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Randomized, double-blind, placebo-controlled trial of vitamin D supplementation in Parkinson disease[1–4]

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ABSTRACT

Background: In our previous study, higher serum 25-hydroxyvitamin D [25(OH)D] concentrations and the vitamin D receptor (*VDR*) *Fok*I CC genotype were associated with milder Parkinson disease (PD).

Objective: We evaluated whether vitamin D_3 supplementation inhibits the progression of PD on the basis of patient *VDR* subgroups.

Design: Patients with PD (n = 114) were randomly assigned to receive vitamin D_3 supplements (n = 56; 1200 IU/d) or a placebo (n = 58) for 12 mo in a double-blind setting. Outcomes were clinical changes from baseline and the percentage of patients who showed no worsening of the modified Hoehn and Yahr (HY) stage and Unified Parkinson's Disease Rating Scale (UPDRS).

Results: Compared with the placebo, vitamin D_3 significantly prevented the deterioration of the HY stage in patients [difference between groups: P = 0.005; mean ± SD change within vitamin D_3 group: +0.02 ± 0.62 (P = 0.79); change within placebo group: +0.33 ± 0.70 (P = 0.0006)]. Interaction analyses showed that *VDR* *Fok*I genotypes modified the effect of vitamin D_3 on changes in the HY stage (P-interaction = 0.045), UPDRS total (P-interaction = 0.039), and UPDRS part II (P-interaction = 0.021). Compared with the placebo, vitamin D_3 significantly prevented deterioration of the HY stage in patients with *Fok*I TT [difference between groups: P = 0.009; change within vitamin D_3 group: −0.38 ± 0.48 (P = 0.91); change within placebo group, +0.63 ± 0.77 (P = 0.009)] and *Fok*I CT [difference between groups: P = 0.020; change within vitamin D_3 group: ±0.00 ± 0.60 (P = 0.78); change within placebo group: +0.37 ± 0.74 (P = 0.014)] but not *Fok*I CC. Similar trends were observed in UPDRS total and part II.

Conclusion: Vitamin D_3 supplementation may stabilize PD for a short period in patients with *Fok*I TT or CT genotypes without triggering hypercalcemia, although this effect may be nonspecific for PD. This trial was registered at UMIN Clinical Trials Registry as UMIN000001841. *Am J Clin Nutr* 2013;97:1004–13.

INTRODUCTION

Parkinson disease (PD)[5] is a debilitating movement disorder. Serum concentrations of 25-hydroxyvitamin D [25(OH)D], which is the primary circulating form of vitamin D and is mostly obtained from sun exposure, are lower in PD patients than in age-matched healthy controls (1, 2). Vitamin D insufficiency is a well-established risk factor for osteoporosis and bone fractures (3). Moreover, in a meta-analysis of randomized controlled trials, supplemental vitamin D reduced risk of falling in older individuals (4), which suggested that vitamin D insufficiency may also increase risk of falls. Indeed, PD patients have a lower bone-mineral density than do age-matched controls (5) and higher risk of falls (6) and hip fractures (7), although these risks may be ascribable in part to factors unrelated to PD, such as older age, a low BMI, and limited exposure to sunlight (8). In a long-term cohort study, PD incidence was 3 times higher in persons with the lowest quartile of serum 25(OH)D concentrations than in those with the highest quartile (9). Moreover, a higher prevalence of low serum 25(OH)D concentrations has been reported in patients with more severe PD compared with in those with milder PD (10, 11). In the United States, mortality rates of idiopathic PD show a north-south gradient that may parallel high-low latitudes and low-high sun exposure (12) and may be consistent with the hypothesis that 25(OH)D insufficiency because of reduced sun exposure may lead to the deterioration of PD. Vitamin D receptor and 1α-hydroxylase, which is the enzyme that converts 25(OH)D to 1,25-dihydroxyvitamin D [1,25(OH)D], the active form of vitamin D, are expressed in large neurons of the substantia nigra where pigmented dopaminergic neurons are selectively lost in PD (13). Furthermore, *VDR* knockout mice show muscular and motor impairment (14). Genetic studies have shown genotypes in the *VDR* gene that are associated with risk of PD (15). Our recent study also showed that the *Fok*I C allele of *VDR*, which is

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[5] Abbreviations used: EQ-5D, EuroQol 5 Dimension; HY, Hoehn and Yahr; LED, levodopa equivalency dose; MMSE, Mini-Mental State Examination; PD, Parkinson disease; PDQ39, Parkinson's Disease Questionnaire-39; UPDRS, Unified Parkinson's Disease Rating Scale; VDR, vitamin D receptor; 1,25(OH)D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

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considered to transduce the signal more efficiently than the *Fok*I T allele, is associated with milder forms of PD (11). These observations suggest that vitamin D and *VDR* genotypes may be associated with the incidence of PD and progression in patients with PD. Therefore, a randomized, double-blind, placebo-controlled trial with the use of vitamin D_3 supplements was conducted to elucidate whether a 12-mo intake of vitamin D supplements can inhibit the progression of PD in a cohort in which *VDR* genotypes were analyzed (11).

PATIENTS AND METHODS

Study design

A randomized, double-blind, placebo-controlled, parallel-group trial was conducted at the Department of Neurology in Katsushika Medical Center, Tokyo, Japan. Both vitamin D_3 and a placebo were purchased from Zenyaku Co Ltd. The study protocol was reviewed and approved by the ethics committee of Jikei University School of Medicine and the clinical study committee of the Katsushika Medical Center. The trial (UMIN000001841) was registered with the UMIN Clinical Trials Registry on 9 March 2009.

