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ORIGINAL PAPER

GC and VDR SNPs and Vitamin D Levels in Parkinson's Disease: The Relevance to Clinical Features

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Abstract Vitamin D deficiency is suggested to be associated with Parkinson's disease (PD). Our aim was to investigate the serum 25-hydroxyvitamin D_3 (25OHD) levels of PD patients in Turkish cohort, to investigate any association of vitamin D binding protein (GC) genotypes with PD due to the significant role of GC in vitamin D transport, to determine whether vitamin D receptor (VDR) haplotype that we previously demonstrated to be a risk haplotype for AD is also a common haplotype for PD and to investigate any relevant consequence of serum 25OHD levels, GC or VDR genotypes on clinical features of PD. Three hundred eighty-two PD patients and 242 healthy subjects were included in this study. The serum 25OHD levels were investigated by *CLI*A, and GC and VDR SNPs were evaluated with LightSnip. Our results indicated a strong relationship between low serum 25OHD levels and PD ($p < 0.001$). rs7041 of GC and *Apa*I of VDR were associated with the PD risk ($p < 0.05$). Minor allele carriers for *Bsm*I of VDR gene in both PD patients and healthy subjects had significantly higher levels of serum 25OHD ($p < 0.05$). The homozygous major allele carriers for rs2282679, rs3755967 and rs2298850 of GC gene in PD patients with slower progression had significantly higher levels of serum 25OHD ($p < 0.05$). Minor allele carriers for *Fok*I of VDR gene were more frequent in patients with advanced-stage PD ($p < 0.05$). Consequently, this is the first study demonstrating GC gene as a risk factor for PD. The relationship between PD's clinical features and low 25OHD or risk genotypes might have effects on PD independently.

Keywords Parkinson's disease · Vitamin D · Vitamin D binding protein · GC · Vitamin D receptor · SNP

Introduction

The vitamin D receptor (VDR) gene is located on chromosome 12, and both linkage and genome-wide association (GWA) studies have identified an additional Alzheimer's disease (AD) risk locus that includes the VDR gene (Poduslo and Yin 2001; Blacker et al. 1998; Hollenbach et al. 1998; Luedecking-Zimmer et al. 2003; Beecham et al. 2009). Our study in 2007 provided the first evidence of a genetic association between a neurodegenerative disorder—namely—AD and VDR; a polymorphism in the VDR gene was identified that might increase the risk of AD by 2.3 times (Gezen-Ak et al. 2007). Single nucleotide polymorphisms (SNPs) in the VDR gene have been studied in various diseases for many years (Uitterlinden et al. 2004) and are thought to have effects on vitamin D-dependent pathways (Gezen-Ak et al. 2012, 2007). A limited number

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of studies have also found associations between VDR polymorphisms and cognitive decline (Kuningas et al. 2009; Beydoun et al. 2012), AD (Lehmann et al. 2011; Wang et al. 2012; Gezen-Ak et al. 2012, 2007), as well as Parkinson's disease (PD) (Butler et al. 2011; Gatto et al. 2015; Han et al. 2012; Kim et al. 2005; Suzuki et al. 2012, 2013; Torok et al. 2013).

Parkinson's disease is an important and widespread neurodegenerative disorder. A limited number of studies have suggested that low vitamin D levels (25-hydroxyvitamin D_3, 25OHD) are associated with PD (Evatt et al. 2008; Sato et al. 1997, Sato et al. 2005, 2001; Knekt et al. 2010). Other studies that investigated VDR SNPs in PD had some controversial results, but most of the negative results involved cohorts from eastern Asian populations (Lin et al. 2014; Butler et al. 2011; Kim et al. 2005; Lv et al. 2013; Petersen et al. 2014; Torok et al. 2013; Liu et al. 2013; Gatto et al. 2015; Han et al. 2012; Suzuki et al. 2012, 2013).

On the other hand, vitamin D binding protein (DBP, VDBP, group-specific component of serum, Gc globulin or GC) is suggested to be a key molecule for understanding the action of vitamin D (Bhan 2014). GC is the major protein whose functions are binding, solubilization and transport of vitamin D and its metabolites (Delanghe et al. 2015; Bhan 2014). It was demonstrated to be co-localized with amyloid beta (Moon et al. 2013) to be elevated in CSF or serum of AD patients (Abdi et al. 2006; Zhang et al. 2008; Bishnoi et al. 2015) and was indicated as an amyloid beta scavenger (Bishnoi et al. 2015) in a limited number of studies. GC was also reported to be elevated in CSF of PD patients (Zhang et al. 2008). Yet the biological relevance of GC to neurodegeneration is still unclear, and there is no study investigating GC SNPs in PD patients.

