Title

Vitamin D receptor gene polymorphisms and cognitive decline in Parkinson's disease

Authors

Gatto, Nicole M

Paul, Kimberly C

Sinsheimer, Janet S

et al.

Publication Date

2016-11-01

DOI

10.1016/j.jns.2016.09.013

Peer reviewed

Journal of the Neurological Sciences 370 (2016) 100–106

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Vitamin D receptor gene polymorphisms and cognitive decline in Parkinson's disease

Nicole M. Gatto, MPH, PhD [a,*], Kimberly C. Paul [b], Janet S. Sinsheimer, PhD [e,f,g], Jeff M. Bronstein, MD, PhD [d], Yvette Bordelon, MD, PhD [d], Rebecca Rausch, PhD [d], Beate Ritz, MD, PhD [b,c,d]

[a] School of Community and Global Health, Claremont Graduate University, Claremont, CA 91711, United States
[b] Department of Epidemiology, UCLA, Los Angeles, CA 90095, United States
[c] Department of Environmental Health Sciences, UCLA, Los Angeles, CA 90095, United States
[d] Department of Neurology, UCLA, Los Angeles, CA 90095, United States
[e] Department of Human Genetics, UCLA, Los Angeles, CA 90095, United States
[f] Department of Biomathematics, UCLA, Los Angeles, CA 90095, United States
[g] Department of Biostatistics, UCLA, Los Angeles, CA 90095, United States

ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form 12 August 2016
Accepted 6 September 2016
Available online 11 September 2016

Keywords:
Vitamin D receptor
Parkinson's disease
MMSE
*Fok*I
Cognitive decline

ABSTRACT

We and others have suggested that vitamin D receptor gene (*VDR*) polymorphisms influence susceptibility for Parkinson's disease (PD), Alzheimer's disease (AD), mild cognitive impairment (MCI) or overall cognitive functioning. Here we examine *VDR* polymorphisms and cognitive decline in patients with PD. Non-Hispanic Caucasian PD patients (n = 190) in the Parkinson Environment Gene (PEG) study were successfully genotyped for seven *VDR* polymorphisms. Cognitive function was assessed with the Mini-Mental State Exam (MMSE) at baseline and at a maximum of three follow-up exams. Using repeated-measures regression we assessed associations between *VDR* SNP genotypes and change in MMSE longitudinally. PD cases were on average 67.4 years old at diagnosis and were followed for an average of 7.1 years into disease. Each additional copy of the *Fok*I A allele was associated with a 0.115 decrease in the total MMSE score per year of follow-up ($\beta = -0.115$, SE(β) = 0.05, p = 0.03) after adjusting for age, sex, education and PD duration. The effect on MMSE by the *Fok*I A allele was comparable in absolute magnitude to the effect for disease duration in years prior to first interview ($\beta = -0.129$ per year, SE(β) = 0.08, p = 0.13), and years of education ($\beta = 0.118$ per year, SE(β) = 0.03, $p < 0.001$). When LD/LED use and PD subtype were added to the model, the effect of the *Fok*I A allele on total MMSE score was magnified ($\beta = -0.141$, SE(β) = 0.05, p = 0.005). Results point to *Fok*I, a functional VDR polymorphism, as being associated with cognitive decline in PD. Future studies examining the contributions of the vitamin D metabolic pathway to cognitive dysfunction in PD are needed.

1. Introduction

Cognitive dysfunction is a common, non-motor complication in Parkinson's disease (PD), a debilitating neurodegenerative disorder, and it is characterized by a spectrum of disability from mild cognitive impairment (PD-MCI) to PD dementia (PDD) [1]. A pooled multi-center cohort study estimated that 26% of non-PDD patients have PD-MCI, with lower proportions (19%) among incident, unmedicated patients and higher proportions in patients with advanced PD (39%) [2]. PDD also increases with disease duration, with prevalence estimates ranging from 28% after 5 years of disease to 80% after 20 years [3,4]. PD-MCI is clinically heterogeneous affecting variable domains of cognition [1]. Previously, age, lower education, motor symptom severity and non-tremor dominant phenotype have been consistently associated with PD-MCI [5] but the former three with levodopa dose accounted for at most 35% of variation in cognition in patients with PD [6] leaving much room to identify other risk or protective factors.

Genetic variability may account for some of the differences in susceptibility to cognitive dysfunction and rates of decline in PD. To date, the focus has been on genes associated with PD or Alzheimer's disease (AD) risk including APOE, SNCA, COMT and MAPT, and results are inconsistent [7,8]. Some studies indicated that carriers of the APOE ε4 allele have lower cognitive performance in multiple domains [7], reduced brain activity in the temporo-parietal region of the brain during memory encoding tasks [8] and are more likely to develop PDD [9]. Identifying additional genetic biomarkers would be of value and possibly contribute to a greater understanding of cognitive dysfunction in PD, help predict

* Corresponding author at: School of Community and Global Health, Claremont Graduate University, 675 W Foothill Blvd, Suite 310, Claremont, CA 91711, United States.
E-mail address: nicole.gatto@cgu.edu (N.M. Gatto).

http://dx.doi.org/10.1016/j.jns.2016.09.013
0022-510X/

PD-MCI conversions to PDD, and possibly point out protective measures based on information gained about gene pathways or products.

Normal neuronal function depends on the absorption and activation of vitamin D [10]. Vitamin D controls calcium and phosphorus metabolism, and has potent anti-oxidant activity, reducing lipid peroxidation and increasing enzymes that protect against oxidation [11–13]. Oxidative stress is thought to contribute to the pathogenesis of AD [14] and to play a role in dopaminergic cell death in PD [10,15]. Damage to the brain from oxidative stress could also contribute to cognitive dysfunction in neurodegenerative diseases [16].

