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A study into genes encoding longevity in humans

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CHAPTER 4

VDR Gene Variants Associate with Cognitive Function and Depressive Symptoms in Old Age

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Abstract

Vitamin D has been recently implicated in brain function. Our objective was to test whether genetic variance in the vitamin D receptor (*VDR*) gene is associated with cognitive functioning and depressive symptoms in old age. The study was carried out in the prospective population-based Leiden 85-plus Study. All 563 participants of the study were genotyped for *Cdx-2*, *FokI*, *BsmI*, *ApaI* and *TaqI* polymorphisms in the *VDR* gene. Our data revealed an overall worse performance on tests measuring cognitive functioning for carriers of *BsmI* ($p=0.013$) and *TaqI* ($p=0.004$) polymorphisms, and of haplotype 2 (BAt) ($p=0.004$). In contrast, carriers of *ApaI* variant-allele and of haplotype 1 (baT) had better cognitive functioning together with less depressive symptoms. These associations could not be explained by differences in calcium levels, and by selective survival, since no associations between the *VDR* gene variants and calcium levels and mortality were observed. In conclusion, our results show that genetic variance in the *VDR* gene influence the susceptibility to age-related changes in cognitive functioning and in depressive symptoms.

Introduction

Ample data provide evidence that vitamin D is involved in brain function. The reported biological processes influenced by vitamin D in the brain include neuroprotection, immunomodulation and detoxification (Brown et al., 2003; Garcion et al., 2002). The neuroprotective effects of vitamin D appear to be exerted via the regulation of calcium homeostasis (Brewer et al., 2001; Brewer et al., 2006; de Viragh et al., 1989), and synthesis of neurotrophins, such as nerve growth factor and neurotrophin 3 (Naveilhan et al., 1996; Neveu et al., 1994a; Neveu et al., 1994b; Saporito et al., 1994; Wang et al., 2000). These biological effects suggest that vitamin D could influence cognitive functioning and the prevalence of depressive symptoms.

The functions of vitamin D are mediated by vitamin D receptor (*VDR*), which belongs to the nuclear hormone receptor (NHR) super-family, and which is ubiquitously expressed in the organism (Kamei et al., 1995; Langub et al., 2001). Defects in the vitamin D signaling system have been associated with multiple sclerosis, and various behavioral- and mood disorders in animals and humans (Cantorna et al., 1996; Garcion et al., 2002; Lansdowne and Provost, 1998; Munger et al., 2004). It has been shown that animals exposed to prenatal vitamin D deficiency have alterations in brain morphology (Eyles et al., 2003), locomotion (Burne et al., 2004; Kesby et al., 2006), and learning and memory (Becker et al., 2005). In addition, mice lacking a functional *VDR* gene appear to suffer from anxiety-like behavior (Kalueff et al., 2004; Kalueff et al., 2006). In humans, vitamin D deficiency has been associated with the presence of an active mood disorder and with worse cognitive functioning (Przybelski and Binkley, 2007; Wilkins et al., 2006). In contrast, little is known about whether and how disturbed function of the *VDR* gene influences these endpoints. The *VDR* gene contains several polymorphisms of which five; *Cdx-2*, *FokI*, *BsmI*, *ApaI* and *TaqI*, have been most often investigated, and associated with a number of phenotypes, such as bone mineral density, and risks for fractures and cancer (Uitterlinden et al., 2004b). In addition, haplotype alleles have been identified that influence the risk of osteoporotic fractures and the expression of the *VDR* gene (Fang et al., 2005; Grundberg et al., 2007). The risk haplotypes that have recently emerged, baT and BAt, are composed of the *BsmI*, *ApaI* and *TaqI* polymorphisms, located in the 3‘ untranslated region (UTR).

The aim of this study was to assess the influence of these five polymorphisms in the VDR gene and the risk haplotypes on cognitive functioning and depressive symptoms in old age. Furthermore, the association with calcium levels, and the incidence of fractures and mortality were assessed for the VDR polymorphisms and the haplotypes. The study was carried out in the Leiden 85-plus Study, a population-based prospective study of the oldest old.

