressive 3 symptoms

4 A.N. Wenzler¹, B. van de Loo^{1,2,3}, N. van der Velde^{2,3}, N.M. Schoor^{1,3}

5 a.n.wenzler@umcg.nl; r.vandeloo@amsterdamumc.nl; n.vandervelde@amsterdamumc.nl;

6 nm.vanschoor@amsterdamumc.nl

7 ¹ Amsterdam UMC location Vrije Universiteit Amsterdam, Epidemiology and Data Science, De Boelelaan 1117,
8 Amsterdam, the Netherlands

9 ² Amsterdam UMC location University of Amsterdam, Internal Medicine, Section of Geriatric Medicine,
10 Meienbergdreef 9, Amsterdam, the Netherlands

11 ³ Amsterdam Public Health Research Institute, Ageing & Later Life, Amsterdam, the Netherlands

12 Corresponding author: Ana N. Wenzler – a.n.wenzler@umcg.nl

13 Abstract

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

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

18 *Methods:* In a sample of older adults from the Longitudinal Ageing Study Amsterdam ($n=922$), depressive
19 symptoms were assessed using the Centre for Epidemiological Studies Depression scale (CES-D scale) at baseline
20 and after 3, 6, and 10 years of follow-up. Blood samples for SNP and serum 25-hydroxyvitamin D₃ (25(OH)D₃)
21 determination were obtained at baseline. The association between 13 SNPs in the VDR gene and the course of
22 depressive symptoms were evaluated using linear mixed models. The interaction between SNPs and serum
23 25(OH)D₃ in relation to depressive symptoms was evaluated using multiple linear regression.

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

27 0.02) lower scores on the CES-D scale for Cdx-2 and 1b-G-886A respectively. This association was not found in
28 persons having the GA or AA genotype.

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

32 Lay abstract

33 The current study aimed at investigating the association between single nucleotide polymorphisms (SNPs) in the
34 vitamin D receptor (VDR) gene and depressive symptoms, as current evidence is inconclusive. Within a sample
35 of older adults from the Longitudinal Ageing Study Amsterdam (n=992), depressive symptoms were assessed at
36 baseline and after 3, 6, and 10 years of follow-up. Serum vitamin D levels and SNPs were determined at baseline.
37 Linear mixed models was used to evaluate the effect of 13 SNPs on the course of depressive symptoms, however
38 no significant effects were found. Multiple linear regression was used to assess the interaction between the 13
39 SNPs and serum vitamin D levels in relation to depressive symptoms. Stratified analysis revealed that within the
40 GG genotype strata, 10 nmol/L higher serum 25(OH)D₃ was associated with 0.27 (95% CI -0.50, -0.04) and 0.23
41 (95% CI -0.48, 0.02) lower scores on the CES-D scale for Cdx-2 and 1b-G-886A respectively. This association was
42 not found in persons having the GA or AA genotype. Future research should elucidate on the function of Cdx-2
43 and 1b-G-886A to describe their effect within the vitamin D metabolism.

44

45 **Abbreviations:**

46 25(OH)D₃: 25-hydroxy-vitamin D₃

47 CES-D: Center for Epidemiologic Studies Depression Scale

48 GDS-15: 15-item geriatric depression scale

49 HWE: Hardy-Weinberg Equilibrium

50 LAPAQ: Longitudinal Ageing Study Amsterdam Physical Activity Questionnaire

51 LASA: Longitudinal Ageing Study Amsterdam

52 LOESS: locally estimated scatterplot smoothing

53 MAF: minor allele frequency

54 SNP: single nucleotide polymorphism

55 VDR: vitamin D receptor

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

57 Introduction

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

65 Different factors (e.g., biological, social, and psychological) play a role in the onset and course of
66 depressive symptoms [12–15]. Vitamin D – a modifiable risk factor of which the status decreases with age [16] –
67 has been associated with the course of depressive symptoms. In a previous study of the Longitudinal Ageing
68 Study Amsterdam, an association between low vitamin D status and an increase in depressive symptoms among
69 women was observed [17]. Two recent meta-analyses found a similar association in both men and women
70 [18,19]. Genetic factors, such as single nucleotide polymorphisms (SNPs) in vitamin D related genes may
71 influence the response to, and therefore the requirement of, vitamin D [20]. The Vitamin D Receptor gene (*VDR*
72 gene) encodes the vitamin D receptor (VDR), the transporter of active vitamin D (1,25(OH)₂D), and thereby
73 influences the availability of 1,25(OH)₂D [21].