A high prevalence of vitamin D deficiency has been reported in patients with PD [17] and PD has been associated with decreased bone mineral density [18], which may be related to deficiencies in vitamin D in patients. Some observational studies report a correlation between serum vitamin D levels and PD disease severity [19–22] and a small randomized trial of vitamin D supplementation in PD was effective in preventing deterioration measured by Hoehn and Yahr staging [23]. In cross-sectional studies, higher vitamin D concentrations were associated with higher scores on cognitive tests of immediate and delayed verbal memory and semantic verbal fluency among non-demented patients with PD [24]. The association between vitamin D and cognitive function and dementia was reviewed in a meta-analysis of 37 studies, which found higher average MMSE scores among participants with higher mean vitamin D serum concentrations (eight studies, 2749 participants) and lower mean vitamin D serum concentrations in patients with AD than in controls (six studies, 888 participants) [25].

The bioactive metabolite and circulating form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), is enzymatically generated through a number of steps known as the vitamin D metabolic pathway. Signaling of bioactive vitamin D occurs through its binding with the vitamin D receptor (VDR), which is widely expressed in the human brain [26]. A number of polymorphisms in the *VDR* gene have been identified [27]. Although findings from epidemiologic studies of PD risk investigating genetic variability in *VDR* have not been consistent [28,29] and have differed across populations studied, we and others have suggested associations between *VDR* polymorphisms including *Bsm*I, *Fok*I, *Apa*I and *Taq*I and PD susceptibility [30–36], and others variants have been suggested to affect PD age of onset [37]. Although some research supports correlations between *VDR* polymorphisms and risk of AD and MCI, or lower cognition function [38–44], to our knowledge, no study has previously examined *VDR* polymorphisms and cognitive decline in Caucasian patients with PD, which is the aim of the current study.

2. Materials and methods

2.1. Study population

The Parkinson Environment Gene (PEG) study is a population-based case-control study of PD in the Central Valley (CV) of California [45], a geographical region with very high ultraviolet radiation (UVR). Briefly, study subjects were recruited between January 2001 and January 2007, resided in Fresno, Tulare, and Kern Counties, and had to have lived in California for at least 5 years prior to diagnosis or interview. Cases were recruited within 3 years of diagnosis and were confirmed as having clinically probable or possible PD by a UCLA movement disorder specialist. Altogether, 28 (90%) of the 31 practicing local neurologists who provided care for PD patients assisted in recruiting cases for this study. We solicited collaboration from Kaiser Permanente, Kern and Visalia Medical Centers and the Veteran's Administration, PD support groups, local newspapers and local radio stations that broadcast public service announcements. The 373 initially confirmed probable or possible PD cases of PEG make up the baseline population for this patient cohort. At first follow-up, 108 patients (29%) could not be reached, 87 of which were Caucasian and thus would have been eligible for this study (52 were deceased, 6 too ill, 18 withdrew, 12 could not be re-contacted). Thus we successfully re-contacted and examined 265 (71%) patients, of whom 13 were re-classified as not having PD and 6 did not complete an interview after examination. Of the 246 patients followed longitudinally, 197 were non-Hispanic Caucasian and eligible for this study; 7 samples failed genotyping, thus 190 participants were included in this analysis. Of these, 46 (26%) completed 2 exams (on average 3.8 years of follow-up) and 144 (71%) completed 3 exams (on average 5.6 years of follow-up).

All subjects provided informed consent; the study was approved by the UCLA Institutional Review Board.

2.2. Selection of SNPs in VDR

Candidate SNPs in *VDR* were selected based on 1) findings from previous studies assessing vitamin D genes in PD [30,31,34,35,37,46–48], 2) previous genetic studies of vitamin D metabolism-related disorders [49,50], 3) potential biological functional relevance of a SNP [51], and/or 4) variants in the coding region of the gene leading to an amino acid change in the protein [52]. We used Build 129 of the NCBI dbSNP, the NIEHS-sponsored GeneSNPs web portal (University of Utah Genome Center, 2007) and the NCBI OMIM database to identify candidate SNPs for genotyping. SNPs were required to have a predicted minor allele frequency >5% in Caucasian populations. Six SNPs were genotyped: rs4334089, rs11568820 (tagging the Cdx2 site), rs1544410 (tagging the *Bsm*I site), rs731236 (tagging the *Taq*I site), rs7975232 (tagging the *Apa*I site) and rs2228570 (tagging the *Fok*I site).

2.3. Biospecimen collection, DNA isolation and genotyping

Biospecimens (either blood or saliva) provided by study participants were used to obtain DNA. For blood samples, a total of 20 ml EDTA preserved blood was collected by venipuncture. Saliva samples were collected using an Oragene kit. DNA extraction from blood or saliva samples was performed by the UCLA Human Genetics Core Facility using Autopure LSTM nucleic acid purification instrument from Gentra Systems (gentra.com) or the best suitable Qiagen kit (i.e. DNeasy). DNA quality, purity, and concentration were measured using a UV spectrophotometer to determine the A260/A280 ratio (range of usually 1.7–2.0). DNA was quantitated using OD 260/280 and diluted for storage to 1:20,100 μl using ddH_2O. Genotyping of SNPs was performed at University of Washington using ABI TaqMan MGB chemistry with an ABI 7900 instrument in 384 well formats. Call rates were >90%.

2.4. Cognitive assessment

UCLA movement disorder specialists (JMB, YB) performed neurological exams at baseline and each follow-up exam, to confirm PD diagnosis and assess disease progression utilizing the Unified Parkinson's Disease Rating Scale Part III (UPDRS-III) (motor examination) [53,54]. Patients were classified by PD subtype [55]. Depressed mood was assessed with the Geriatric Depression Scale (GDS) [56]. Additionally, trained interviewers recorded information on demographic, use and dosage of levodopa (LD) or levodopa equivalents (LED) and other medications, and risk factors throughout follow-up. Screening for global cognitive function was conducted at each exam with the Mini-Mental State Exam (MMSE), a widely used 30-point test assessing cognitive function, including tests of orientation, attention, memory, language, and visual-spatial skills [57]. A 26-point telephone version of the MMSE, validated to estimate the in-person MMSE, was administered for 3 patients at baseline exams and 6 at the first follow-up. For these participants, validated weights were applied to make scores comparable with the 30-point in-person interview [58]. For patients with an MMSE score ≥26, in-depth assessment of cognitive function was performed at the next two follow-up exams with a detailed neuropsychological battery that included tests of global cognition, executive function, language, memory, and visuospatial skills described in greater detail elsewhere [59,60].