Our aim in this study was: (1) to investigate the serum 25OHD levels of PD patients in Turkish cohort, (2) to investigate any association of GC genotype with PD, due to significant role of GC in vitamin D transport, (3) to determine whether VDR haplotype that we previously demonstrated to be a risk haplotype for AD (Gezen-Ak et al. 2012) is unique to AD or a common haplotype associated with another neurodegenerative disorder, PD, (4) to investigate any relevant consequence of serum 25OHD levels, VDR or GC genotypes on clinical features of PD.

Materials and Methods

Samples

Three hundred eighty-two PD patients and 242 age-matched controls free from any neurodegenerative disorder were included in the study. Patients were clinically diagnosed according to UK PD Society Brain Bank criteria at the Department of Neurology, Cerrahpasa Faculty of Medicine and at the Behavioral Neurology and Movement Disorders Unit, Department of Neurology, Istanbul Faculty of Medicine of Istanbul University. The age at examination, gender, PD duration at examination, affected side and predominant symptoms at onset were gathered from the medical records. The severity of PD was assessed according to Hoehn–Yahr staging. The Hoehn and Yahr scale is a widely used clinical rating scale in PD staging (Hoehn and Yahr 1967). It defines broad categories of motor function in PD by mainly focusing on unilaterality versus bilaterality and the presence of postural reflex impairment. The scale includes stages 1 through 5 with higher stages correlating to motor decline and deterioration in quality of life. It is rated as follows: 1.0: unilateral involvement only; 1.5: unilateral and axial involvement; 2.0: bilateral involvement without impairment of balance; 2.5: mild bilateral disease with recovery on pull test; 3.0: mild to moderate bilateral disease; some postural instability; physically independent; 4.0: severe disability; still able to walk or stand unassisted; 5.0: wheelchair bound or bedridden unless aided (Goetz et al. 2004).

Assessment of clinical features of the disease was based on 6 clinical domains including: initial predominant symptoms (TD: tremor dominant; AR: akinetic rigid or both), the course of the predominant symptoms (TT: tremor-dominant onset with ongoing tremor-dominant symptoms; TB: tremor-dominant onset with ongoing bradykinesia symptoms; BB: bradykinesia-dominant onset with ongoing bradykinesia-dominant symptoms), the duration of PD, disease stage (1 through 5, the Hoehn and Yahr scale), freezing of gait (FoG, assessed by yes/no) and the falls (assessed by yes/no). While freezing of gait and falls are associated with advanced stages of the disease, tremor is a marker of relatively benign course. Tremor-dominant PD patients progress more slowly than patients with akinetic-rigid-dominant PD. Disease stage was evaluated as <3 or ≥ 3, in order to define more severe cases given that postural instability which is associated with falls and loss of independence emerges at stage 3, whereas <3 stages are defined as mild disease. Stage 3 is an important turning point in the progression of the disease. The duration of PD was evaluated as <7 or ≥ 7 years since the median duration of illness at stage 3 is about 7 years although the progression of the disease is quite variable (Hoehn and Yahr 1967).

In our cohort, all of the PD patients were under dopaminergic treatment, which was calculated as the levodopa-equivalent daily dose (LEDD). As the dose of dopaminergic medications may need to be increased or new medications added over the course of the disease,

higher doses of LEDD may be related to later stages of the disease.

Patients were also analyzed by separating into subgroups according to age of onset and family history (patients with first- or second-degree relatives with neurodegenerative disorders). All demographics of patient and healthy subjects are given in Table 1.

After all results were obtained for the patients and control groups, an additional group called "*VDR profile group*" was established, consisting of 58 individuals (Table 1). There were two reasons for including this group. First, although several Turkish studies reported similar mean 25OHD values (van der Meer et al. 2011; Hekimsoy et al. 2010), our 25OHD levels were lower than the mean levels of other populations (Evatt et al. 2008; Sato et al. 1997, 2001, 2005; Meamar et al. 2015). Thus, we wanted to test the levels of healthy, educated (at least graduated), young and working individuals whose blood samples were collected within 1 week in April. Second, we wanted to test the effect of VDR genotype on VDR mRNA levels, given that the *Apa*I genotype distributions indicated that the same genotype and a very similar haplotype were risk factors for PD in the present study and for AD in our previous study. Though, the "VDR profile group" was not selected randomly for the *Apa*I genotype distributions. Given that the frequency of the "aa" genotype is low in the population that genotype was preferentially included in this group to determine the effect of "aa" on VDR mRNA levels.