Participants and methods

Participants

The Leiden 85-plus Study is a prospective population based study in which all 85-year-old inhabitants of the city Leiden, in The Netherlands, were invited to take part. There were no selection criteria related to health or demographic characteristics. The population under study

consists of 599 subjects, all Caucasians and members of the 1912-1914 birth cohort, enrolled in the month of their 85th birthday between 1997 and 1999 (Bootsma-van der Wiel et al., 2002). For the present study, DNA was available for 563 participants. All participants of the Leiden 85-plus Study were followed for mortality until August 1, 2005. Primary causes of death were obtained from the Dutch Central Bureau of Statistics and categorized according to the 10th International Classification of Diseases (ICD-10). The Medical Ethical Committee of the Leiden University Medical Centre approved the study and informed consent was obtained from all participants or in case of severe cognitive impairment, from their guardian.

Calcium levels at baseline

Calcium and albumin concentrations were determined in serum using fully automated analyzers (Hitachi 747 and 911; Hitachi, Ltd, Tokyo, Japan). Total calcium levels were adjusted for albumin using the following formula: corrected calcium = uncorrected calcium - [(40 – albumin) × 0.02] (Palmer et al., 1988).

Cognitive function and depressive symptoms

Overall cognitive function was measured with the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). From the specific domains of cognitive functioning, attention was assessed with the Stroop Test (Klein et al., 1997), processing speed with the Letter Digit Coding Test (LDT) (Houx et al., 2002) and memory with the 12-Word Learning Test, which assesses immediate recall (WLTI) and delayed recall (WLTD)(Brand and Jolles, 1985). The prevalence of depressive symptoms was assessed with the 15-item Geriatric Depression Scale (GDS-15) (De Craen et al., 2003). The tests assessing specific domains of cognitive functioning could not be administered to 92 participants because of severe cognitive impairment (MMSE score < 19 points). All participants were visited annually for re-measurement of cognitive functioning and depressive symptoms during a mean follow-up period of 4.2 years. During the study, parallel versions of the tests were used and details of testing are described elsewhere (Houx et al., 2002). In addition to the specific tests, a composite cognitive score was calculated by converting the scores of the individual tests (Stroop Test, LDT, WLTI and WLTD) into a z-score ((individual level – mean level)/SD), and computing the average. A higher composite cognitive score reflects better performance on the tests measuring cognitive functioning.

The incidence of fractures

The incidence of fractures was assessed yearly during the five-year follow-up period. The number of fractures was obtained by self-reporting using a standardized yes/no format. When the participant was severely cognitively impaired, a guardian was asked for the information. In addition, the general practitioner, or the nursing home physician in case of institutionalization, was interviewed concerning fracture related contacts with the participant. The composite of self-reported and physician reported fractures were used. Fractures included hip, wrist and other fractures.

Possible confounders

Socio-demographic characteristics, such as sex and level of education were considered as possible confounders. Education was divided into two levels: a lower education level, including individuals without schooling or with only primary school education (less than 6 years of schooling), and a higher education level (6 years or more of schooling).

Genotyping

The *Cdx-2* G/A (rs11568820) and *BsmI* C/T (rs1544410) single nucleotide polymorphisms (SNPs) were genotyped using an Assay-by-Design (Applied Biosystems, Foster City, CA, USA), consisting of PCR primers and TaqMan MGB probes, on an ABI 7900 HT real-time PCR (Applied Biosystems, Foster City, CA, USA). Amplification reactions were made at standard conditions except for the following modifications. A qPCR core kit was used (Eurogentec, Liege, Belgium) and one-third of the amount of assay mix. *FokI* G/A (rs10735810), *ApaI* A/C (rs7975232) and *TaqI* A/G (rs731236) polymorphisms were genotyped using MassArray platform according to the protocols of the manufacturer (Sequenom Inc., San Diego, CA, USA).