74 To date, three studies investigated this topic. In the Leiden 85-plus study, a Dutch cohort study ($n=563$),
75 it was found that the alternative allele on Apa1 (A>C) was associated with a decreased score on the 15-item
76 geriatric depression scale (GDS-15) after 4.2 years of follow-up, while other SNPs (Cdx-2, Fok1, Bsm1 and Taq1)
77 were not associated with these scores [22]. In the B-Vitamins for the Prevention Of Osteoporotic Fractures (B-
78 PROOF) study, another Dutch cohort study ($n=2839$), no associations were found between SNPs in the *VDR* gene
79 (Cdx-2, Fok1, Bsm1 and Taq1) nor were interactions with serum 25-hydroxy-vitamin D₃ (25(OH)D₃) in relation
80 to depressive symptoms identified [23]. In a third study based on a sample of German older adults ($n=101$), solely
81 Fok1 was investigated in relation to depression. The researchers found the alternative allele (G>A) to be
82 associated with a lower prevalence of depression [24].

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

86 over time. Therefore, the primary aim of the study was to investigate whether 13 SNPs (Cdx-2, GATA, 1b-C-2481A,
87 1b-G-886A, 1b-C-673T, 1b-C25A, Fok1, Bsm1, Apa1, Taq1, 291M, 444K and 282W) within the *VDR* gene were
88 associated with the course of depressive symptoms. A secondary aim was to investigate whether there was an
89 interaction effect of serum 25(OH)D₃ in the association between these SNPs and depressive symptoms.

90 Methods

91 *Study design and population*

92 Data from a representative sample of Dutch older adults, obtained from an ongoing population-based cohort
93 study, the Longitudinal Ageing Study Amsterdam (LASA), was used. Details of the study are described elsewhere
94 and summarised below [25,26]. The LASA study is conducted in line with the Declaration of Helsinki and received
95 approval by the medical ethics committee of the VU University Medical Centre (IRB numbers: 92/138, 2002/141,
96 2012/361 and 2016.301). All included participants signed an informed consent. In 1992, participants were
97 recruited from municipal registries in three different regions in the Netherlands. LASA focusses on physical,
98 cognitive, emotional, and social functioning and has follow-up measurements every three years, including a main
99 interview, a medical interview with clinical measurements, and a self-administered questionnaire. For this study
100 data from 1995/1996 were used as baseline measurement ($n=2546$); the reason being that blood (i.e., needed
101 for serum 25 (OH)D₃ determinations) was not collected in all three regions in 1992/1993. Follow-up
102 measurements were performed in 1998/1999, 2001/2002 and 2005/2006. Participants with a non-Dutch
103 ethnicity ($n=26$), and persons not participating in the medical interview and the consecutive blood-collection
104 ($n=1598$) were excluded, leaving $n=922$ in the study sample. For the longitudinal analyses, participants with
105 missing information on the exposure for three or more timepoints were excluded ($n=195$), resulting in $n=727$ in
106 the sample for analysis. For the cross-sectional interaction analyses, participants with missing information on
107 baseline depressive symptoms ($n=41$) and serum 25(OH)D₃ concentration ($n=2$) were excluded, resulting in
108 $n=879$ in the sample for analysis. Visual representation of the study sample is presented in **Figure 1**.