CLIA Assay

Vitamin D levels were measured from serum samples of patients and controls by chemiluminescent immunoassay (CLIA) method with DiaSorin LIAISON 25 OH Vitamin D Total Assay (Cat. No. 310600). Each 25OHD test run included case–control sets, two blinded quality control (QC) specimens from our study as well as two inter assay controls and six calibrators. The test detection range was 4–150 ng/mL. Both the low and high controls were in the range of the calibrators in each run. Assay calibrators for the assay were as follows: low control, 17.6 ng/mL (normal range 7.50–25.5 ng/mL); high control, 55.8 ng/mL (normal range 31.2–82.8 ng/mL).

Table 1 Demographics of patient and control groups

Groups	*n* (number of individuals)	Age (mean ± SD)	Age onset/PD duration (years)/LEDD
Patient groups			
All PD (independent from age of onset and familial background)	382 (42.1 % female)	61.7 ± 11.4 (aged between 24 and 89)	Age at PD onset 14 to 86/6.0 ± 5.6/713.5 ± 413.5
Subgroups according to age of onset and familial background			
PD sporadic No familial background	292 (40.8 % female)	62.9 ± 11.0 (aged between 33 and 89)	Age at PD onset 25 to 86/5.8 ± 5.0/720.5 ± 422.9
PD sporadic Age of onset >50	205 (42.9 % female)	67.9 ± 7.9 (aged between 51 and 89)	>50 (age at PD onset 50 to 86)/5.0 ± 4.5/656.4 ± 409.7
PD sporadic Age of onset <50	87 (35.6 % female)	50.9 ± 7.8 (aged between 33 and 74)	<50 (age at PD onset 25 to 49)/[†] 7.6 ± 5.7/[¥]872.4 ± 417.5
PD with familial background	90 (46.7 % female)	58.0 ± 11.9 (aged between 24 and 87)	Age at PD onset 14 to 85/6.6 ± 7.1/689.9 ± 381.7
Control groups			
Healthy subjects Age matched with PD sporadic group*	242 (51.2 % female)	64.6 ± 10.3 (aged between 36 and 87)	–
VDR profile group (independent healthy subjects)	58 (72.4 % female)	33.2 ± 8.6 (aged between 23 and 52)	–

The groups were sex matched ($p = 0.09$), except VDR profile group. VDR profile group was independent from the present case–control study. The aim for establishing this group was given in method section

* Healthy subjects and PD sporadic group were age matched ($p = 0.07$)

[†] The mean PD duration of PD sporadic patients with age of onset <50 years was significantly higher than that of PD sporadic patients with age of onset >50 years (95 % CI 0.6–4.3; $p < 0.01$, Dunn's multiple comparison) [¥] The mean LEDD of PD sporadic patients with age of onset <50 years was significantly higher than that of All PD patients with age of onset >50 years (95 % CI 17.4–300.4; $p < 0.01$, Dunn's multiple comparision), PD sporadic patients (95 % CI 6.6–297.3; $p < 0.05$, Dunn's multiple comparison), PD sporadic patients with age of onset >50 years (95 % CI 63.8–368.2; $p < 0.001$, Dunn's multiple comparison)

Genotyping

DNA was isolated from peripheral blood samples using *QIA*amp DNA Blood Mini Kit (Cat. No. 51304, Qiagen). Genotypes of rs2282679, rs3755967, rs2298850 and rs7041 SNPs within *GC* gene and rs2228570 (*Fok*I) SNP within *VDR* gene were determined by real-time polymerase chain reaction (RT-PCR) method using simple probes (LightSNiP, TibMolBiol) and LightCycler FastStart DNA Master HybProbe Kit (Cat. No. 12239272001, Roche) with LightCycler 480 Instrument II (Roche). Melting curve analysis was performed for genotyping. PCR and restriction fragment length polymorphism (RFLP) methods were performed in order to determine the genotypes of rs7975232 (*Apa*I), rs7731236 (*Taq*I), rs757343 (*Tru*9I) and rs1544410 (*Bsm*I) SNPs within *VDR* gene as previously described (Gezen-Ak et al. 2012, 2007).