Statistical analysis

The program Haploview (Barrett et al., 2005) was used to estimate allele frequencies, test for Hardy-Weinberg equilibrium and to estimate pair-wise linkage disequilibrium (LD) between the SNPs. Haplotypes and haplotype frequencies were calculated using the program SNPHAP (*http://www-gene.cimr.cam.ac.uk/clayton/software*). In order to take into account the uncertainty in haplotype probabilities, the multiple imputation approach was used (Rubin, 1987), and with SNPHAP ten datasets were generated by randomly assigning a haplotype to each subject according to its haplotype probabilities. All statistical analyses were performed with the ten datasets. The haplotype specific estimates were calculated by averaging the ten dataset-specific estimates, and the standard errors were estimated using the estimated variance within and across the datasets. The associations between baseline calcium levels and *VDR* polymorphisms and haplotypes were tested using sex adjusted linear regression. Associations between cognitive functioning, depressive symptoms and *VDR* polymorphisms and haplotypes were analyzed using a sex and education adjusted linear mixed model, estimating the overall mean difference in cognitive functioning or depressive symptoms during follow-up. Cox proportional hazard model, measuring time-to event was used to estimate the risk of incident fractures, and mortality during the follow-up period, in relation to the polymorphisms or haplotypes. The reference group contained zero-copies of a risk allele or haplotype. All analyses were performed with SPSS, version 12.0 (SPSS Inc., Chicago, IL, USA) statistical software.

Results

Demographic characteristics and baseline measures of cognitive functioning and depressive symptoms of the 563 participants of the Leiden 85-plus Study are presented in Table 1. All

study participants were genotyped for *Cdx-2*, *FokI*, *BsmI*, *ApaI* and *TaqI* polymorphisms in the VDR gene. The genotype frequencies of the SNPs (Table 1) were in agreement with Hardy-Weinberg equilibrium and similar to those reported in other Caucasian populations (Uitterlinden et al., 2004a). The *BsmI*, *ApaI* and *TaqI* polymorphisms were in strong linkage disequilibrium (LD) (D'> 0.99) (Figure 1), and defined five haplotypes, of which the first three had frequencies > 5 %. These haplotypes have previously been described as baT, BAt and bAT, respectively. The haplotype frequencies were similar to those reported in other Caucasian populations (Fang et al., 2005; Grundberg et al., 2007).

Global cognitive functioning, attention, processing speed, memory and the prevalence of depressive symptoms were assessed at baseline, age 85 years, and re-examined annually during a mean follow-up period of 4.2 years. During follow-up, a significant decline in cognitive functioning, and an increase in depressive symptoms were observed in all participants (all $p<0.001$) (Vinkers et al., 2005). These changes were not attributable to the *Cdx-2* or *FokI* polymorphisms, since during follow-up no differences in cognitive functioning and depressive symptoms were observed for carriers of these polymorphisms (data not shown). On the other hand, carriers of the *BsmI* and *TaqI* polymorphisms performed worse on all tests measuring cognitive functioning (Table 2). This worse performance was reflected by a lower composite cognitive score (*BsmI* $p_{trend}=0.013$; *TaqI* $p_{trend}=0.004$), but not by a lower MMSE, which measures global cognitive functioning (*BsmI* $p_{trend}=0.999$; *TaqI* $p_{trend}=0.899$). From specific domains of cognitive functioning, attention, immediate- and delayed memory were affected most, whereas for the prevalence

Table 1. Characteristics of study participants

Number	563
Age[1]	85 (-)
Female (%)	375 (67 %)
Low level of education (%)	362 (65 %)
Calcium (mmol/l)[1]	2.23 (2.16-2.29)
MMSE (points)[1]	26 (22-28)
MMSE >18 points (%)	471 (84 %)
Specific domains of cognitive functioning[1]	
Stroop Test (seconds)	74 (60-97)
LDT (digits)	16 (12-21)
WLTI (pictures)	25 (20-28)
WLTD (pictures)	9 (7-11)
GDS-15 (points)[1]	2 (1-3)
Polymorphisms[2]	
Cdx-2 (G/A)	0.19
FokI (G/A)	0.34
BsmI (C/T)	0.43
ApaI (A/C)	0.46
TaqI (A/G)	0.42

[1] Data are presented as medians with interquartile ranges; [2] Data are presented as minor allele frequencies; MMSE - Mini-Mental State Examination; LDT - Letter Digit Coding Test; WLTI - Word Learning Test Immediate Recall; WLTD - Word Learning Test Delayed Recall; GDS-15 - 15-item Geriatric Depression Scale