Study population, eligibility, and consent

Consecutive patients with PD, in accordance with clinical diagnostic criteria of the UK Parkinson's Disease Society Brain Bank (16), who showed the pattern of PD in 3-dimensional stereotactic surface projection analysis with the use of single-photon emission computed tomography were included (17). Inclusion criteria were patients who *1*) were diagnosed with PD by ≥2 neurologists, *2*) were aged 45–85 y, and *3*) did not have first- or second-degree relatives with PD. Exclusion criteria were patients who *1*) had a history of stones in the urinary tract, *2*) were already taking vitamin D_3 or activated vitamin D, *3*) were diagnosed with osteoporosis or bone fractures, *4*) had severe dementia or depression, *5*) had severe psychosis and hallucinations, or *6*) were considered incapable of taking part in the study by the neurologists in charge. When patients satisfied these criteria, they and their families were asked to provide written, informed consent after the neurologists explained the study to them at the Center.

Randomization, blinding, and intervention

A central computerized procedure was used to randomly assign patients in permutated blocks of 4 to receive either vitamin D_3 or the placebo. In adults, an intake ≥1000 IU vitamin D_3/d is needed to bring vitamin D concentrations in ≥50% of the population to 30 ng/mL, which is considered the threshold to reduce risk of falls, fractures, and colorectal cancer (19). Thus, a dose of 1200 IU/d was selected. Active and placebo tablets were identical in appearance. Blinding of the study to patients, their families, and doctors who evaluated the severity of the disease was performed by MU, who does not see patients with PD.

Clinical evaluation at entry

To assess primary outcomes, the modified Hoehn and Yahr (HY) stage (19), Unified Parkinson's Disease Rating Stage (UPDRS) total score as well as 4 subscores (part I: nonmotor experiences of daily living; part II: motor experiences of daily living; part III: motor examination; and part IV: motor complications) (20), and the Japanese version of the Mini-Mental State Examination (MMSE) validated by Ideno et al (21) were scored by the neurologists in charge. All scores were assessed on therapy. To assess secondary outcomes, the Japanese version of the Parkinson's Disease Questionnaire-39 (PDQ39; total and 8 dimensions) validated by Kohmoto et al (22), the EuroQol 5 Dimension (EQ-5D), and the visual analog scale validated by Tsuchiya et al (23) were completed by the patients.

Basic data

At entry and 12 mo, BMI, blood pressure, levodopa equivalency dose (LED) (24), and laboratory data of peripheral blood calcium (normal range: 8.5–10.4 mg/dL), high-sensitive parathyroid hormone (normal range: 160–520 pg/mL), and liver and renal function were measured. Serum concentrations of 25(OH)D (ng/mL) and 1,25(OH)D (pg/mL) were measured at SRL Inc as described previously (25).

Polymerase chain reaction and direct sequencing were used to analyze *VDR* genotypes according to the following polymorphisms identified with restriction enzymes: *Fok*I (rs10735810), *Bsm*I (rs1544410), *Cdx*2 (rs11568820), *Apa*I (rs7976091), *Taq*I (rs731236), and vitamin D binding protein *GC*1 (rs7041)/*GC*2 (rs4588) as described previously (11).

Follow-up procedures and determination of outcomes

Patients were followed up every 0.5–3 mo at the Center to monitor disease progression and adjust drug doses. At each follow-up, participants were asked to bring the bottle of supplement to check their compliance. After 12 mo of taking the supplement, the HY stage, UPDRS, MMSE, EQ-5D, PDQ39, basic data including serum concentrations of 25(OH)D and 1,25(OH)D, and LED were measured again. Primary outcomes were defined as changes from baseline and the proportion of patients who showed no worsening or improvement after taking the study supplement for 12 mo as measured by the modified the HY stage, UPDRS (total and parts I–IV), and MMSE, which were scored by the same neurologists. Secondary outcomes were changes and the proportion of patients who showed no worsening or improvement in PDQ39 and EQ-5D as answered by patients. No patients underwent deep brain stimulation.

Statistical analysis

It was estimated that the primary outcome [ie, HY stage (no worsening or improvement)] would be seen in 30% of patients in the vitamin D_3 group and 10% of patients in the placebo group. An equally divided sample of 120 subjects was required to detect this difference, with a type I error (2 sided) of 5% and a power of 80%. The accrual period was planned from April 2009 to March 2011.

Efficacy was assessed by using intention-to-treat analysis. Endpoint changes were calculated as the score after intake of the study supplement minus the score before intake of the study supplement. When the change in the HY stage, UPDRS, PDQ39,

or EQ5D was ≤0, patients were considered not worsened or improved. In contrast, when the change was >0, the patient was considered worsened. For the MMSE and visual analog scale, if the change was ≥0, the patient was considered not worsened or improved and vice versa. Finally, the percentage of patients who were not worsened or improved was compared between vitamin D_3 and placebo groups.

If data were normally distributed, data were analyzed with paired or unpaired *t* tests for comparisons within or between groups before and after the consumption of supplements, respectively. If data were not normally distributed, they were analyzed by using the Wilcoxon's signed-rank or Mann-Whitney test. Categorical variables were assessed by using the chi-square test. The percentage of subjects who were not worsened or improved in both primary and secondary outcomes was compared between the 2 groups by using the RR as well as risk difference with a 95% CI and the number needed to treat. To avoid an increase in type I errors introduced by multiple testing in the Mann-Whitney test, Monte Carlo permutation statistics were used for comparison between the 2 groups in changes from baseline of both total and subgroup analyses by using 7 kinds of *VDR* and *GC* genotypes. The distribution of maximum *t* statistics on the basis of 10,000 random permutations was compared with observed values to determine the *P* value and its 95% CI. To identify an interaction between the intervention group and genotypes, a new variable was constructed by multiplying the intervention group and genotypes, and then this new variable was analyzed with linear regression models for changes and a homogeneity test for not worsening or improvement, which was represented as the *P*-interaction (26). All reported *P* values are 2 sided. $P < 0.05$ was considered significant. All analyses were performed with Stata 12.0 software (StataCorp LP).