Table 1
Characteristics of non-Hispanic Caucasian Parkinson's disease cases by inclusion or exclusion in analyses, PEG Study.

Characteristic: mean ± standard deviation or n(%)	Cases with follow-up MMSE (n = 190)		Cases lost to follow-up (n = 87)	
	Baseline MMSE			
	≥26 (n = 172)	<26 (n = 18)	≥26 (n = 65)	<26 (n = 22)
Age, years				
At diagnosis	67.1 ± 10.3	70.4 ± 8.7	71.3 ± 11.2	76.5 ± 4.4
At baseline interview	68.8 ± 10.1	73.0 ± 8.3	73.3 ± 11.2	78.7 ± 5.1
At final interview	74.3 ± 9.8	77.4 ± 7.4	–	–
Duration of disease, years				
At baseline interview	1.9 ± 1.4	2.3 ± 1.6	2.3 ± 1.5	2.2 ± 1.2
At final interview	7.2 ± 2.8	6.9 ± 2.8	–	–
Sex				
Male	94 (0.55)	12 (0.67)	33 (0.51)	17 (0.77)
Female	78 (0.45)	6 (0.33)	32 (0.49)	5 (0.23)
Education, years				
Some high school or less	13 (0.08)	2 (0.11)	10 (0.15)	5 (0.23)
High school graduate	51 (0.30)	6 (0.33)	18 (0.28)	8 (0.36)
More than high school	108 (0.63)	10 (0.56)	37 (0.57)	9 (0.41)
Smoking status				
Never	97 (0.56)	13 (0.72)	29 (0.45)	14 (0.64)
Ever	75 (0.44)	5 (0.28)	36 (0.55)	8 (0.36)
Family history of PD				
No	144 (0.84)	15 (0.83)	58 (0.89)	20 (0.91)
Yes	28 (0.16)	3 (0.17)	7 (0.11)	2 (0.09)
Baseline MMSE score	28.8 ± 1.1	23.1 ± 2.8	28.3 ± 1.2	22.4 ± 2.3
Number of MMSE assessments, range	2–4	2–4	1	1
Baseline UPDRS[a]	18.6 ± 8.9	22.5 ± 10.7	23.5 ± 10.2	27.8 ± 12.3
Baseline GDS[b]	3.2 ± 3.3	3.4 ± 3.2	3.6 ± 2.6	5.5 ± 3.4
PD subtype				
PIGD[c]	110 (0.64)	14 (0.78)	51 (0.78)	17 (0.77)
Tremor dominant	43 (0.25)	2 (0.11)	5 (0.08)	3 (0.14)
Intermediate	19 (0.11)	2 (0.11)	9 (0.14)	2 (0.09)
Levodopa (LD) or levodopa equivalent (LED) use				
Baseline, yes	114 (0.67)	14 (0.82)	44 (0.69)	17 (0.81)
LD (mg/day)	263.2 ± 243.7	524.0 ± 127.1	265.6 ± 251.2	333.3 ± 238.9
LED (mg/day)	339.7 ± 253.0	568.4 ± 530.8	319.5 ± 274.2	359.6 ± 238.8

[a] Unified Parkinson's Disease Rating Scale.
[b] Geriatric Depression Scale.
[c] Postural instability and gait difficulty.

2.5. Statistical analyses

VDR polymorphisms were assessed for Hardy-Weinberg Equilibrium using a chi-square test in PEG controls [30]. Haplotypes and haplotype frequencies were calculated for the *Fok*I, *Taq*I, *Apa*I and *Bsm*I polymorphisms using PHASE 1.0 software [61]. Analyses of SNPs used additive genetic models, which indicate that the expected outcome changes by β for heterozygotes and 2β for homozygotes of the minor allele compared to homozygotes of the major allele. To assess associations between *VDR* SNP genotypes and haplotypes and progression of cognitive decline (MMSE scores) longitudinally, we used repeated-measures linear regression, which estimates between- and within-subject (time-dependent) associations between genotypes and MMSE scores across follow-up. Within-subject associations assumed a variance components correlation structure for the repeated measurements, which we selected over an unstructured correlation structure based on lower AIC scores. To estimate the effect of *VDR* genotypes and haplotypes on MMSE over time, we included an interaction term between *VDR* and follow-up time, which represents the change in MMSE score per year of follow-up by genotypes or haplotypes. In order to address variable length of follow-up time for subjects, variation in time between exam, and differences in baseline exam scores, we treated both the intercept and regression coefficient for time as random effects. Multivariable models included age at diagnosis (years), sex, education (years) and disease duration prior to first interview (0–3 years). Full models also included LD/LED use (mg/day) and PD subtype [postural instability and gait difficulty (PIGD), tremor dominant, intermediate]. We also used lower AIC scores to determine which genotype or haplotype model was the best fit for our data. For *VDR* SNPs for which the interaction term was statistically significant in the analyses of MMSE scores over follow-up, we used simple univariate analyses to examine cross-sectionally at two follow-up times differences in scores on the individual tests in the neuropsychological battery by SNP genotypes also assuming additive genetic models. A p-value <0.05 was considered statistically significant. All analyses used SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

PD cases included in the analysis were on average 67.1 years at diagnosis, and were followed for an average of 7.2 years after baseline, and participated in 2–4 MMSE assessments during follow-up (Table 1). There were more male than female cases, more never than ever smokers, and more cases with greater than a high school education. The average baseline MMSE score was 28.3, indicating no cognitive impairment. Baseline UPDRS-III was 18.9 demonstrating low overall levels of motor disability in the PD cases shortly after diagnosis (mean 2 years), and baseline GDS scores were in the normal range. Cases who were lost to follow-up were on average 5.2 years older at diagnosis, had slightly longer disease duration prior to baseline, were more likely to have some high school education or less and to have higher UPDRS-III and GDS at baseline. Cases excluded from in-depth cognitive assessment (i.e., with baseline MMSE <26) were older at baseline, more likely to be male, have lower education and higher UPDRS-III, were more likely the PIGD subtype and to have taken levodopa or an equivalent, but did not differ in family history of PD.