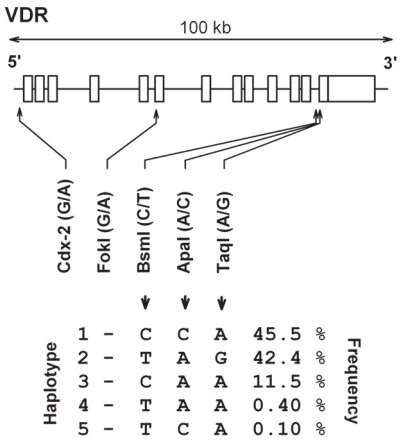

Figure 1. The VDR gene structure and haplotypes. The VDR gene spans a genomic region of 100 kb and contains 14 exons (indicated with boxes). The approximate positions of the five polymorphisms analyzed in this study are indicated with arrows. The BsmI, ApaI and TaqI polymorphisms are in strong linkage disequilibrium (LD) and define five haplotypes. The first three haplotypes, haplotype 1, haplotype 2 and haplotype 3 have previously been described as baT, BAt and bAT, respectively.

Table 2. Cognitive functioning and depressive symptoms during follow-up dependent on the *VDR* polymorphisms

	VDR genotypes			
	wt/wt	wt/var	var/var	
	Mean (SE)	Difference (SE)	Difference (SE)	p_{trend}
BsmI (C/T)				
Composite cognitive score	-0.08 (0.06)	-0.12 (0.08)	-0.25 (0.10)*	0.013*
MMSE (points)	22.6 (0.48)	0.47 (0.61)	-0.15 (0.78)	0.999
Stroop Test (seconds)	84.3 (2.31)	2.00 (2.95)	10.4 (3.78)*	0.010*
LDT (digits)	16.2 (0.51)	-1.04 (0.65)	-0.34 (0.83)	0.471
WLTI (pictures)	21.3 (0.48)	-1.07 (0.61)	-2.18 (0.77)*	0.004*
WLTD (pictures)	7.33 (0.23)	-0.30 (0.29)	-0.78 (0.39)*	0.037*
GDS-15 (points)	2.93 (0.21)	-0.05 (0.26)	0.55 (0.34)	0.158
ApaI (A/C)				
Composite cognitive score	-0.22 (0.07)	0.00 (0.08)	0.16 (0.10)	0.135
MMSE (points)	23.1 (0.51)	-0.32 (0.63)	-0.56 (0.76)	0.456
Stroop Test (seconds)	89.9 (2.45)	-2.65 (3.05)	-6.80 (3.68)	0.068
LDT (digits)	16.4 (0.53)	-1.40 (0.66)*	-0.06 (0.80)	0.737
WLTI (pictures)	19.8 (0.51)	0.49 (0.63)	1.61 (0.76)*	0.041*
WLTD (pictures)	6.83 (0.24)	0.23 (0.30)	0.44 (0.36)	0.222
GDS-15 (points)	3.42 (0.21)	-0.56 (0.26)*	-0.72 (0.32)*	0.019*
TaqI (A/G)				
Composite cognitive score	-0.07 (0.06)	-0.13 (0.08)	-2.94 (0.10)*	0.004*
MMSE (points)	22.7 (0.48)	0.55 (0.60)	-0.30 (0.78)	0.899
Stroop Test (seconds)	84.0 (2.29)	2.12 (2.92)	11.0 (3.80)*	0.008*
LDT (digits)	16.3 (0.50)	-0.96 (0.64)	-0.63 (0.84)	0.310
WLTI (pictures)	21.4 (0.47)	-1.15 (0.60)	-2.51 (0.78)*	0.001*
WLTD (pictures)	7.40 (0.23)	-0.37 (0.29)	-0.99 (0.37)*	0.009*
GDS-15 (points)	2.93 (0.20)	-0.06 (0.26)	0.60 (0.33)	0.135

*$p < 0.05$; SE – standard error; MMSE - Mini-Mental State Examination; LDT - Letter Digit Coding Test; WLTI - Word Learning Test Immediate Recall; WLTD - Word Learning Test Delayed Recall; GDS-15 - 15-item Geriatric Depression Scale

of depressive symptoms no differences were observed (Table 2). In contrast, carriers of the *ApaI* variant-allele tended to have less depressive symptoms than non-carriers during follow-up (p_{trend}=*0.019*) (Table 2). These differences were observed for both heterozygous (-0.56 points, 95 % CI:-1.07 to -0.04, *p=0.036*) and homozygous (-0.72 points, 95 % CI: -1.35 to -0.09, *p=0.026*) *ApaI* variant-allele carriers. In addition, these participants performed better, although not statistically significant, on tests measuring processing speed, attention and memory (Table 2).