RESULTS

Participants

A total of 137 patients were screened from April 2009 to March 2011, which led to the enrollment of 114 patients; 56 patients were randomly assigned to receive vitamin D_3, and 58 patients were randomly assigned to receive a placebo for 12 mo, and the trial ended in March 2012 as scheduled (**Figure 1**). Ten patients (8.8%) withdrew from the study (7 patients in the vitamin D_3 group and 3 patients in the placebo group; a difference that was not significant). One patient in the vitamin D_3 group developed a cerebral infarction as a serious adverse event, and he was subsequently lost to follow-up. One patient in the placebo group was lost to follow-up for unknown reasons. Consequently, 55 patients in the vitamin D_3 group (98.2%) and 57 patients in the placebo group (98.3%) were analyzed with intention-to-treat analyses. One patient in the vitamin D_3 group (because of a decubitus ulcer) and one patient in the placebo group (because of the exacerbation of PD) stopped taking the test supplement as advised by their doctor in charge. In analyzed patients, compliance was 89.1% (49 of 55 patients) in the vitamin D_3 group and 96.5% (55 of 57 patients) in the placebo group, which was a difference that was not significant. After 12 mo, one diagnosis of PD in the vitamin D_3 group was changed to the Parkinsonian variant of multiple system atrophy, and one diagnosis was changed to progressive supranuclear palsy. One diagnosis in the placebo group was switched to progressive supranuclear palsy. These 3 patients worsened during the study period. However, all of these cases were included in the intention-to-treat analyses. All other PD patients responded to standard therapy including levodopa.

Patient characteristics were similar in the 2 groups (**Table 1**). The mean age of the study population was 72 y. Disease duration, LED, and primary and secondary endpoints (data not shown), as well as

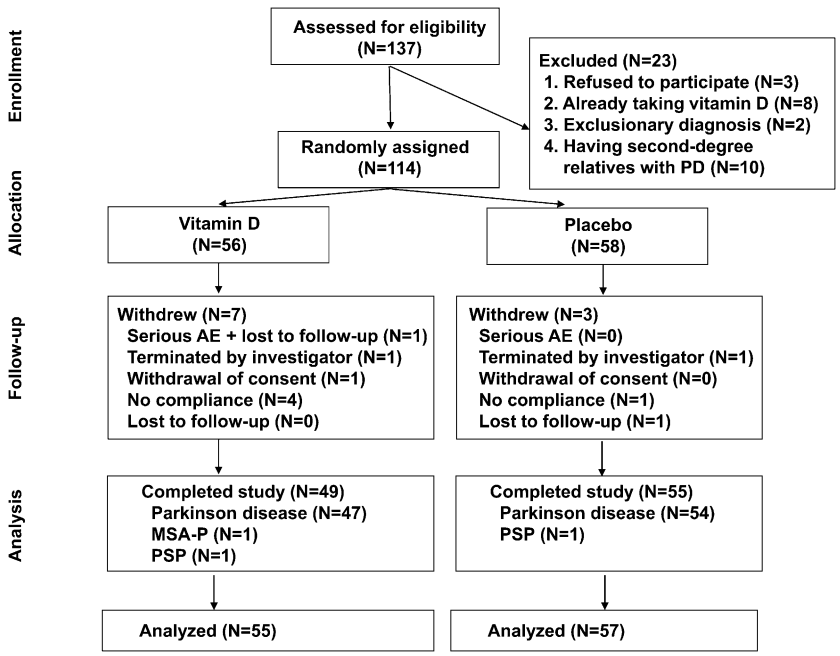

FIGURE 1. Participant flowchart. AE, adverse event; MSA-P, Parkinsonian variant of multiple system atrophy; PD, Parkinson disease; PSP, progressive supranuclear palsy.

TABLE 1
Baseline characteristics by study group[1]

	Vitamin D_3 (n = 56)	Placebo (n = 58)
Age (y)	72.5 ± 6.6[2]	71.2 (6.9)
M [*n* (%)]	31 (52)	29 (53)
BMI (kg/m^2)	22.7 ± 2.8	22.8 ± 3.7
Disease duration (mo)	24 (2–60)[3]	13 (3–42)
Levodopa dose equivalency (mg)	300 (150–550)	300 (150–600)
Modified Hoehn and Yahr stage [*n* (%)]		
1/1.5	5/1 (10.7)	10/2 (20.6)
2/2.5	26/13 (69.6)	23/9 (55.2)
3	9 (16.1)	12 (20.7)
4	1 (1.8)	2 (3.4)
5	1 (1.8)	0 (0)
UPDRS		
Total	34 (22.5–48.5)	32 (20–44)
Part I: mentation, behavior, and mood	1 (0–2)	0.5 (0–1)
Part II: activities of daily living	9 (6.5–13.5)	8 (5–12)
Part III: motor examination	22 (13–32)	20 (14–29)
Part IV: complications of therapy	0 (0–1)	0 (0–1)
MMSE	28 (26–30)	28 (26–30)
25(OH)D (ng/mL)	22.5 ± 9.7	21.1 ± 8.8
1,25(OH)D (pg/mL)	61.3 ± 17.1	60.4 ± 16.8
*Fok*I CC/CT/TT [*n* (%)]	21 (38)/31 (55)/4 (7)	18 (31)/28 (48)/12 (21)
*Bsm*I GG/AG/AA [*n* (%)]	46 (82)/9 (16)/1 (2)	51 (88)/5 (9)/2 (3)
*Cdx*2 GG/GA/AA [*n* (%)]	20 (36)/26 (46)/10 (18)	19 (33)/33 (57)/6 (10)
*Apa*I GG/GT/TT [*n* (%)]	33 (59)/17 (30)/6 (11)	39 (67)/13 (22)/6 (10)
*Taq*I TT/TC/CC [*n* (%)]	46 (82)/9 (16)/1 (2)	50 (86)/5 (9)/3 (5)
*GC*1 TT/TG/GG [*n* (%)]	22 (39)/19 (34)/15 (27)	20 (34)/28 (48)/10 (7)
*GC*2 CC/CA/AA [*n* (%)]	37 (66)/16 (29)/3 (5)	33 (57)/21 (36)/4 (7)