VDR mRNA Expression

RNA was isolated from peripheral blood samples from "*VDR profile group*" using *QIA*amp RNA Blood Mini Kit (Cat. No. 52304 QIAGEN). cDNA synthesis were performed with VDR mRNA-specific primer (5′TCGGCTAGCTTCTGGATCAT3′) and Transcriptor First Strand cDNA Synthesis Kit (Roche 04379012001). The levels of expression of the VDR mRNA were analyzed by quantitative RT-PCR (qRT-PCR) using Universal Probe Library (UPL) probes and a LightCycler 480 Probe Master Mix Kit (Roche 04707494001) with a LightCycler 480. All of the primers and probes were chosen from Roche's UPL system as follows: VDR, UPL Probe REF 04688660001, #Probe 67 and Actin beta (ACTB), Real time ready catalog assay ACTB Assay ID 143636. ACTB used as housekeeping gene. Each PCR was performed in triplicate.

Statistical Analysis

25OHD values were season adjusted according to the cosinor model of Sachs et al. (2013). No significant difference was observed between the adjusted and unadjusted 25OHD values; thus, all comparisons were made with the adjusted values. Data are presented as the mean ± standard deviation (SD) in the text and figure legends. Group comparisons for serum 25OHD levels were performed by the Kruskal–Wallis test followed by Dunn's multiple comparison test. The groups were then adjusted for age and sex by univariate analysis of variance.

The distributions of alleles and genotypes of SNPs within GC and VDR genes were analyzed with Chi-square (χ^2) test, *df*: 2 (for genotypes), *df*: 1 (for alleles). All data were given as mean ± SD. *p* values lower than 0.05 were considered statistically significant. Significance also confirmed with Cramer's V/Kendall's tau-c. An exact test for Hardy–Weinberg Equilibrium (HWE) was also performed.

Mean 25OHD and LEDD values of genotypes in groups were compared with one-way ANOVA with Tukey C post hoc multiple comparisons test. Mean 25OHD and LEDD values of minor allele carriers and homozygote major allele carriers in groups were compared with one-way ANOVA with Welch correction.

The serum 25OHD levels were also compared, depending on the clinical features of the patients by one-way ANOVA with Welch correction. The genotypes for each SNPs in groups were also compared, depending on the clinical features of the patients by with Chi-square (χ^2) test. The tests were repeated in groups with sample size greater than 200 by stratifying data for disease stage or PD duration or both.

Statistical analyses were performed by "SPSS 20.0" software. Haplotype analysis was performed for GC and VDR genes independent from each other by "Haploview 4.2" software.

Relative mRNA expressions were calculated as previously described (Gezen-Ak et al. 2011; Dursun et al. 2011). Raw data of each group were analyzed on GraphPad InStat DTCG 3.06 (GraphPad Software, Inc.) using one-way ANOVA method, and *p* values less than 0.05 were considered statistically significant. qRT-PCR data were given as mean and SD.

Results

Serum Vitamin D Levels

The 25OHD levels of patient groups were significantly lower than control groups. When the groups were adjusted for age and sex, the significance became $p = 0.003$ and observed power was 93 %. The detailed comparisons for adjusted and unadjusted 25OHD levels were given in Table 2. The mean level of serum 25OHD in the *VDR profile group* was 19.271 ± 12.95 and did not differ from the 25OHD levels of healthy controls ($p = 0.71$).

Multivariable linear regression analysis of 25OHD of groups showed that 25OHD levels of PD sporadic group were positively correlated with age and age of onset ($p = 0.039$, $p = 0.013$; respectively, $r = 0.134$); 25OHD levels of all PD group were nearly significant positively correlated with age and age of onset ($p = 0.059$, $p = 0.060$; respectively, $r = 0.85$). No significant correlation was observed for the remaining parameters. Data were not given.

Allele and Genotype Data

The alleles of the controls were in Hardy–Weinberg Equilibrium, except for the *Bsm*I polymorphic site.