Each additional copy of the *Fok*I A allele was associated with a 0.115 decrease in the total MMSE score per year of follow-up (β = −0.115,

Table 2
Associations[a] between VDR SNPs and decreases in total MMSE score in points per year of follow-up.

VDR SNP	Major/minor allele	MAF[b]	VDR SNP			Follow-up time			VDR SNP × time		
			β[a]	SE (β)	p-Value	β[a]	SE (β)	p-Value	β[a]	SE (β)	p-Value
rs11568820 (Cdx2)	C/T	0.19	−0.132	0.24	0.58	−0.203	0.05	<0.0001	0.077	0.07	0.25
rs1544410 (*Bsm*I)	C/T	0.45	0.066	0.21	0.75	−0.174	0.06	0.01	−0.012	0.06	0.83
rs2228570 (*Fok*I)	G/A	0.40	0.317	0.20	0.11	−0.078	0.06	0.17	−0.115	0.05	0.03
rs4334089	G/A	0.24	−0.248	0.23	0.29	−0.226	0.05	<0.0001	0.107	0.06	0.09
rs731236 (*Taq*I)	A/G	0.47	−0.023	0.20	0.91	−0.180	0.06	0.005	0.009	0.06	0.88
rs7975232 (*Apa*I)	A/C	0.42	0.169	0.21	0.41	−0.099	0.06	0.10	−0.086	0.05	0.11

[a] From repeated measures linear regression assuming additive genetic models; adjusted for age at diagnosis (years), sex, education (years), PD duration prior to first interview (0–3 years); β indicates the effect per copy of the minor allele compared to homozygotes of the major allele.
[b] Minor allele frequency.

SE(β) = 0.05, p = 0.03) after adjusting for age, sex, education and PD duration prior to baseline (Table 2). The effect on cognitive decline associated with the *Fok*I A allele was greater in absolute magnitude than the effect of aging on MMSE scores per year over follow-up time (β = −0.078, SE(β) = 0.06, p = 0.17), and comparable in absolute magnitude to the effect of increasing years of disease duration prior to baseline (β = −0.129, SE(β) = 0.085, p = 0.13), and the association with number of years of education (β = 0.118 per year, SE(β) = 0.034, p < 0.001) (Table 3). These results for genotypes would be equivalent to a 0.975 decrease in MMSE score over 5 years for the AG genotype and a decrease of 1.55 points for the AA genotype (Fig. 1). In contrast, the mean 5-year decrease in MMSE score for individuals with the GG genotype would be 0.39 points. When we added LD/LED use and PD subtype to our model, the effect of the *Fok*I A allele became stronger (β = −0.141, SE(β) = 0.05, p = 0.005), equivalent to a 0.141 decrease in the total MMSE score per year of follow-up.

The A allele of rs4334089 showed a tendency towards higher MMSE, but this association was not statistically significant at a cut off of p = 0.05 (β = 0.107, SE(β) = 0.06, p = 0.09). The SNPs Cdx2, *Bsm*I, *Taq*I and *Apa*I were not associated with declines in MMSE scores over follow-up at a significance cut off of 0.05. Excluding four patients who reported use of anticholinergic medications and three who reported taking benzodiazepines did not change the results of analyses.

Data from in-depth neuropsychological testing were available for 122 participants with an MMSE ≥26 and genotyping data. Generally, compared to the GG genotype, performance on tests was lower for patients with the GA and lowest with the AA *Fok*I genotype at both follow-up exams (Supplemental Table 1). Of the 38 total sub-scales comprising the individual tests, patients with the *Fok*I AA genotype had lower performance on 34 (90%) at the first follow-up and on 30 (79%) at the second follow-up. Performance significantly decreased on the Similarities WAIS-III sub-scale and Boston Naming Test at the second follow-up among patients as the number of *Fok*I A alleles increased, indicating faster decline (Supplemental Table 1).

Three-SNP (containing *Taq*I, *Apa*I and *Bsm*I polymorphisms) and 4-SNP (containing *Fok*I, *Taq*I, *Apa*I and *Bsm*I polymorphisms) haplotypes were defined; those with frequencies >5% were included in analyses of change in total MMSE score (Table 4). Although results suggested effects of a combination of alleles from these SNPs, model fit statistics indicated that the model containing *Fok*I alone (AIC = 4405.3) best fit the data compared to the 3 SNP (AIC = 4409.1) or 4 SNP (AIC = 4412) haplotype models.

Table 3
Association[a] between *Fok*I and change in total MMSE score in points per year of follow-up.

Effect	β[a]	SE (β)	p-Value
*Fok*I	0.317	0.195	0.106
Follow-up time, years	−0.078	0.056	0.166
Interaction term (*Fok*I × follow-up time)	−0.115	0.052	0.027
Age at diagnosis, years	−0.039	0.012	0.002
Sex, male	−0.538	0.246	0.030
Female	*ref*	–	–
Education, years	0.118	0.034	0.001
Disease duration at baseline, years	−0.129	0.085	0.130

Model fit statistics: −2 log likelihood = 4383.3; AIC = 4405.3.
[a] From repeated-measures regression assuming additive genetic models; β indicates the effect per copy of the minor allele compared to homozygotes of the major allele.

4. Discussion

This is the first study to suggest that *VDR* polymorphisms influence cognitive decline in individuals affected with PD; specifically, we found that the *Fok*I polymorphism was associated with decreases in total MMSE score over follow-up time after accounting for age, sex, education, and PD duration at baseline. The decrease in MMSE associated with each additional copy of the *Fok*I A allele had an effect size that is comparable in magnitude to the positive effect of education and the negative effect of disease duration prior to baseline. The effect of the *Fok*I A allele on MMSE score was magnified when LD/LED use and PD subtype were taken into consideration. Our results indicate that, on average, individuals with the AA *Fok*I genotype who begin follow-up with otherwise normal cognitive function (MMSE ≥26) had faster declines in cognition. This implies that carriers of this genotype may become cognitively impaired, i.e., reach MMSE ≤23 sooner than other *Fok*I genotypes. *Fok*I genotypes with the A allele also generally performed lower on individual tests in the neuropsychological battery compared to the GG genotype. Model fit analyses indicated that genotyping the *Fok*I SNP was sufficient to capture the effects on cognition associated with this region of the VDR.