The haplotype analyses revealed similar results as those with the individual polymorphisms. Carriers of at least one copy of haplotype 1 (baT), which contains the *ApaI* polymorphism, had less depressive symptoms (p_{trend}=*0.026*), and performed better, although statistically not significantly, on tests measuring attention, immediate memory and delayed memory compared to non-carriers (Table 3). The opposite was observed for carriers of haplotype 2 (BAt), which combines the variant alleles of *BsmI* and *TaqI* polymorphisms (Table 3). For all these associations, an allele dosage dependent effect was observed, which was more pronounced for haplotype 2 (BAt) carriers, who had mainly impairments in attention (p_{trend}=*0.008*), immediate memory (p_{trend}=*0.001*) and delayed memory (p_{trend}=*0.009*) (Figure 2).

Table 3. Cognitive functioning and depressive symptoms during follow-up dependent on the *VDR* haplotypes

	VDR haplotypes			
	0-copies Mean (SE)	1-copy Difference (SE)	2-copies Difference (SE)	p-trend
Haplotype 1 (baT)				
Composite score	-0.09 (0.07)	-0.01 (0.08)	0.13 (0.10)	0.215
MMSE (points)	22.5 (0.55)	-0.32 (0.62)	-0.60 (0.76)	0.424
Stroop Test (seconds)	84.3 (2.72)	-1.72 (3.01)	-5.78 (3.67)	0.124
LDT (digits)	15.3 (0.59)	-1.44 (0.66)*	-0.23 (0.80)	0.576
WLTI (pictures)	22.6 (0.56)	0.52 (0.62)	1.47 (0.76)	0.056
WLTD (pictures)	7.95 (0.27)	0.26 (0.29)	0.39 (0.36)	0.254
GDS-15 (points)	2.60 (0.24)	-0.48 (0.26)	-0.69 (0.33)*	0.026*
Haplotype 2 (BAt)				
Composite score	0.05 (0.07)	-0.13 (0.08)	-0.29 (0.10)*	0.004*
MMSE (points)	22.0 (0.53)	0.57 (0.60)	-0.29 (0.77)	0.912
Stroop Test (seconds)	79.3 (2.70)	2.12 (2.92)	10.7 (3.77)*	0.008*
LDT (digits)	15.2 (0.59)	-1.01 (0.64)	0.63 (0.83)	0.298
WLTI (pictures)	24.1 (0.55)	-1.15 (0.60)	-2.50 (0.77)*	0.001*
WLTD (pictures)	8.51 (0.26)	-0.37 (0.28)	-0.98 (0.37)*	0.009*
GDS-15 (points)	2.14 (0.24)	-0.03 (0.26)	0.56 (0.33)	0.147
Haplotype 3 (bAT)				
Composite score	-0.10 (0.05)	0.12 (0.09)	0.56 (0.28)*	0.032*
MMSE (points)	22.0 (0.40)	0.76 (0.68)	2.77 (2.25)	0.124
Stroop Test (seconds)	83.3 (2.05)	-3.17 (3.30)	-13.3 (10.5)	0.153
LDT (digits)	14.2 (0.44)	1.25 (0.72)	3.74 (2.29)	0.024*
WLTI (pictures)	22.9 (0.42)	0.76 (0.68)	3.84 (2.20)	0.074
WLTD (pictures)	8.05 (0.20)	0.32 (0.32)	2.13 (1.04)*	0.067
GDS-15 (points)	2.14 (0.18)	0.39 (0.30)	0.48 (0.94)	0.171

* p < 0.05; SE – standard error; MMSE - Mini-Mental State Examination; LDT - Letter Digit Coding Test; WLTI - Word Learning Test Immediate Recall; WLTD - Word Learning Test Delayed Recall; GDS-15 - 15-item Geriatric Depression Scale