[1] MMSE, Mini-Mental State Examination; UPDRS, Unified Parkinson's Disease Rating Scale; 1,25(OH)D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.
[2] Mean ± SD (all such values).
[3] Median; interquartile range in parentheses (all such values).

25(OH)D and 1,25(OH)D concentrations and *VDR* and *GC* genotypes, were not significantly different between vitamin D_3 and placebo groups. However, 22% and 48% of total participants were considered vitamin D sufficient [≥30 ng 25(OH)D/mL] and deficient [<20 ng 25(OH)D/mL at baseline (3), respectively].

Changes in serum 25(OH)D concentrations and related variables

In the vitamin D_3 group, 25(OH)D concentrations increased from a mean ± SD of 22.5 ± 9.7 ng/mL at baseline to a mean of 41.7 ± 12.6 ng/mL after 12 mo ($P < 0.0001$), whereas 25(OH)D

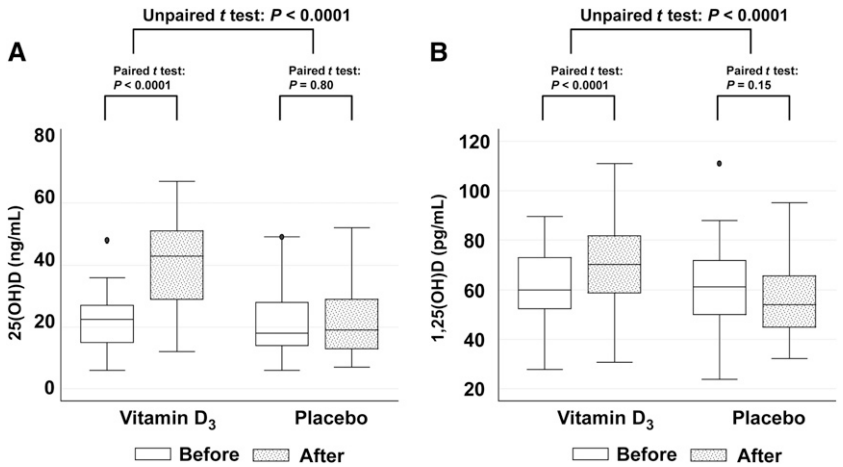

FIGURE 2. Box plots in vitamin D–related serum variables before and after the intake of test supplements for 12 mo. The lines projecting out from the boxes extend to the adjacent values, which are the most extreme observations that are not more than 1.5 times the height of the box beyond either quartile. A: 25(OH)D concentrations (normal range: 30–60 ng/mL). B: 1,25(OH)D concentrations (normal range: 20–60 pg/mL). 25(OH)D and 1,25(OH)D were considered to be normally distributed on the basis of skewness and kurtosis tests. Statistical analyses were performed by using a paired t test within the group. When changes from baseline were compared between vitamin D_3 (n = 55) and placebo (n = 57) groups, Student's t tests were used. 1,25(OH)D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

TABLE 2
Changes from baseline and proportion of patients who showed no worsening or improvement in primary and secondary endpoints within the vitamin D_3 or placebo group and between the vitamin D_3 and placebo groups[1]