The VDR is a member of the steroid/thyroid hormone super family of transcription regulation factors [27]. Actions of the bioactive metabolite and circulating form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), are initiated when it binds to the VDR [62]. The *VDR* SNP rs2228570 tags *Fok*I, a restriction fragment length polymorphism

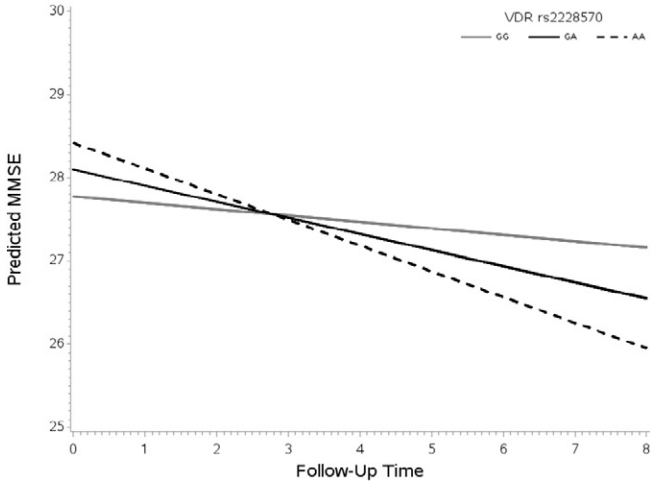

Fig. 1. Predicted decline in total MMSE score over follow-up time in years by *Fok*I genotype.

Table 4
Associations[a] between VDR haplotypes and changes in total MMSE score in points per year of follow-up.

Haplotype number	Haplotype frequency (%)	β[a]	SE (β)	p-Value
3 SNP haplotype[b] (SNP order: *Taq*I, *Apa*I, *Bsm*I)				
3:AAC	11.39	0.206	0.09	0.02
2:GAT	42.88	0.054	0.06	0.33
1:ACC	44.13	*ref*	–	–
4 SNP haplotype[c] (SNP order: *Taq*I, *Apa*I, *Bsm*I, *Fok*I)				
6:AACA	4.27	0.054	0.13	0.68
5:AACG	7.12	0.280	0.11	0.01
4:ACCA	10.85	-0.044	0.09	0.62
3:GATG	19.57	0.149	0.07	0.05
2:GATA	23.31	-0.046	0.07	0.51
1:ACCG	33.27	*ref*	–	–

[a] For VDR haplotype × follow-up time, adjusted for age at diagnosis (years), sex, education (years), PD duration prior to first interview (0–3 years); β indicates the effect per copy of the minor allele compared to homozygotes of the major allele.
[b] Model fit statistics: −2 log likelihood = 4383.1; AIC = 4409.1.
[c] Model fit statistics: −2 log likelihood = 4374.0; AIC = 4412.0.

(RFLP) of a T to C variation at translation initiation codon (AGT) in exon 2 [63]. To date, the *Fok*I polymorphism is the only known functional polymorphism in the *VDR* [27,49,64]. It results in an altered ACG codon upstream from the translation initiation codon and the generation of an additional start codon [65]. The A variant results in a longer VDR protein [27,66] with less transcriptional activity [67], while the G variant allele is 1.7 times more active [68–70]. Carriers of the GG variant are thus expected to have more VDR activity than carriers of the GA or AA variant. We found the *Fok*I A genotypes with presumably less VDR activity to be associated with greater decline in MMSE scores over follow-up time compared with the GG genotype.

In two studies of Eastern and Western European populations, SNPs tagging the *Taq*I [43] and *Apa*I loci [39,42,43], but not those tagging the *Fok*I locus [39,44] were associated with risk of AD. *Apa*I and *Bsm*I polymorphisms were associated with an increased risk of MCI in elderly Uygur adults [40]. Carriers of variant *Bsm*I and *Taq*I genotypes had worse overall cognitive performance in the Leiden-85 cohort [41], and a greater decline in cognitive function was associated with *Apa*I, *Bsm*I and *Taq*I polymorphisms among female non-Hispanic white participants in the Baltimore Longitudinal Study of Aging [38]. Taken together, results from these studies suggest the *VDR* contributes to differences in cognitive function in older adults. In contrast to AD, in which deficits in memory predominate, deficits in attention, construction and praxis, and visuospatial functions are more severe in PDD. In addition, executive dysfunction is more pronounced in PDD particularly compared with early and moderate stages of AD [71]. The clinical profile of PD-MCI may be heterogeneous; most studies report that memory and attention/executive impairment are common and others find that executive dysfunction is more common than amnestic deficits [71]. Impaired nigrostriatal dopaminergic function is associated with cognitive deficits in early PD. In addition to the presence of diffuse amyloid-beta plaques (which are a hallmark of AD), the neuropathology of PDD is characterized by inclusions of alpha-synuclein containing Lewy bodies [71]. Thus, there may be some overlap and interrelationship in mechanisms underlying cognitive decline in PD and AD apart from disease-specific pathogenic processes [72,73].

The *VDR* is widely expressed in neurons and glial cells of adult human brains in the cortex, thalamus, amygdala, throughout the olfactory system, in hippocampal regions, and most prominently in the substantia nigra and hypothalamus [26]. *VDR*-mediated transcription can be induced by dopamine, which might imply that gene expression normally regulated by *VDR* could be influenced by dopamine levels [74], suggesting a possible interrelationship between vitamin D and PD. In addition, several mechanisms by which vitamin D may impact brain function have been proposed. Vitamin D regulates brain neurotrophic factors such as nerve growth factor (NGF) [75], neurotrophin 3 (NT3) and glial cell line-derived neurotrophic factor (GDNF), and increases neurite outgrowth in cultured embryonic hippocampal cells of rats [76]. In rat cortical neurons, vitamin D has been shown to upregulate the expression of proteins such as microtubule-associated protein-2, growth-associated protein-43 and synapsin-1 which may be important for neuronal plasticity [77]. Vitamin D may also act as a neuroprotective agent, as it can also induce the synthesis of Ca^{2+}-binding proteins in the cortex and hippocampus of rats [78]. It is involved in the regulation of a key enzyme involved in glutathione metabolism important for detoxification pathways in the brain, in which reactive oxidant species are eliminated [11], and can directly inhibit the synthesis of nitric oxide synthase [79,80].