109 *Data collection*

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

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

115 Morning blood samples obtained at the healthcare centre in 1995/1996 were centrifuged and frozen at
116 -80°C until determination of the *VDR* gene in 2004/2005, and at -20°C until determination of the serum $25(\text{OH})\text{D}_3$
117 concentrations (nmol/L) in 1997/1998. Genotyping was performed in Rotterdam in cooperation with the
118 endocrinology laboratory of the VUmc using the Taqman allelic discrimination assay. Samples with pipetting
119 errors, sex mismatch, inconsistencies, and too low DNA were excluded. During this project 13 SNPs in the *VDR*
120 gene were investigated [30]: Cdx-2, GATA, 1b-C-2481A, 1b-G-886A, 1b-C-673T, 1b-C25A, Fok1, Bsm1, Apa1, Taq1,
121 291M, 444K and 282W. The analysis of $25(\text{OH})\text{D}_3$ was performed at the Endocrine Laboratory of the VUmc by
122 competitive protein-binding assay (Nichols Diagnostic Capistrano, CA, USA). All measures of serum $25(\text{OH})\text{D}_3$
123 concentrations were standardised in 2015 [31] using the Vitamin D Standardisation Program protocol [32].
124 Seasonality in serum $25(\text{OH})\text{D}_3$ concentrations was removed by performing locally estimated scatterplot
125 smoothing (LOESS) (**Supplementary Figure 1**)[33]

126 Covariables and known confounders of serum $25(\text{OH})\text{D}_3$ concentrations included were: sex, age,
127 physical activity, body mass index (BMI), educational status, smoking status, alcohol consumption and chronic
128 diseases [18]. All variables were measured at baseline in 1995/1996, except sex and educational status, which
129 were measured in 1992/1993 as part of the main interview. Sex was included as a binary variable (male or
130 female). Educational status was split in three categories; low (elementary school or less), middle (lower
131 vocational, intermediate vocational, general intermediate or general secondary school), or high (higher
132 vocational, college or university). Age at date of main interview was included as baseline age. Information on
133 physical activity was measured with the LASA Physical Activity Questionnaire (LAPAQ) [34]. The LAPAQ covers
134 activities such as walking, bicycling, gardening, light to heavy household activities, and sport activities. Physical
135 activity was included as a continuous variable of minutes of physical activity per week. Self-report of chronic
136 diseases was obtained during the main interview and included among others arthritis, cardiac disease, cancer,
137 chronic non-specific lung disease, diabetes, peripheral arterial disease, and stroke; incontinence and head
138 trauma were not included. Chronic diseases were included in three categories; participants having 0 chronic
139 diseases, 1 chronic diseases or ≥ 2 chronic diseases. Anthropometric measures for calculating the BMI (kg/m^2),
140 smoking status, alcohol consumption and anti-depressant use were obtained during the medical interview.
141 Smoking status was defined as former, current, and never. Self-reported alcohol consumption was categorised

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

149 *Statistical analysis*

150 Descriptive statistics was used to describe the baseline characteristics of the study population. In a non-response
151 analysis, a T-test, Wilcoxon signed-rank-test and a two proportions z-test were used to evaluate whether the
152 respective mean, median or proportion differed between responders ($n=922$) and non-responders ($n=1598$).
153 Genetic characteristics of the SNPs were described as MAF and Hardy-Weinberg Equilibrium (HWE). Seasonality
154 in serum 25(OH)D₃ concentrations was removed by performing locally estimated scatterplot smoothing (LOESS)
155 [38]. SNPs in the *VDR* gene were included as dichotomised variables, having no (=0) or at least 1 (=1) alternative
156 allele. In the main analysis variables were dichotomised as the sample sizes for heterogeneous alternative allele
157 subgroups for all the SNPs were too small ($n=1$ to $n=24$) to include it as a separate category (**Supplementary**
158 **Table 1**). All analyses were complete case analysis. The results of this study will be compared with previously
159 conducted studies, which did not use correction for multiple testing. Therefore, unadjusted p-values will be
160 presented in the results. However, when multiple tests are conducted, controlling for the False Discovery Rate
161 (FDR) is important. Therefore, the Benjamini-Hochberg corrected p-values were presented as well, to investigate
162 whether the found effect measures were still significant after controlling for multiple testing [39].