Outcome	Vitamin D_3 (n = 55)	Placebo (n = 57)	Difference between groups	
			RR (95% CI); RD (95% CI)	P (95% CI) or P
HY stage (stages 1–5)				
Change (after − before)	0.02 ± 0.62[2]	0.33 ± 0.70		0.005 (0.003, 0.006)[3]
Within-group P	0.79	0.0006		
Not worsened or improved [n (%)]	16 (29.1)	7 (12.3)	2.37 (1.06, 5.31); 0.17 (0.02, 0.32)	0.028[4]
UPDRS total (0–195)				
Change (after − before)	−0.87 ± 12.8	4.20 ± 14.5		0.11 (0.10, 0.11)[3]
Within-group P	0.85	0.05		
Not worsened or improved [n (%)]	21 (38.2)	22 (38.6)	0.99 (0.62, 1.58); −0.00 (−0.18, 0.18)	0.96[4]
UPDRS part I (0–16)				
Change (after − before)	0.11 ± 1.30	0.49 ± 1.63		0.28 (0.27, 0.29)[3]
Within-group P	0.66	0.06		
Not worsened or improved [n (%)]	12 (21.8)	12 (21.1)	1.04 (0.51, 2.11); 0.01 (−0.14, 0.16)	0.92[4]
UPDRS part II (0–48)				
Change (after − before)	−0.87 ± 12.8	4.37 ± 14.6		0.004 (0.003, 0.006)[3]
Within-group P	0.32	0.004		
Not worsened or improved [n (%)]	26 (47.3)	16 (28.1)	1.68 (1.02, 2.78); 0.19 (0.02, 0.37)	0.036[4]
UPDRS part III (0–108)				
Change (after − before)	−1.05 ± 10.0	1.05 ± 9.09		0.26 (0.25, 0.27)[3]
Within-group P	0.37	0.58		
Not worsened or improved [n (%)]	27 (49.1)	27 (47.4)	1.04 (0.71, 1.52); 0.02 (−0.17, 0.20)	0.86[4]
UPDRS part IV (0–23)				
Change (after − before)	0.35 ± 1.54	0.44 ± 1.32		0.48 (0.47, 0.49)[3]
Within-group P	0.07	0.006		
Not worsened or improved [n (%)]	9 (16.4)	8 (14.0)	1.17 (0.48, 2.80); 0.02 (−0.11, 0.16)	0.73[4]
MMSE				
Change (after − before)	−0.33 ± 2.16	0.27 ± 1.74		0.12 (0.12, 0.13)[3]
Within-group P	0.42	0.11		
Not worsened or improved [n (%)]	31 (63.3)	43 (78.2)	0.81 (0.63, 1.04)−0.15 (−0.32, 0.02)	0.09[4]
PDQ39 total				
Change (after − before)	−5.41 ± 17.4	−3.15 ± 17.5		0.32 (0.31, 0.32)[3]
Within-group P	0.04	0.30		
Not worsened or improved [n (%)]	33 (67.3)	31 (56.4)	1.19 (0.88, 1.62); 0.11 (−0.08, 0.30)	0.25[4]
PDQ39 mobility				
Change (after − before)	−3.80 ± 25.3	−0.77 ± 26.6		0.49 (0.48, 0.50)[3]
Within-group P	0.29	0.95		
Not worsened or improved [n (%)]	24 (50.0)	24 (43.6)	1.15 (0.76, 1.73); 0.06 (−0.13, 0.26)	0.52[4]
PDQ39 activities of daily living				
Change (after − before)	−2.47 ± 23.9	−0.83 ± 24.7		0.29 (0.28, 0.30)[3]
Within-group P	0.22	0.94		
Not worsened or improved [n (%)]	29 (59.2)	21 (38.2)	1.55 (1.03, 2.33); 0.21 (0.02, 0.40)	0.032[4]
PDQ39 emotional well-being				
Change (after − before)	−5.27 ± 22.6	−3.56 ± 21.8		0.41 (0.40, 0.42)[3]
Within-group P	0.06	0.29		
Not worsened or improved [n (%)]	31 (63.3)	24 (43.6)	1.45 (1.00, 2.10); 0.20 (0.01, 0.38)	0.045[4]
PDQ39 stigma				
Change (after − before)	0.30 ± 23.9	−5.45 ± 16.5		0.29 (0.28, 0.30)[3]
Within-group P	0.91	0.05		
Not worsened or improved [n (%)]	18 (36.7)	23 (41.8)	0.88 (0.54, 1.42); −0.05 (−0.24, 0.14)	0.60[4]
PDQ39 communication				
Change (after − before)	−5.73 ± 18.81	−1.21 ± 21.2		0.37 (0.36, 0.38)[3]
Within-group P	0.10	0.67		
Not worsened or improved [n (%)]	21 (43.8)	21 (38.2)	1.15 (0.72, 1.82); 0.06 (−0.13, 0.25)	0.57[4]
PDQ39 bodily support				
Change (after − before)	−7.64 ± 20.8	−1.97 ± 22.2		0.09 (0.08, 0.09)[3]
Within-group P	0.007	0.51		
Not worsened or improved [n (%)]	29 (60.4)	23 (41.8)	1.44 (0.98, 2.13); 0.19 (−0.00, 0.38)	0.06[4]

(Continued)

TABLE 2 (*Continued*)

Outcome	Vitamin D_3 (n = 55)	Placebo (n = 57)	Difference between groups: RR (95% CI); RD (95% CI)	Difference between groups: P (95% CI) or P
PDQ39 social support				
Change (after − before)	−3.65 ± 19.7	0.00 ± 17.3		0.21 (0.20, 0.22)[3]
Within-group P	0.24	0.58		
Not worsened or improved [n (%)]	13 (27.1)	12 (21.8)	1.24 (0.63, 2.46); 0.05 (−0.11, 0.22)	0.53[4]
PDQ39 cognitive impairment				
Change (after − before)	−2.86 ± 17.0	−1.36 ± 18.5		0.99 (0.99, 1.00)[3]
Within-group P	0.41	0.44		
Not worsened or improved [n (%)]	18 (37.5)	25 (45.5)	0.83 (0.52, 1.31); −0.08 (−0.27, 0.11)	0.41[4]
EQ-5D				
Change (after − before)	0.01 ± 0.20	−0.04 ± 0.31		0.78 (0.77, 0.79)[3]
Within-group P	0.29	0.86		
Not worsened or improved [n (%)]	12 (25.0)	18 (32.7)	0.76 (0.41, 1.42); −0.08 (−0.25, 0.10)	0.39[4]
Visual analog scale				
Change (after − before)	−4.58 ± 16.0	−1.51 ± 20.0		0.25 (0.24, 0.25)[3]
Within-group P	0.076	0.89		
Not worsened or improved [n (%)]	25 (52.1)	34 (61.8)	0.84 (0.60, 1.19); −0.10 (−0.29, 0.09)	0.32[4]

[1] Within-group P values are for differences in outcomes before and after intervention within the group and were assessed by using Wilcoxon's signed-rank test. EQ-5D, EuroQol 5 Dimension; HY, Hoehn and Yahr; MMSE, Mini-Mental State Examination; PDQ39, Parkinson's Disease Questionnaire-39; RD, risk (absolute) difference; UPDRS, Unified Parkinson's Disease Rating Scale.