We did not have current measurements of serum vitamin D nor levels prior to diagnosis to examine cognitive decline and the relationship between possibly more and less sensitive VDRs under different physiologic vitamin D levels. Serum vitamin D has been shown to be correlated with multiple measures of cognitive function, not including the MMSE, in non-demented patients with PD [24]. Our population was highly exposed to UVR, which stimulates the physiological production of bioactive vitamin D in the body [81]. The MMSE is a widely used, standardized method for assessing global mental status [57]. When administering the instrument to cognitively "normal" individuals, there is an expected "ceiling effect", limiting the MMSE in its ability to discriminate between cognitive performance in the upper range of functioning. Cognitive decline in PD may not be linear [2], yet we used linear models to estimate gene effects, and extrapolated within the range of our patient's MMSE scores. We thus could not estimate the effect of VDR polymorphisms in more severely impaired patients, since few declined to these levels while under observation and we excluded those with low MMSE scores at the beginning of follow-up. We were able to examine cognitive domain-specific associations with the *VDR Fok*I polymorphism. However, these analyses were limited by our small sample size and multiple testing, the former of which also limited our ability to detect small effects of *VDR* polymorphisms on cognition. We did not include a variable for co-morbid disease in regression models, so effect estimates did not take into account a potential contribution of this factor. The study also lacked a comparable unaffected control group, so it is not possible to conclude that the decline in MMSE associated with *Fok*I is specific to individuals with PD. However, among elderly adults without severe cognitive impairment (MMSE ≤ 18 points) in the Leiden-85 study, *Fok*I was not associated with worse overall cognitive performance [41]; additional studies should examine the association between *Fok*I and cognitive decline in otherwise healthy adults. All analyses were restricted to non-Hispanic Caucasian participants in order to avoid population stratification bias.

In conclusion, our study suggests that *Fok*I, a functional *VDR* polymorphism, may be associated with cognitive decline in PD. Additional investigations should examine the vitamin D metabolic pathway in cognitive dysfunction in PD; especially interesting might be studies of vitamin D levels in susceptible carriers of this functional receptor variant.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jns.2016.09.013.

Authors' roles

NMG, KCP, JSS, JMB, YB, RR and BR were involved in the conception, organization, and execution of the research project. NMG, KCP, JSS and BR were involved in the design and execution of the statistical analysis; NMG, KCP, JSS, RR and BR reviewed and critiqued the statistical analysis. NMG, KCP, JSS and BR were involved in writing of the first draft of the manuscript; NMG, KCP, JSS, JMB, YB, RR and BR were involved in the review and critique.

Financial disclosures of all authors

None.

Funding sources for study

This work was supported by the NIH (grant numbers 1R03ES017139 and GM053275 (JSS)) and by a Burroughs Wellcome Fund Population and Laboratory Based Sciences Fellowship Award. The sponsor had no role in the study design; collection, analysis and interpretation of data; writing of the report; or decision to submit the article for publication.