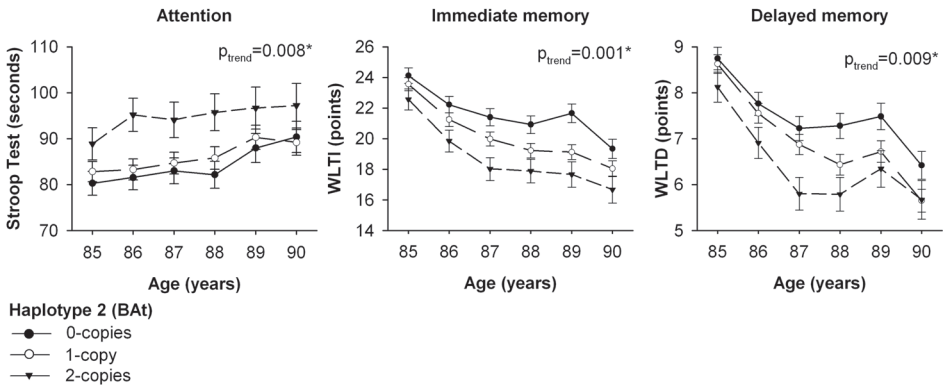

Figure 2. Differences in cognitive functioning between participants carrying 0-, 1- or 2-copies of the haplotype 2 (BAt). The p-value represents the overall mean difference in cognitive functioning during follow-up. * $p < 0.05$

In order to explore whether differences in calcium levels or selective survival have influenced the associations observed with cognitive functioning, we analyzed the relation between these phenotypes and *VDR* gene variants. In cross-sectional analyses at age 85 years, serum calcium levels were not associated with the *VDR* polymorphisms (data not shown), except the *Cdx-2* polymorphism. Homozygous (-0.05 mmol/l, 95 % CI: -0.10 to -0.00, *p=0.032*) but not heterozygous (0.001 mmol/l, 95 % CI: -0.02 to 0.02, *p=0.955*) *Cdx-2* variant allele carriers had lower serum calcium levels compared to non-carriers. No associations between the *VDR* haplotypes and calcium levels were observed. Likewise, the mortality risks did not differ between the different *VDR* polymorphism and haplotype carriers during mean 4.2-year follow-up period (data not shown).

Since in other studies the same polymorphisms and haplotypes as analyzed in this study have been associated with the risk of fractures, we assessed the influence of *VDR* polymorphisms and haplotypes on the incidence of fractures during follow-up. There were no differences in the incidence of fractures between the different polymorphism carriers (data not shown). However, the additional haplotype analyses revealed a trend for increased incidence of fractures for haplotype 1 (baT) carriers (1-copy HR: 1.46, 95 % CI: 0.86 to 2.49; 2-copies HR: 1.52, 95 % CI: 0.81 to 2.83).

Discussion

The main finding of this study is that genetic variance in the *VDR* gene contributes to differences in cognitive functioning and depressive symptoms in old age. An overall better performance on tests measuring attention, processing speed and memory, together with a lower prevalence of depressive symptoms were observed for carriers of *ApaI* variant-allele and of haplotype 1 (baT), which contains the *ApaI* variant-allele. In contrast, carriers of the *BsmI* and *TaqI* polymorphisms had impairments in attention and memory. Similar associations were observed

with haplotype 2 (BAt), which combines the variant alleles of *BsmI* and *TaqI*.

The research on the role of vitamin D in the human brain has so far focused mainly on the influence of vitamin D on mood disorders. Vitamin D deficiency is considered as a possible contributor to seasonal affective disorder (SAD), since SAD has been associated with winter months and sunlight deprivation (Rosenthal et al., 1984; Schlager et al., 1993; Spoont et al., 1991). In addition, there are studies showing associations between low vitamin D levels and mood disorders, accompanied with worse cognitive functioning (Lansdowne and Provost, 1998; Przybelski and Binkley, 2007; Wilkins et al., 2006). In accordance with the latter studies, we observed in this study that genetic variance in the *VDR* gene influences both cognitive functioning and depressive symptoms. From the specific domains of cognitive functioning, attention and memory were affected most. These two cognitive domains are most vulnerable and tend to decline constantly across adult lifespan, in contrast to cognitive abilities such as autobiographical memory and emotional processing, which stay unchanged throughout life (Hedden and Gabrieli, 2004). Our data suggest that carriers of the *BsmI* and *TaqI* polymorphisms are more susceptible for the age-related deterioration of cognitive functioning, whereas the *ApaI* polymorphism contributes to a protective effect.