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

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

172 For the interaction analysis, multiple linear regression was used to examine the interaction between
173 SNPs in the *VDR* gene and serum 25(OH)D₃ concentrations, in relation to depressive symptoms at baseline.
174 Levene's Test was used to test for equal variance among the genotypes. The serum 25(OH)D₃ concentration was
175 divided by ten, so a one-step increase in serum 25(OH)D₃ was equivalent to 10 nmol/L. The interaction of the
176 SNP with serum 25(OH)D₃ concentration was tested by adding an interaction term to the model (SNP*25(OH)D₃).
177 Exact p-values were presented with a p-value of $p < 0.10$ deemed statistically significant. In case of a significant
178 interaction, stratified analysis was performed. Three models were used: Model 1; adjusted for age and sex, Model
179 2; additionally adjusted for lifestyle factors (BMI, physical activity, smoking, and alcohol use) Model 3;
180 additionally adjusted for educational status and comorbidities. Exact p-values were presented, with a p-value
181 $p < 0.05$ deemed statistically significant.

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

186 Results

187 The study sample consisted of 922 participants who were on average 75.6 (SD 6.6) years old, were 51% women,
188 and had a median CES-D score of 13.6 (IQR, 11.6-15.6). The prevalence of depressive symptom [40] and anti-
189 depressant use at baseline was 24% and 2.5%, respectively. Mean serum 25(OH)D₃ concentration was 46.6
190 nmol/L (SD 17.7), mean BMI was 26.6 kg/m² (SD 5.2) and median physical activity in minutes per week was 128.6
191 (IQR 67.7 – 189.7). The basic characteristics are summarized in **Table 1**. Compared to non-responders,
192 participants in the study sample were older and more often women, never smokers, moderate to excessive
193 alcohol users and more often had two or more comorbidities (all $p < 0.05$; **Supplementary Table 2**). The Minor
194 Allele Frequency (MAF) was smallest for 282W (n=63/919; 3%), and no SNPs departed from HWE
195 (**Supplementary Table 1**).

196

197 *The effect of SNPs on the course of depressive symptoms*

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

211
212 *The interaction between SNPs and serum 25(OH)D₃ in relation to depressive symptoms*

213 An increase of 10 nmol/L serum 25(OH)D₃ was significantly ($p < 0.001$) associated with a decrease of -
214 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
215 serum 25(OH)D₃ in the association with depressive symptoms multiple linear regression was used. The
216 interaction was significant for Cdx-2 ($p = 0.008$) and 1b-886A ($p=0.03$) before Benjamini-Hochberg correction for
217 the fully adjusted model, Model 3 (**Supplementary Table 5**). After Benjamini-Hochberg correction the interaction
218 in the fully adjusted model (i.e., Model 3) for Cdx-2 remained significant ($p=0.10$), however the interaction for
219 1b-G-886A did not ($p=0.20$). Stratified analysis was performed for both SNPs, and for Cdx-2 it showed that within
220 the stratum of the homozygous reference allele genotype (GG), an increase of 10 nmol/L in the model was
221 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%
222 CI, -0.50, -0.04) on the CES-D scale, in Models 1, 2 and 3, respectively (**Table 3**). This association was not found
223 in the stratum of the GA+AA genotype. For 1b-G-886A it showed that in the stratum of homozygous reference
224 allele genotype (GG), an increase of 10 nmol/L in the model was significantly associated with lower scores of
225 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)

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

228

229 *Sensitivity analysis*

230 As a sensitivity analysis, the prior analysis were repeated using an additive model, so three genotypes of each
231 SNP (reference allele homozygous, heterozygous, and alternative allele homozygous). Sensitivity analysis for the
232 linear mixed model yielded no different results (**Supplementary Table 6**). The stratified analysis showed that for
233 Cdx-2, as well as within the stratum of homozygous reference allele genotype (GG) as in the stratum of
234 homozygous alternative allele genotype (AA), higher serum 25(OH)D₃ concentrations were significantly
235 associated with a lower CES-D score (**Supplementary Table 7**).