References

[1] I. Litvan, et al., Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines, Mov. Disord. 27 (3) (2012) 349–356.
[2] D. Aarsland, et al., Mild cognitive impairment in Parkinson disease: a multicenter pooled analysis, Neurology 75 (12) (2010) 1062–1069.
[3] M.A. Hely, et al., The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years, Mov. Disord. 23 (6) (2008) 837–844.
[4] D. Aarsland, et al., Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study, Arch. Neurol. 60 (3) (2003) 387–392.
[5] A.J. Yarnall, L. Rochester, D.J. Burn, Mild cognitive impairment in Parkinson's disease, Age Ageing 42 (5) (2013) 567–576.
[6] D. Verbaan, et al., Cognitive impairment in Parkinson's disease, J. Neurol. Neurosurg. Psychiatry 78 (11) (2007) 1182–1187.
[7] I.F. Mata, et al., APOE, MAPT, and SNCA genes and cognitive performance in Parkinson disease, JAMA Neurol. 71 (11) (2014) 1405–1412.
[8] C. Nombela, et al., Genetic impact on cognition and brain function in newly diagnosed Parkinson's disease: ICICLE-PD study, Brain 137 (Pt 10) (2014) 2743–2758.
[9] X. Huang, et al., Apolipoprotein E and dementia in Parkinson disease: a meta-analysis, Arch. Neurol. 63 (2) (2006) 189–193.
[10] H.L. Newmark, J. Newmark, Vitamin D and Parkinson's disease–a hypothesis, Mov. Disord. 22 (4) (2007) 461–468.
[11] S.J. Kiraly, et al., Vitamin D as a neuroactive substance: review, Sci. World J. 6 (2006) 125–139.
[12] S. Sardar, A. Chakraborty, M. Chatterjee, Comparative effectiveness of vitamin D3 and dietary vitamin E on peroxidation of lipids and enzymes of the hepatic antioxidant system in Sprague–Dawley rats, Int. J. Vitam. Nutr. Res. 66 (1) (1996) 39–45.
[13] H. Wiseman, Vitamin D is a membrane antioxidant. Ability to inhibit iron-dependent lipid peroxidation in liposomes compared to cholesterol, ergosterol and tamoxifen and relevance to anticancer action, FEBS Lett. 326 (1–3) (1993) 285–288.
[14] G. Perry, A.D. Cash, M.A. Smith, Alzheimer disease and oxidative stress, Am. J. Clin. Nutr. 2 (3) (2002) 120–123.
[15] P. Meredith, T. Sarna, The physical and chemical properties of eumelanin, Pigment Cell Res. 19 (6) (2006) 572–594.
[16] E. Mariani, et al., Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 827 (1) (2005) 65–75.
[17] Y. Sato, M. Kikuyama, K. Oizumi, High prevalence of vitamin D deficiency and reduced bone mass in Parkinson's disease, Neurology 49 (5) (1997) 1273–1278.
[18] F. van den Bos, et al., Bone mineral density and vitamin D status in Parkinson's disease patients, J. Neurol. 260 (3) (2013) 754–760.
[19] M. Suzuki, et al., 25-hydroxyvitamin D, vitamin D receptor gene polymorphisms, and severity of Parkinson's disease, Mov. Disord. (2011).
[20] A.L. Peterson, M. Mancini, F.B. Horak, The relationship between balance control and vitamin D in Parkinson's disease-a pilot study, Mov. Disord. 28 (8) (2013) 1133–1137.
[21] Y. Sato, Y. Honda, J. Iwamoto, Risedronate and ergocalciferol prevent hip fracture in elderly men with Parkinson disease, Neurology 68 (12) (2007) 911–915.
[22] Y. Sato, et al., Abnormal bone and calcium metabolism in immobilized Parkinson's disease patients, Mov. Disord. 20 (12) (2005) 1598–1603.
[23] M. Suzuki, et al., Randomized, double-blind, placebo-controlled trial of vitamin D supplementation in Parkinson disease, Am. J. Clin. Nutr. 97 (2013) 1004–1013.
[24] A.L. Peterson, et al., Memory, mood, and vitamin D in persons with Parkinson's disease, J. Parkinsons Dis. 3 (4) (2013) 547–555.
[25] C. Balion, et al., Vitamin D, cognition, and dementia: a systematic review and meta-analysis, Neurology 79 (13) (2012) 1397–1405.
[26] D.W. Eyles, et al., Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain, J. Chem. Neuroanat. 29 (1) (2005) 21–30.
[27] A.G. Uitterlinden, et al., Genetics and biology of vitamin D receptor polymorphisms, Gene 338 (2) (2004) 143–156.
[28] S.Y. Kang, et al., Vitamin D receptor polymorphisms and Parkinson's disease in a Korean population: revisited, Neurosci. Lett. 628 (2016) 230–235.
[29] M.Y. Niu, L. Wang, A.M. Xie, *Apa*I, *Bsm*I, *Fok*I, and *Taq*I polymorphisms in the vitamin D receptor gene and Parkinson's disease, Chin. Med. J. 128 (13) (2015) 1809–1814.
[30] N.M. Gatto, et al., Vitamin D receptor gene polymorphisms and Parkinson's disease in a population with high ultraviolet radiation exposure, J. Neurol. Sci. 352 (1–2) (2015) 88–93.
[31] X. Han, et al., Vitamin D receptor gene polymorphism and its association with Parkinson's disease in Chinese Han population, Neurosci. Lett. 525 (1) (2012) 29–33.
[32] Y.H. Lee, J.H. Kim, G.G. Song, Vitamin D receptor polymorphisms and susceptibility to Parkinson's disease and Alzheimer's disease: a meta-analysis, Neurol. Sci. 35 (12) (2014) 1947–1953.
[33] C. Li, et al., Vitamin D receptor gene polymorphisms and the risk of Parkinson's disease, Neurol. Sci. 36 (2) (2015) 247–255.
[34] M.S. Petersen, et al., The role of vitamin D levels and vitamin D receptor polymorphism on Parkinson's disease in the Faroe Islands, Neurosci. Lett. 561 (2014) 74–79.
[35] R. Torok, et al., Association of vitamin D receptor gene polymorphisms and Parkinson's disease in Hungarians, Neurosci. Lett. 551 (2013) 70–74.
[36] Z.T. Zhang, et al., Association between vitamin D receptor gene polymorphisms and susceptibility to Parkinson's disease: a meta-analysis, Neurosci. Lett. 578 (2014) 122–127.
[37] M.W. Butler, et al., Vitamin D receptor gene as a candidate gene for Parkinson disease, Ann. Hum. Genet. 75 (2) (2011) 201–210.
[38] M.A. Beydoun, et al., Vitamin D receptor and megalin gene polymorphisms and their associations with longitudinal cognitive change in US adults, Am. J. Clin. Nutr. 95 (1) (2012) 163–178.
[39] D. Gezen-Ak, et al., Association between vitamin D receptor gene polymorphism and Alzheimer's disease, Tohoku J. Exp. Med. 212 (3) (2007) 275–282.
[40] K. Keyimu, et al., Relationship between vitamin D receptor gene polymorphism and mild cognitive impairment in elderly Uygur people, Int. J. Clin. Exp. Med. 7 (12) (2014) 5282–5288.
[41] M. Kuningas, et al., VDR gene variants associate with cognitive function and depressive symptoms in old age, Neurobiol. Aging 30 (3) (2009) 466–473.
[42] L. Laczmanski, et al., Vitamin D receptor gene polymorphisms in Alzheimer's disease patients, Exp. Gerontol. 69 (2015) 142–147.
[43] D.J. Lehmann, et al., The vitamin D receptor gene is associated with Alzheimer's disease, Neurosci. Lett. 504 (2) (2011) 79–82.
[44] E. Luedecking-Zimmer, et al., Association of the 3′ UTR transcription factor LBP-1c/CP2/LSF polymorphism with late-onset Alzheimer's disease, Am. J. Med. Genet. B Neuropsychiatr. Genet. 117b (1) (2003) 114–117.
[45] G.A. Kang, et al., Clinical characteristics in early Parkinson's disease in a central California population-based study, Mov. Disord. 20 (9) (2005) 1133–1142.
[46] J.S. Kim, et al., Association of vitamin D receptor gene polymorphism and Parkinson's disease in Koreans, J. Korean Med. Sci. 20 (3) (2005) 495–498.
[47] C.H. Lin, et al., Vitamin D receptor genetic variants and Parkinson's disease in a Taiwanese population, Neurobiol. Aging 35 (5) (2014) 1212.e11–1212.e13.
[48] Z. Lv, et al., Association study between vitamin d receptor gene polymorphisms and patients with Parkinson disease in Chinese Han population, Int. J. Neurosci. 123 (1) (2013) 60–64.
[49] A.R. Baker, et al., Cloning and expression of full-length cDNA encoding human vitamin D receptor, Proc. Natl. Acad. Sci. U. S. A. 85 (10) (1988) 3294–3298.
[50] N.A. Morrison, et al., Prediction of bone density from vitamin D receptor alleles, Nature 367 (6460) (1994) 284–287.
[51] J.J. McGrath, et al., A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations, J. Steroid Biochem. Mol. Biol. 121 (1–2) (2010) 471–477.
[52] S.A. Abrams, et al., Vitamin D receptor *Fok*1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty, J. Bone Miner. Res. 20 (6) (2005) 945–953.
[53] S. Fahn, R. Elton, Members of the UPDRS Development Committee, in: C.D. Marsden, S. Fahn, D.B. Calne, M. Goldstein (Eds.), Recent Developments in Parkinson's Disease, Macmillan Health Care Information, Florham Park, NJ 1987, pp. 153–163 (293–304).
[54] Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, The Unified Parkinson's Disease Rating Scale (UPDRS): status and recommendations, Mov. Disord. 18 (7) (2003) 738–750.
[55] J. Jankovic, et al., Variable expression of Parkinson's disease: a base-line analysis of the DATATOP cohort. The Parkinson Study Group, Neurology 40 (10) (1990) 1529–1534.
[56] J.A. Yesavage, et al., Development and validation of a geriatric depression screening scale: a preliminary report, J. Psychiatr. Res. 17 (1) (1982) 37–49.
[57] M.F. Folstein, S.E. Folstein, P.R. McHugh, "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician, J. Psychiatr. Res. 12 (3) (1975) 189–198.
[58] L.A. Newkirk, et al., Validation of a 26-point telephone version of the Mini-Mental State Examination, J. Geriatr. Psychiatry Neurol. 17 (2) (2004) 81–87.
[59] D.J. Burdick, et al., People with Parkinson's disease and normal MMSE score have a broad range of cognitive performance, Mov. Disord. 29 (10) (2014) 1258–1264.
[60] K. Paul, et al., APOE, MAPT, and COMT and Parkinson's disease susceptibility and cognitive symptom progression, J. Parkinson's Dis. (2016).
[61] M. Stephens, P.P. Donnelly, A comparison of bayesian methods for haplotype reconstruction from population genotype data, Am. J. Hum. Genet. 73 (5) (2003) 1162–1169.
[62] H.M. Macdonald, Contributions of sunlight and diet to vitamin D status, Calcif. Tissue Int. 92 (2) (2013) 163–176.
[63] T. Saijo, et al., A unique mutation in the vitamin D receptor gene in three Japanese patients with vitamin D-dependent rickets type II: utility of single-strand conformation polymorphism analysis for heterozygous carrier detection, Am. J. Hum. Genet. 49 (3) (1991) 668–673.
[64] D. Bodiwala, et al., Polymorphisms in the vitamin D receptor gene, ultraviolet radiation, and susceptibility to prostate cancer, Environ. Mol. Mutagen. 43 (2) (2004) 121–127.