Table 2 25OHD comparisons in groups

Groups	Unadjusted 25OHD levels*			Season-adjusted 25OHD levels*		
	Mean ± SD	95 % CI	*p*	Mean ± SD	95 % CI	*p*
Patient groups						
All PD	14.0 ± 11.7	12.8–15.2	<0.001**	14.7 ± 11.4	13.6–15.8	<0.001**
$n = 382$				(14.7 ± 11.7)†	(13.5–15.9)†	
Subgroups according to age of onset and familial background						
PD sporadic	13.8 ± 12.3	12.4–15.2	<0.001**	14.6 ± 12.0	13.3–16.0	<0.001**
No familial background				(14.6 ± 12.0)†	(13.2–15.9)†	
$n = 292$						
Age onset >50	14.4 ± 14.1	12.5–16.3	<0.001**	15.4 ± 13.8	13.5–17.3	<0.001**
$n = 205$				(15.0 ± 11.5)†	(13.4–16.7)†	
Age onset <50	12.5 ± 6.3	11.1–13.8	<0.001**	12.9 ± 5.4	11.8–14.1	<0.001**
$n = 87$				(13.7 ± 12.1)†	(11.1–16.3)†	
PD with familial background	14.7 ± 9.4	12.7–16.7	<0.001**	14.9 ± 9.3	12.9–16.8	<0.001**
$n = 90$				(15.2 ± 11.4)†	(12.8–17.6)†	
Control group						
Healthy subjects	19.2 ± 8.6	18.1–20.3		18.6 ± 8.5	17.5–19.6	
$n = 242$				(18.4 ± 12.8)†	(16.8–20.0)†	

* $p < 0.0001$ according to Kruskal–Wallis test

† The age and sex were adjusted mean ± SD and 95 % CI values. When the groups were adjusted for age and sex the significance became $p = 0.003$ and observed power was 93 %

** Compared to healthy subjects according to Dunn's multiple comparison test. The 25OHD levels did not differ between patient groups. The 25OHD level of VDR profile group was given in Supplemental Table 5

According to the *Bsm*I genotyping results for the controls, all three genotypes had very similar frequencies. Moreover, the distribution of the *Bsm*I alleles did not match Hardy–Weinberg equilibrium. Likewise, studies have reported that the B allele is strongly underrepresented in Japanese women and other Asian women, and the results differ from Hardy–Weinberg (Kostner et al. 2009; Lurie et al. 2007).

When PD patients and controls were compared for genotype frequencies of rs7975232 (*Apa*I) within the VDR gene, higher frequencies of "**aa**" and "**Aa**" genotypes were found in the sporadic PD patients and "**AA**" genotypes in healthy subjects (Table 3). When the allelic distributions of *Apa*I were compared, the "**a**" allele was significantly higher in all PD patients. The genotype and allele distributions of VDR gene were given in detail in Table 3.

When PD patients and controls were compared for genotype frequencies of rs7041 within the GC gene, higher frequencies of "**TT**" genotypes were found in the PD patients. The genotype and allele distributions of GC were given in detail in Table 3.

Haplotype Data

There were no significant differences in the frequencies of GC gene haplotypes between any patient and control groups (Supplementary Table 1). The frequency of the "**TCGGC (TaUbF)**" haplotype was nearly significantly higher in all PD groups, PD sporadic group or PD sporadic group with age onset >50, compared to all healthy subjects ($p = 0.07$; 0.06; 0.07, respectively). The frequency of the **TAGGC (TAUbF)** haplotype was extremely low in all PD groups, PD sporadic group or PD sporadic group with age onset >50, compared to all healthy subjects ($p < 0.0001$) (Supplementary Table 2). Removing the most distant SNP (rs2228570, *Fok*I) increased the significance of associated haplotypes. "**TCGG (TaUb)**" haplotype became significantly higher in foretold groups and **TAGG (TAUb)** haplotype remained extremely significant (Supplementary Table 3).

25OHD Levels Comparisons Depending on the VDR or GC Genotypes

Minor allele carriers for rs2282679, rs3755967 and rs2298850 of GC gene in PD sporadic patients with age of onset >50 had significantly lower levels of serum 25OHD compared to homozygous major allele carriers of the corresponding SNPs. Minor allele carriers for rs7041 of GC gene in PD sporadic patients with age of onset <50 had significantly lower levels of serum 25OHD compared to homozygous major allele carriers.