236 Discussion

237 The objective of this study was to investigate whether 13 SNPs in the *VDR* gene were associated with the course
238 of depressive symptoms among older adults. In this study, no SNPs were associated with the course of depressive
239 symptoms. We found no significant interactions between the SNPs and time in relation to depressive symptoms,
240 suggesting that the course of depressive symptoms was the same for genetic predisposition of the SNPs. We also
241 investigated the interaction of SNPs in the *VDR* gene and serum 25(OH)D₃ in relation to depressive symptoms.
242 An interaction was found for Cdx-2 and 1b-G-886A, although no longer statistically significant after Benjamini-
243 Hochberg correction for 1b-G-886A. Stratified analyses showed that for both Cdx-2 and 1b-G-886A, within the
244 stratum of homozygous reference allele genotype a higher concentration of serum 25(OH)D₃ was associated with
245 a lower score of depressive symptoms on the CES-D scale, whilst this association was not found within the
246 stratum of genotype with at least one alternative allele.

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

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

268 In our study we found interactions between serum 25(OH)D₃ concentrations, Cdx-2 and 1b-G-886A
269 before Benjamini-Hochberg correction, which is not in line with prior research[23]. The interaction of serum
270 25(OH)D₃ and Cdx-2 remained significant after Benjamini-Hochberg correction. The B-PROOF study investigated
271 the interaction between seven SNPs, including Cdx-2 and serum 25(OH)D₃ concentrations without adjusting for
272 multiple testing. This study did not observe the interaction between Cdx-2 and serum 25(OH)D₃ concentrations,
273 we observed within our study [23]. To our knowledge 1b-G-886A has not been studied in relation to serum
274 25(OH)D₃ and depressive symptoms priorly. Results of our study suggested that the genotype with at least one
275 alternative allele for Cdx-2 and 1b-G-886A alters the beneficial effect of serum 25(OH)D₃ concentrations on
276 depressive symptoms. The sensitivity analysis showed conflicting results within the homozygous alternative
277 allele genotype stratum for Cdx-2, where higher levels of serum 25(OH)D₃ concentrations were associated with
278 lower depressive symptoms on the CES-D scale. However, this stratum consisted of 32 participants, and had a
279 wide CI and therefore most likely does not reflect the true association. The other results of our study are in line
280 with the B-PROOF study, finding no interaction between Fok1, Bsm1 and Taq1 and serum 25(OH)D₃
281 concentrations in relation to depressive symptoms [23].

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

284 however, there are different hypothesis. Firstly, SNPs could alter the impact of vitamin D on depressive symptoms
285 by influencing inflammation, calcium homeostasis or mono-amine neurotransmission in the brain [43–46]. GATA,
286 Cdx-2 and 1b-G-886A lie within the promoter region of the *VDR* gene [47–49]. SNPs in this region may alter the
287 *VDR* gene expression or translation capacity, impacting vitamin D signalling efficiency or the levels of the *VDR*
288 present in different areas of the brain [50–52]. Prior research mentioned that the alternative allele in GATA
289 increases the *VDR* expression [53]; possibly leading to more 1,25(OH)₂D₃ in the brain [54]. Higher concentrations
290 of 1,25(OH)₂D₃ might be the reason we observed a slower increase of depressive symptoms among the genotype
291 with at least one alternative allele for GATA. It can also be hypothesised that the SNPs Cdx-2 and 1b-G-886A could
292 affect the *VDR* levels among different genotypes, so that higher vitamin D levels would not be associated with
293 less depressive symptoms due to less opportunity to transport vitamin D. The SNPs that were found to have an
294 effect could also be in linkage disequilibrium with another SNP which influences the course of depressive
295 symptoms or serum 25(OH)D₃ concentrations [55]. Secondly, vitamin D might not play a role in the course of
296 depressive symptoms but might be a marker of depressive symptoms among older adults. Older adults could be
297 less active and go outside less often, for example due to the presence of chronic diseases, which could influence
298 the vitamin D status. Results of the effect of vitamin D on depressive symptoms is inconclusive. Results from a
299 recent study based on longitudinal data from 139,128 participants from the UK biobank found that vitamin D
300 status predated depressive symptoms in older adults [56]. More studies found positive findings [57] where others
301 found no association [58–60]. A causal role for vitamin D as assessed in a randomized controlled trial has not
302 been established yet; longitudinal studies with long follow-up time including multiple measures of vitamin D and
303 depressive symptoms over time might elucidate on this relationship.