[2] Mean ± SD (all such values).

[3] Calculated by using Monte Carlo permutation statistics of the Mann-Whitney test.

[4] Calculated by using the chi-square test.

concentrations changed little in the placebo group from a mean of 21.1 ± 8.8 ng/mL at baseline to a mean of 21.4 ± 9.8 ng/mL after 12 mo (**Figure 2**A). Moreover, 1,25(OH)D concentrations increased significantly from a mean of 61.3 ± 17.1 ng/mL at baseline to a mean of 69.9 ± 18.0 ng/mL after 12 mo in the vitamin D_3 group ($P < 0.0001$); 1,25(OH)D concentrations values were unchanged in the placebo group from a mean of 60.4 ± 16.8 ng/mL at baseline to a mean of 56.0 ± 14.7 ng/mL after 12 mo (Figure 2B). LED was reduced in 5 of 49 (10%) patients in the vitamin D_3 group and 3 of 55 (5%) patients in the placebo group, which was a difference that was not significant.

Efficacy

Changes from baseline and the proportion of patients who showed no worsening or improvement in primary and secondary endpoints were compared between vitamin D_3 and placebo groups as well as within the vitamin D_3 or placebo group (**Table 2**). Modified HY stages were essentially unchanged within the vitamin D_3 group, whereas they worsened significantly within the placebo group ($P = 0.0006$). The difference between groups was significant ($P = 0.005$). The HY stage did not worsen or improve in 16 of 55 patients who received vitamin D_3 and in 7 of 57 patients who received the placebo ($P = 0.028$). The risk difference was 17% (95% CI: 2%, 32%), and thus, the number needed to treat was considered to be 6 patients (95% CI: 48, 3 patients). Even with the assumption that one lost patient in the vitamin D_3 group and one lost patient in the placebo group worsened, the difference was still significant ($P = 0.032$).

Similar to the HY stages, UPDRS part II scores remained unchanged in the vitamin D_3 group, but worsened significantly in the placebo group ($P = 0.004$). The difference between groups was also significant ($P = 0.004$). No worsening or improvement in part II occurred in 26 of 55 patients in the vitamin D_3 group and 16 of 57 patients in the placebo group ($P = 0.036$). In contrast, there were no differences between vitamin D_3 and placebo groups in UPDRS total or parts I, III, or IV. MMSE scores were not different within each group and between groups.

The total score of the PDQ39 ($P = 0.04$) and bodily support in the PDQ39 ($P = 0.007$) improved in the vitamin D_3 group but did not change in the placebo group. In the activities of daily living part of the PDQ39, 59.2% of patients in the vitamin D_3 group did not worsen or improve, which was significantly more than the 38.2% of patients in the placebo group ($P = 0.032$). Similarly, in the emotional well-being part of the PDQ39, 63.3% of patients in the vitamin D_3 group did not worsen or improve, which was significantly more than the 43.6% of patients in the placebo group ($P = 0.045$). No other significant differences in other outcomes were shown within each group or between groups.

Effect modification by *VDR* and *GC* genotypes

Interaction analyses based on *VDR* and *GC* genotypes were performed. Effects of vitamin D_3 compared with the placebo were significantly modified by *Fok*I genotypes as measured with the HY stage (P-interaction = 0.045) and the UPDRS total (P-interaction = 0.039) and part II (P-interaction = 0.035) but not by the other *VDR* and *GC* genotypes tested. Proportions of patients who did not worsen or improve in the HY stage (P-interaction = 0.54), UPDRS total (P-interaction = 0.021), and UPDRS part II (P-interaction = 0.0039) are shown in **Table 3**. In patients with *Fok*I TT, the HY stages were unchanged within the vitamin D_3 group, whereas they worsened markedly within the placebo group ($P = 0.009$). The difference between groups was also significant ($P = 0.009$). Similarly, in patients with *Fok*I CT, HY stages were unchanged within the vitamin D_3 group,

TABLE 3
Interaction analyses of *Fok*I genotypes[1]