Table 3 Distribution of GC gene rs2282679, rs3755967, rs2298850, rs7041 and VDR gene rs731236 (*Taq*I), rs7975232 (*Apa*I), rs757343 (*Tru*9I), rs1544410 (*Bsm*I), rs2228570 (*Fok*I) genotypes and alleles

GC SNPs	Groups	Genotype			*p* value	Allele		*p* value
		CC	CA	AA		C	A	
rs2282679	All PD ($n = 382$)	220 (57.6 %)	140 (36.6 %)	22 (5.8 %)	0.19	580 (75.9 %)	184 (24.1 %)	0.67
	PD sporadic ($n = 292$)	168 (57.5 %)	106 (36.3 %)	18 (6.2 %)	0.29	442 (75.7 %)	142 (24.3 %)	0.63
	PD sporadic age of onset >50 ($n = 205$)	118 (57.6 %)	72 (35.1 %)	15 (7.3 %)	0.54	308 (75.1 %)	102 (24.9 %)	0.52
	PD sporadic age of onset <50 ($n = 87$)	50 (57.5 %)	34 (39.1 %)	3 (3.4 %)	0.16	134 (77.0 %)	40 (23.0 %)	0.99
	PD with familial background ($n = 90$)	52 (57.8 %)	34 (37.8 %)	4 (4.4 %)	0.29	138 (76.7 %)	42 (23.3 %)	0.93
	Healthy subjects ($n = 226$)	140 (61.9 %)	68 (30.1 %)	18 (8 %)		348 (77.0 %)	104 (23.0 %)	
		GG	GA	AA		G	A	
rs3755967	All PD ($n = 382$)	218 (57.1 %)	142 (37.2 %)	22 (5.8 %)	0.25	578 (75.7 %)	186 (24.3 %)	0.45
	PD sporadic ($n = 292$)	166 (56.8 %)	108 (37.0 %)	18 (6.2 %)	0.32	440 (75.3 %)	144 (24.7 %)	0.41
	PD sporadic age of onset >50 ($n = 205$)	117 (57.1 %)	73 (35.6 %)	15 (7.3 %)	0.53	307 (74.9 %)	103 (25.1 %)	0.36
	PD sporadic age of onset <50 ($n = 87$)	49 (56.3 %)	35 (40.2 %)	3 (3.4 %)	0.18	133 (76.4 %)	41 (23.6 %)	0.77
	PD with familial background ($n = 90$)	52 (57.8 %)	34 (37.8 %)	4 (4.4 %)	0.38	138 (76.7 %)	42 (23.3 %)	0.81
	Healthy subjects ($n = 226$)	140 (62.2 %)	69 (30.7 %)	16 (7.1 %)		349 (77.6 %)	101 (22.4 %)	
		CC	CG	GG		A	G	
rs2298850	All PD ($n = 382$)	219 (57.3 %)	141 (36.9 %)	22 (5.8 %)	0.74	579 (75.8 %)	185 (24.2 %)	0.66
	PD sporadic ($n = 292$)	167 (57.2 %)	107 (36.6 %)	18 (6.2 %)	0.79	441 (75.5 %)	143 (24.5 %)	0.61
	PD sporadic age of onset >50 ($n = 205$)	117 (57.1 %)	73 (35.6 %)	15 (7.3 %)	0.79	307 (74.9 %)	103 (25.1 %)	0.49
	PD sporadic age of onset <50 ($n = 87$)	50 (57.5 %)	34 (39.1 %)	3 (3.4 %)	0.48	134 (77.0 %)	40 (23.0 %)	0.97
	PD with familial background ($n = 90$)	52 (57.8 %)	34 (37.8 %)	4 (4.4 %)	0.70	138 (76.7 %)	42 (23.3 %)	0.95
	Healthy subjects ($n = 226$)	135 (60.0 %)	76 (33.8 %)	14 (6.2 %)		346 (76.9 %)	104 (23.1 %)	
		GG	GT	TT		G	T	
rs7041	All PD ($n = 382$)	125 (32.7 %)	175 (45.8 %)	82 (21.5 %)*	0.043* OR 1.8 95 % CI 1.1–2.8	425 (55.6 %)	339 (44.4 %)	0.09
	PD sporadic ($n = 292$)	100 (34.2 %)	129 (44.2 %)	63 (21.6 %)	0.040* OR 1.8 95 % CI 1.1–2.9	329 (56.3 %)	255 (43.7 %)	0.18
	PD sporadic age of onset >50 ($n = 205$)	70 (34.1 %)	90 (43.9 %)	45 (22.0 %)	0.049* OR 1.8 95 % CI 1.1–3.0	230 (56.1 %)	180 (43.9 %)	0.19
	PD sporadic age of onset <50 ($n = 87$)	30 (34.5 %)	39 (44.8 %)	18 (20.7 %)	0.24	99 (56.9 %)	75 (43.1 %)	0.41
	PD with familial background ($n = 90$)	25 (27.8 %)	46 (51.1 %)	19 (21.1 %)	0.19	96 (53.3 %)	84 (46.7 %)	0.10
	Healthy subjects ($n = 225$)	77 (34.4 %)	117 (52.2 %)	30 (13.4 %)		271 (60.5 %)	177 (39.5 %)	