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

313 more comorbidities, therefore representing an unhealthier sample of Dutch older adults. This likely does not
314 have any implications for the genetic analyses, however, it could have impacted the analyses including serum
315 25(OH)D₃ as lifestyle does impact vitamin D status [61]. Also, results are not generalisable to other ethnicities,
316 as solely Dutch participants were included in the study. Secondly, vitamin D supplementation was not considered,
317 therefore participants could have had a higher total of vitamin D (i.e., 25(OH)D₃ + 25(OH)D₂) status, possibly
318 weakening, or strengthening the found interaction effects. Vitamin D3 supplementation is directly linked to
319 serum 25(OH)D₃ levels [62]; meaning that 25(OH)D₃ status is a result from supplementation, sunlight and diet.
320 Vitamin D2 supplementation could have decreased the levels of 25(OH)D₃ and increased the levels of 25(OH)D₂
321 [63], thereby possibly leading to an underestimation of the real effect. However, as vitamin D supplementation
322 often consists vitamin D3, compared to vitamin D2, and the Dutch nutrition guidelines also recommend taking
323 vitamin D3 [64], it is not likely that potential vitamin D supplementation would have changed the outcomes of
324 this study much. Future research should include a more representative study sample representing the general
325 Dutch older adult population, to improve study results and being able to generalise the results. Furthermore, the
326 previously found U-shaped relationship of depressive symptoms with age [1,65] was not evaluated during this
327 study as we did not include enough time-points to test for non-linearity [66]. The association we found between
328 time and depressive symptoms might become stronger, as the non-linear prediction curve would fit the data
329 better. To be able to test for non-linearity, future research should focus on increasing the sample size or time-
330 point of the exposure variable. Lastly, other vitamin D related genes such as *CYP27A1*, *CYP27B1* and *RXR* could
331 be investigated [67]. To elucidate the underlying biological mechanisms of the SNPs, functional research is
332 needed to test whether *VDR* gene activity or *VDR* levels are different among genotypes.

333 *Conclusion*

334 To conclude, no SNPs in the *VDR* gene were associated with the course of depressive symptoms. Two SNPs in the
335 *VDR* gene interacted with serum 25(OH)D₃ concentrations in relation to depressive symptoms. Stratified analysis
336 revealed that within the GG genotype strata for the SNPs 1b-G-886A and Cdx-2, higher serum 25(OH)D₃ was
337 associated with lower scores on the CES-D scale. This association was not found in stratum of the GA+AA
338 genotype. Future research should focus on increasing the sample size, taking effect estimates of SNPs in the *VDR*
339 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)

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

539 ^b Depressive symptom assessed with CES-D scale (0-60) at baseline

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

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

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

544 ^f Serum vitamin D (25(OH)D₃) concentration was seasonally adjusted with locally estimated scatterplot
545 smoothing (LOESS)

546 **Table 2** Longitudinal analysis with linear mixed models of the association between SNPs in de VDR gene and
547 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

548

549 ^a Centre for Epidemiologic Studies - Depression Scale (CES-D scale) with continuous score ranging from 0 – 60550 ^b Associations are adjusted for age and sex551 ^c P-values not adjusted for multiple testing with Benjamini-Hochberg

552 ^d P-values adjusted for multiple correction with Benjamini-Hochberg

553 * p-values $p < 0.05$ were deemed statistically significant for the main effects, and $p < 0.10$ were deemed

554 statistically significant for the interaction effects

555

556 **Table 3** Stratified cross-sectional analysis of the association between serum 25(OH)D₃ concentrations and

557 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			

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

559 ^b Model 1 adjusted for age and sex

560 ^c Model 2 adjusted for age, sex, BMI, physical activity, smoking and alcohol use

561 ^d Model 3 adjusted for age, sex, BMI, physical activity, smoking alcohol use, educational status and

562 comorbidities

563 * p-value <0.05 were deemed statistically significant

564

565 **Figure 1** Flowchart of study participants

566 Declarations

567 Conflict of interest

568 The authors declare that they have no conflict of interest.

569 Acknowledgement

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574 feedback was provided by BvdL, NS, and NvdV. Finally, ANW, BvdL, NMS and NvdV prepared the completed
575 manuscript. All authors read and approved the final manuscript.