		*Fok*I CC			*Fok*I CT			*Fok*I TT		
	P-interaction	Vitamin D_3 (n = 21)	Placebo (n = 18)	*P* (95% CI) or *P*	Vitamin D_3 (n = 30)	Placebo (n = 27)	*P* (95% CI) or *P*	Vitamin D_3 (n = 4)	Placebo (n = 12)	*P* (95% CI) or *P*
HY stage										
Change (after – before)	0.045[2]	0.12 ± 0.67[3]	0.08 ± 0.49	0.89 (0.89–0.90)[4]	0.00 ± 0.60	0.37 ± 0.74	0.020 (0.018–0.023)[4]	−0.38 ± 0.48	0.63 ± 0.77	0.009 (0.007–0.011)[4]
P[5]		0.62	0.47		0.78	0.014		0.91	0.009	
Not worsened or improved [*n* (%)]	0.54[6]	—	—	—	—	—	—	—	—	—
UPDRS total										
Change (after – before)	0.039[2]	1.48 ± 11.4	−0.89 ± 14.1	0.60 (0.60–0.61)[4]	−1.20 ± 13.1	4.74 ± 12.4	0.22 (0.21–0.23)[4]	−10.8 ± 15.2	10.6 ± 17.4	0.018 (0.016–0.021)[4]
P[5]		0.57	0.71		0.80	0.14		0.14	0.041	
Not worsened or improved [*n* (%)]	0.021[6]	6 (28)	9 (50)		12 (40)	11 (41)		3 (75)	2 (17)	
RR (95% CI)		0.57 (0.25–1.30)		0.17[7]	0.98 (0.52–1.85)		0.95[7]	4.50 (1.13–18.0)		0.029[7]
RD (95% CI)		−0.21 (−0.52–0.09)			−0.01 (−0.26–0.24)			0.58 (0.11–1.05)		
UPDRS part II										
Change (after – before)	0.035[2]	0.71 ± 4.42	0.28 ± 3.51	0.60 (0.59–0.61)[4]	−0.63 (4.68)	2.63 ± 4.75	0.19 (0.19–0.20)[4]	−3.25 ± 2.06	4.00 ± 9.19	0.019 (0.016–0.022)[4]
P[5]		0.56	0.84		0.28	0.016		0.066	0.037	
Not worsened or improved [*n* (%)]	0.0039[6]	7 (33)	9 (50)		15 (50)	11 (22)		4 (100)	1 (8)	
RR (95% CI)		0.67 (0.31–1.43)		0.29[7]	2.25 (1.02–4.96)		0.030[7]	12.0 (1.84–78.4)		0.0006[7]
RD (95% CI)		−0.17 (0.47–0.13)			0.28 (0.04–0.52)			0.92 (0.76–1.07)		

[1] HY, Hoehn and Yahr; UPDRS, Unified Parkinson's Disease Rating Scale.
[2] Linear regression models were applied to evaluate an interaction effect and calculate the *P* value between the intervention group and *Fok*I genotypes.
[3] Mean ± SD (all such values).
[4] Calculated by using Monte Carlo permutation statistics of the Mann-Whitney test.
[5] Difference in the outcome before and after intervention within the group was assessed by using Wilcoxon's signed-rank test.
[6] Calculated by using the test of homogeneity.
[7] Calculated by using the chi-square test.

TABLE 4
Changes in serum calcium, hsPTH, and ALP concentrations[1]

	Vitamin D_3 (n = 55)			Placebo (n = 57)			
	Preintervention	Postintervention	P^2	Preintervention	Postintervention	P^2	P^3
Calcium (mg/dL)[4]	9.2 (8.8, 9.4)[5]	9.1 (9.0, 9.5)	0.21	9.2 (9.0, 9.5)	9.2 (9.0, 9.4)	0.67	
Change (after – before)	0.0 (−0.1, −0.3)			0.0 (−0.2, −0.2)			0.07
hsPTH (pg/mL)[6]	430 (340, 500)	390 (320, 460)	0.045	375 (310, 480)	405 (310, 520)	0.07	
Change (after – before)	−30 (−110, −40)			30 (−50, 90)			0.007
ALP (U/L)[7]	235 (178, 267)	238 (211, 274)	0.07	213 (167, 259)	238 (201, 304)	0.0001	
Change (after – before)	13 (−8, 38)			30 (−1, 71)			0.09

[1] ALP, alkaline phosphatase; hsPTH, high-sensitivity parathyroid hormone.
[2] Difference in the outcome before and after intervention within the group was assessed by using Wilcoxon's signed-rank test.
[3] Calculated by using Monte Carlo permutation statistics of the Mann-Whitney test.
[4] Laboratory data of peripheral blood calcium (normal range: 8.5–10.4 mg/dL).
[5] Median; IQR in parentheses (all such values).
[6] Normal range: 160–520 pg/mL.
[7] Normal range: 115–359 U/L.

whereas they worsened moderately within the placebo group (P = 0.014). The difference between groups was also significant (P = 0.020). However, in patients with *Fok*I CC, vitamin D had no significant effects on changes in the HY stage either within the group or between groups. Similarly, vitamin D_3 significantly prevented deterioration in the UPDRS total and part II in patients with *Fok*I TT but not *Fok*I CC. The percentage of patients who showed no worsening or improvement in the UPDRS total was significantly higher in the vitamin D_3 group than in the placebo group in patients with *Fok*I TT (P = 0.029), but not *Fok*I CT or CC. Similarly, the percentage of patients who showed no worsening or improvement in the UPDRS part II was significantly higher in the vitamin D_3 group than in the placebo group in patients with *Fok*I TT (P = 0.0006) and CT (P = 0.030) but not CC.

Safety

There were no obvious adverse events associated with hypercalcemia. Serum calcium concentrations remained within the normal range (**Table 4**). However, one patient in the placebo group showed an increase to 11.0 mg/dL, which was above the upper limit. Highly sensitive parathyroid hormone concentrations decreased significantly (P = 0.045) in the vitamin D_3 group but were not altered in the placebo group. These changes from baseline were significantly different between the 2 groups (P = 0.007). Alkaline phosphatase was significantly increased (P = 0.0001) in the placebo group but not in the vitamin D_3 group. In contrast, there were no significant changes before and after intake of vitamin D_3 as well as between vitamin D_3 and placebo groups, in body weight, blood pressure, liver or renal function, and inflammation or metabolic variables as assessed by using blood chemistry (liver transaminases, blood urea nitrogen, serum creatinine, high-sensitivity C-reactive protein, blood sugar, hemoglobin A1c, total cholesterol, LDL and HDL cholesterol, and triglycerides).