[65] K. Kostner, et al., The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature, Anticancer Res. 29 (9) (2009) 3511–3536.
[66] E. van Etten, et al., The vitamin D receptor gene *FokI* polymorphism: functional impact on the immune system, Eur. J. Immunol. 37 (2) (2007) 395–405.
[67] E.M. John, et al., Sun exposure, vitamin D receptor gene polymorphisms, and risk of advanced prostate cancer, Cancer Res. 65 (12) (2005) 5470–5479.
[68] S.Z. Abdel-Rahman, et al., A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies, Cancer Lett. 107 (2) (1996) 229–233.
[69] H. Arai, et al., A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women, J. Bone Miner. Res. 12 (6) (1997) 915–921.
[70] M.L. McCullough, et al., Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: a nested case-control study, Breast Cancer Res. 9 (1) (2007) R9.
[71] K.A. Jellinger, Neurobiology of cognitive impairment in Parkinson's disease, Expert. Rev. Neurother. 12 (12) (2012) 1451–1466.
[72] D. Aarsland, Cognitive impairment in Parkinson's disease and dementia with Lewy bodies, Parkinsonism Relat. Disord. (2015).
[73] H. Hildebrandt, et al., Cognitive profiles of patients with mild cognitive impairment or dementia in Alzheimer's or Parkinson's disease, Dement. Geriatr. Cogn. Dis. Extra 3 (1) (2013) 102–112.
[74] T. Matkovits, S. Christakos, Ligand occupancy is not required for vitamin D receptor and retinoid receptor-mediated transcriptional activation, Mol. Endocrinol. 9 (2) (1995) 232–242.
[75] D. Wion, et al., 1,25-Dihydroxyvitamin D3 is a potent inducer of nerve growth factor synthesis, J. Neurosci. Res. 28 (1) (1991) 110–114.
[76] D.A. Fernandes de Abreu, D. Eyles, F. Feron, Vitamin D, a neuro-immunomodulator: implications for neurodegenerative and autoimmune diseases, Psychoneuroendocrinology 34 (Suppl. 1) (2009) S265–S277.
[77] H. Taniura, et al., Chronic vitamin D3 treatment protects against neurotoxicity by glutamate in association with upregulation of vitamin D receptor mRNA expression in cultured rat cortical neurons, J. Neurosci. Res. 83 (7) (2006) 1179–1189.
[78] P.A. de Viragh, K.G. Haglid, M.R. Celio, Parvalbumin increases in the caudate putamen of rats with vitamin D hypervitaminosis, Proc. Natl. Acad. Sci. U. S. A. 86 (10) (1989) 3887–3890.
[79] E. Garcion, et al., 1,25-Dihydroxyvitamin D3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis, Brain Res. Mol. Brain Res. 45 (2) (1997) 255–267.
[80] E. Garcion, et al., Expression of inducible nitric oxide synthase during rat brain inflammation: regulation by 1,25-dihydroxyvitamin D3, Glia 22 (3) (1998) 282–294.
[81] D.E. Lawson, et al., Relative contributions of diet and sunlight to vitamin D state in the elderly, Br. Med. J. 2 (6185) (1979) 303–305.