VDR SNPs	Groups	Genotype			*p* value	Allele		*p* value
		TT (TT)	TC (Tt)	CC (tt)		T (T)	C (t)	
rs731236 (*Taq*I)	All PD ($n = 381$)	154 (40.4 %)	182 (47.8 %)	45 (11.8 %)	0.24	490 (64.3 %)	272 (35.7 %)	0.58
	PD sporadic ($n = 292$)	118 (40.4 %)	142 (48.6 %)	32 (11.0 %)	0.18	378 (64.7 %)	206 (35.3 %)	0.71
	PD sporadic age of onset >50 ($n = 205$)	86 (42.0 %)	98 (47.8 %)	21 (10.2 %)	0.27	270 (65.9 %)	140 (34.1 %)	0.99
	PD sporadic age of onset <50 ($n = 87$)	32 (36.8 %)	44 (50.6 %)	11 (12.6 %)	0.28	108 (62.1 %)	66 (37.9 %)	0.37
	PD with familial background ($n = 89$)	36 (40.4 %)	40 (44.9 %)	13 (14.6 %)	0.72	112 (62.9 %)	66 (37.1 %)	0.48
	Healthy subjects ($n = 240$)	109 (45.4 %)	98 (40.8 %)	33 (13.8 %)		316 (65.8 %)	164 (34.2 %)	

Table 3 continued

		AA (AA)	AC (Aa)	CC (aa)		A (A)	C (a)	
rs7975232 (*Apa*I)	All PD ($n = 381$)	130 (34.1 %)	194 (50.9 %)	57 (15 %)	0.077	454 (59.6 %)	308 (40.4 %)*	0.029* OR 1.3 95 % CI 1.03–1.65
	PD sporadic ($n = 292$)	95 (32.5 %)	152 (52.1 %)	45 (15.4 %)	0.045* OR 1.6 95 % CI 1.0–2.7	342 (58.6 %)	242 (41.4 %)	0.016* OR 1.4 95 % CI 1.1–1.7
	PD sporadic age of onset >50 ($n = 205$)	68 (33.2 %)	101 (49.3 %)	36 (17.6 %)	0.039* OR 1.8 95 % CI 1.1–3.2	237 (57.8 %)	173 (42.2 %)	0.015* OR 1.4 95 % CI 1.1–1.8
	PD sporadic age of onset <50 ($n = 87$)	27 (31.0 %)	51 (58.6 %)	9 (10.3 %)	0.18	105 (60.3 %)	69 (39.7 %)	0.20
	PD with familial background ($n = 89$)	35 (39.3 %)	42 (47.2 %)	12 (13.5 %)	0.71	112 (62.9 %)	66 (37.1 %)	0.49
	Healthy subjects ($n = 241$)	101 (41.9 %)	115 (47.7 %)	25 (10.4 %)		317 (65.8 %)	165 (34.2 %)	

		GG (UU)	GA (Uu)	AA (uu)		G (U)	A (u)	
rs757343 (*Tru*9I)	All PD ($n = 381$)	245 (64.3 %)	119 (31.2 %)	17 (4.5 %)	0.41	609 (79.9 %)	153 (20.1 %)	0.18
	PD sporadic ($n = 292$)	190 (65.1 %)	90 (30.8 %)	12 (4.1 %)	0.33	470 (80.5 %)	114 (19.5 %)	0.13
	PD sporadic age of onset >50 ($n = 205$)	136 (66.3 %)	60 (29.3 %)	9 (4.4 %)	0.31	332 (81.0 %)	78 (19.0 %)	0.12
	PD sporadic age of onset <50 ($n = 87$)	54 (62.1 %)	30 (34.5 %)	3 (3.4 %)	0.64	138 (79.3 %)	36 (20.7 %)	0.47
	PD with familial background ($n = 89$)	55 (61.8 %)	29 (32.6 %)	5 (5.6 %)	0.92	139 (78.1 %)	39 (21.9 %)	0.69
	Healthy subjects ($n = 229$)	136 (59.4 %)	79 (34.5 %)	14 (6.1 %)		351 (76.6 %)	107 (23.4 %)	