DISCUSSION

In this randomized, double-blind, placebo-controlled trial, daily supplementation with 1200 IU vitamin D_3 for 12 mo significantly prevented the deterioration of PD as measured with the HY stage, UPDRS part II and total, and some domains of the PDQ39, with no apparent increase in risk of hypercalcemia or other adverse events during the study period. A point estimate of the number needed to treat was 6 patients for no worsening or improvement in the HY stage, which was considered very effective. To the best of our knowledge, this is the first randomized trial to examine the effects of vitamin D_3 in patients with PD. However, a meta-analysis showed that supplemental vitamin D for older adults who participated in randomized controlled trials consistently showed beneficial muscle effects on strength and balance (27). Therefore, it cannot be distinguished whether vitamin D supplementation specifically delays the progression of PD or whether it just nonspecifically improves muscle strength and balance in older adults.

Next, we examined whether the effect of vitamin D supplementation was modified by *VDR* or *GC* genotypes. Ethnic variations in *VDR Fok*I polymorphism ratios for CC:CT:TT are as follows: white, 40:47:13, respectively; African, 42:48:10, respectively; Hispanic, 46:46:8, respectively; Pacific, 29:58:13, respectively; and Asian, 32:5:18, respectively (28). These variations were not significantly different from those in our study population, which showed polymorphism ratios for CC:CT:TT of 34:52:14. We showed that individuals with the *VDR Fok*I TT genotype had significant and consistent responses to vitamin D_3 supplementation, and individuals with the *Fok*I CT genotype had moderate responses. In contrast, patients with the *Fok*I CC genotype had no significant response. By switching from codon ATG (*Fok*I T) to ACG (*Fok*I C), the first potential start site of methionine for transcription moves in the 3′ direction, which results in proteins that are 3 amino acids shorter and more functional even at the same vitamin D concentrations in an experimental setting (29). Thus, patients with the *Fok*I CC, CT, and TT genotypes tend to have better outcomes in this order, suggesting that the *Fok*I TT genotype may be less efficient than the CC genotype is in responding to the same concentrations of serum vitamin D; *Fok*I CT may be the intermediate between TT and CC genotypes. In a prospective cohort study that examined 25(OH)D, *VDR* polymorphisms, and prostate cancer, there were significant interactions between 25(OH)D concentrations and

VDR FokI genotypes. In men with the *FokI* TT genotype, a higher 25(OH)D concentration was related to significant 60–70% lower risk of total and aggressive prostate cancer than in patients with lower 25(OH)D concentrations (30). This observation was consistent with our results, although the diseases were different. Thus, we hypothesize that the raising of 25(OH)D and/or 1,25(OH)D concentrations with vitamin D_3 supplementation is effective in the prevention of deterioration in the subgroup of patients with *FokI* TT, somewhat effective in patients with *FokI* CT, and not effective in patients with the *FokI* CC genotype. *VDR* TaqI genotypes modify the effect of vitamin D supplementation by shortening the time to sputum culture conversion in patients with sputum smear-positive pulmonary tuberculosis (31), which is an example of another disease that shows that *VDR* genotypes may modify the reaction to vitamin D supplementation.

There were several limitations to this study. First, the number of patients analyzed may have been too small to detect small differences in some endpoints or in *VDR* subgroups. Second, the dose of vitamin D_3 was set at 1200 IU, which raised concentrations of 25(OH)D to 19.2 ng/mL and concentrations of 1,25(OH)D to 8.6 pg/mL. However, this dose may not have been enough to maximize the effect of vitamin D_3 supplementation to improve PD. Third, 10 patients did not complete the intake of the study supplement, which could have biased the conclusion toward the null hypothesis. Even with these limitations, vitamin D prevented the deterioration of PD in this study. Fourth, the UPDRS was not measured off therapy, which may have underestimated the efficiency of vitamin D. Fifth, prevalences of 25(OH)D insufficiency and deficiency in this study population were higher than in another cohort (1). Thus, the current results may not be generalizable to other ethnic groups or people living at different latitudes. Sixth, the HY stage was used as a primary endpoint, and other sensitive and accurate measures of outcome, such as a change in the number of steps during the stand-walk-sit test, were not used (32). However, even with the use of insensitive endpoints such as the HY stage, vitamin D supplementation appeared to prevent the deterioration of PD. Seventh, some patients had advanced PD, whereas some patient had early PD, which made the study population rather insensitive to changes in PD, especially over such a short period and in a degenerative disease that lasts for decades. However, even the inclusion of these patients with both early and advanced PD, vitamin D supplementation appeared to inhibit the progression of PD. Eighth, we could not say that vitamin D supplementation was perfectly safe and tolerated in PD because the observation period was only 1 y, and minor clinical signs and symptoms may have been missed. In addition, the sample size was primarily calculated to detect the efficacy of vitamin D_3 and not side effects. One case of cerebral infarction and one withdrawal of consent occurred in the vitamin D_3 group. Four patients were not compliant in taking vitamin D_3, whereas only one patient was not compliant in taking the placebo, although these differences were not significant in such a small study population.

In conclusion, the results of the current study showed a significant effect of 12-mo vitamin D_3 supplementation in the stabilization of the severity of PD in patients with *FokI* CT and TT genotypes for a short period but not inpatients with the *FokI* CC genotype, with no apparent increase in risk of hypercalcemia. However, we are not certain that this effect is specific for PD.

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The authors' responsibilities were as follows—MS and MU: conception and design of the study; MU: drafting of the manuscript and statistical analyses; MS, MY, MH, MM, and MN: conducting the study; DT: *VDR* interpretation; and MS, MN, DT, and MU: interpretation of data. All authors approved the final version of the manuscript. None of the authors had a conflict of interest.

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