576

577 Author information

578 Ana N. Wenzler

579 Bob van de Loo

580 Nathalie van der Velde

581 Natasja M. Schoor

582 Corresponding author: Ana N. Wenzler

583 Ethics declarations

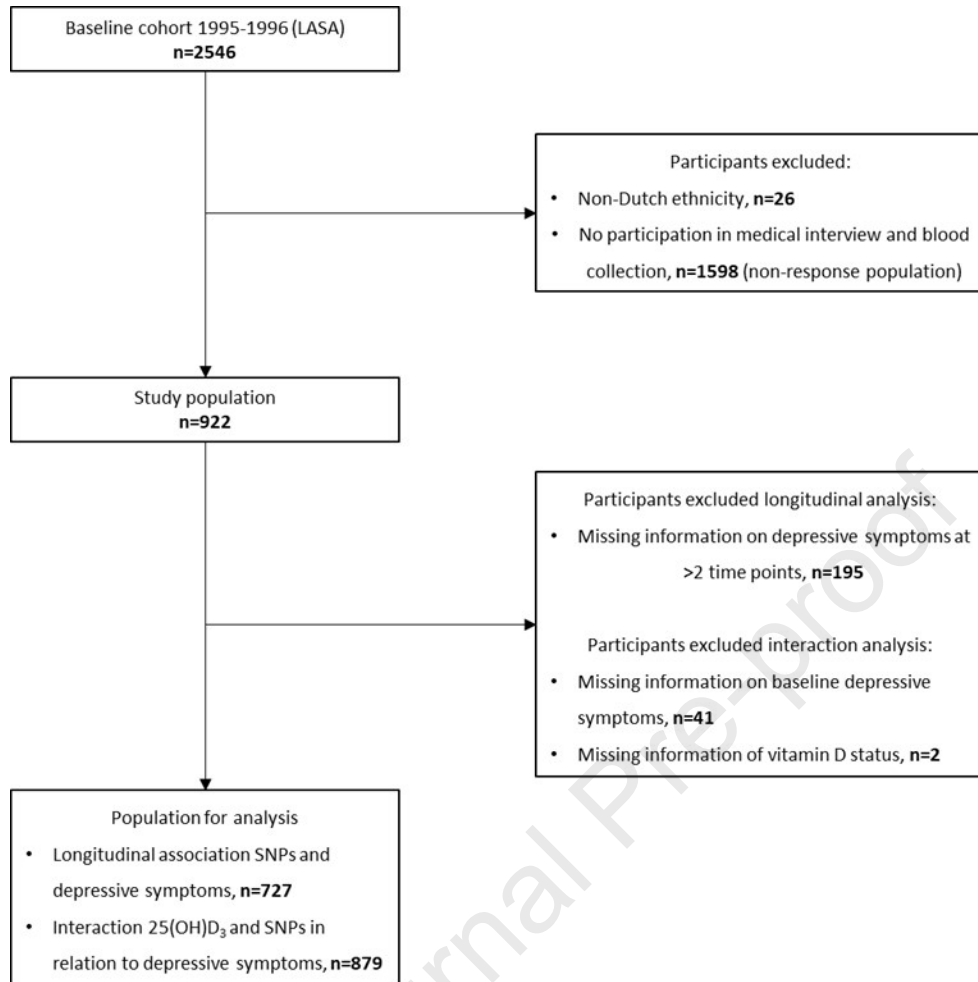
584 The LASA study is conducted in line with the Declaration of Helsinki and received approval by the medical ethics
585 committee of the VU University Medical Centre (IRB numbers: 92/138, 2002/141, 2012/361 and 2016.301). All
586 included participants signed an informed consent.

587 Data accessibility

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

592 Supplementary information

593 Below is the link to the SI Online Resources.



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