In the assessment of overall influence of the *VDR* gene polymorphisms on cognitive functioning, we observed differences with composite cognitive score but not with MMSE. The composite cognitive score used in this study was calculated from four individual tests that have been designed to measure changes in specific domains of cognitive functioning. Therefore, the composite cognitive score might be more sensitive in detecting cognitive impairments than MMSE, which has been designed to assess global cognitive functioning, and contains only few items from the specific cognitive tests. It also might be that due to the 'ceiling' effect of the MMSE, mild impairments in cognitive functioning are not detectable (Houx et al., 2002).

There are several mechanisms through which vitamin D can affect mental performance. The downregulation of the expression of L-type voltage-sensitive calcium-channels (L-VSCC) by vitamin D in hippocampal neurons, has been shown to reduce the influx and excitotoxic effects of calcium to neurons (Brewer et al., 2001). The detrimental role of excessive calcium for memory formation and overall cognitive functioning is widely acknowledged (Sattler and Tymianski, 2000; Thibault et al., 2001; Veng et al., 2003). However, the differences in cognitive functioning between the *VDR* polymorphism and haplotype carriers were unlikely caused by increased or decreased calcium levels, since none of these polymorphisms and haplotypes were associated with calcium levels. However, it is unknown how well the peripheral calcium levels reflect those in the brain. The vitamin D endocrine system plays an essential role in overall calcium homeostasis and therefore we expected to see differences also in peripheral calcium levels within the polymorphism and haplotype carriers. Possibly, other brain specific functions of vitamin D are responsible for the observed effects. It has been shown that in the brain, vitamin D increases the production of neurotrophins, which support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses (Naveilhan et al., 1996; Neveu et al., 1994a; Neveu et al., 1994b; Saporito et al., 1994; Wang et al., 2000). These effects provide protection to, and diminish cognitive impairment underlying neurodegenerative disorders.

In previous studies, several polymorphisms and haplotypes in the *VDR* gene have been associated with bone mineral density and risk of fractures (Fang et al., 2005; Uitterlinden et al., 2004b). The risk haplotypes that have been identified include haplotype 1 (baT) and haplotype

2 (BAt), which were reported to contribute to increased and decreased risk of fractures, respectively (Fang et al., 2005; Uitterlinden et al., 2004b). In this study, we analyzed the same risk haplotypes, and observed a trend for increased risk of fractures for haplotype 1 (baT) carriers, which is in accordance with the other studies. However, for the same haplotype carriers we also observed better performance on test measuring cognitive functioning. An explanation for the apparent contradiction could be that people with better cognitive functioning are more active and therefore may face a higher risk for an incident fall and fracture. The lack of associations between the *VDR* polymorphisms and haplotypes and mortality suggest that selective survival has not influenced the associations observed with cognitive functioning. We speculate that the better cognitive functioning in the haplotype 1 (baT) carriers is due to a higher expression of the haplotype, since increased vitamin D levels have previously been associated with better cognitive functioning. The evidence for the functionality of haplotype 1 (baT), however, is contradictory. In one study it was reported that the haplotype 1 (baT) results in lower *VDR* gene expression and increased mRNA decay (Fang et al., 2005), whereas in another study it was shown that the haplotype 1 (baT) is overexpressed in human trabecular bone samples (Grundberg et al., 2007). It might also be that the associations observed in this study are resulted by other polymorphisms that are in LD with those analyzed in this study. Recently, several new polymorphisms in the *VDR* gene have been identified together with a complete description of the LD and haplo-block structure (Fang et al., 2005).

The strengths of the present study include the population-based sample of the oldest old with a high incidence of depression and cognitive decline, and the annual repeated assessment of depressive symptoms and the functioning of various cognitive domains. The limitations of the study include the lack of vitamin D levels, and information on environmental factors, such as (dietary) calcium and vitamin D intake, which could have influenced the associations. Another limitation is the ascertainment of incident fractures through self-reporting by a questionnaire. This might have led to ascertainment errors, but random errors would only underestimate associations. To our knowledge, this is the first and only report of a relationship between genetic variance in the *VDR* gene and cognitive functioning and depressive symptoms, and therefore until further replication, the possibility of a chance finding cannot be excluded.

In conclusion, our results show that genetic variance in the *VDR* gene influences cognitive functioning and the prevalence of depressive symptoms in old age.

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