		AA (BB)	AG (Bb)	GG (bb)		A (B)	G (b)	
rs1544410 (*Bsm*I)	All PD ($n = 380$)	110 (28.9 %)	134 (35.3 %)	136 (35.8 %)	0.66	354 (46.6 %)	406 (53.4 %)	0.44
	PD sporadic ($n = 292$)	84 (28.8 %)	102 (34.9 %)	106 (36.3 %)	0.76	270 (46.2 %)	314 (53.8 %)	0.54
	PD sporadic age of onset >50 ($n = 205$)	60 (29.3 %)	70 (34.1 %)	75 (36.6 %)	0.84	190 (46.3 %)	220 (53.7 %)	0.55
	PD sporadic age of onset <50 ($n = 87$)	24 (27.6 %)	32 (36.8 %)	31 (35.6 %)	0.76	80 (46.0 %)	94 (54.0 %)	0.71
	PD with familial background ($n = 88$)	26 (29.5 %)	32 (36.4 %)	30 (34.1 %)	0.68	84 (47.7 %)	92 (52.3 %)	0.44
	Healthy subjects ($n = 239$)	67 (28.0 %)	78 (32.6 %)	94 (39.3 %)		212 (44.4 %)	266 (55.6 %)	

		CC (FF)	CT (Ff)	TT (ff)		C (F)	T (f)	
rs2228570 (*Fok*I)	All PD ($n = 382$)	181 (47.4 %)	164 (42.9 %)	37 (9.7 %)	0.75	526 (68.8 %)	238 (31.2 %)	0.47
	PD sporadic ($n = 292$)	133 (45.5 %)	133 (45.5 %)	26 (8.9 %)	0.81	399 (68.3 %)	185 (31.7 %)	0.62
	PD sporadic age of onset >50 ($n = 205$)	96 (46.8 %)	93 (45.4 %)	16 (7.8 %)	0.59	285 (69.5 %)	125 (30.5 %)	0.40
	PD sporadic age of onset <50 ($n = 87$)	37 (42.5 %)	40 (46.0 %)	10 (11.5 %)	0.98	114 (65.5 %)	60 (34.5 %)	0.75
	PD with familial background ($n = 90$)	48 (53.3 %)	31 (34.4 %)	11 (12.2 %)	0.22	127 (70.6 %)	53 (29.4 %)	0.37
	Healthy subjects ($n = 237$)	105 (44.3 %)	107 (45.1 %)	25 (10.5 %)		317 (66.9 %)	157 (33.1 %)	

* Compared with health subjects, Chi-square, *df*: 2 (for genotypes), *df*: 1 (for alleles), significance 2-sided; significance also confirmed with Cramer's V/Kendall's tau-c

Minor allele carriers for *Bsm*I (BB+Bb; B allele is the minor allele in Turkish cohort) of VDR gene in PD patients or healthy subjects had significantly higher levels of serum 25OHD compared to homozygous major allele (bb) carriers. In addition, minor allele carriers for *Taq*I of VDR gene in healthy subjects also had significantly higher levels of serum 25OHD compared to homozygous major allele carriers. Detailed comparisons in subgroups were given in Supplementary Table 4.

LEDD Comparisons Depending on the VDR or GC Genotypes

Minor allele carriers for *Taq*I of VDR gene in PD patients with familial background used significantly lower LEDD compared to homozygous major allele carriers. "aa" carriers for *Apa*I SNP of VDR gene in PD patients with familial background used significantly higher LEDD compared to other genotypes of *Apa*I. Detailed comparisons in subgroups were given in Supplementary Table 4.

Comparisons of Clinical Features Depending on the VDR or GC Genotypes

The homozygous major allele for GC gene rs2282679, rs3755967, rs2298850 SNPs was more frequent in all PD patients with PD duration ≥7 years compared with the patients with PD duration <7 years (Table 4). The significance observed for rs2282679, rs3755967 was originated from the individuals whose disease stage was less than 3 but PD duration ≥7 years ($p = 0.04$, OR 0.4, 95 % CI 0.2–0.9 and $p = 0.06$; respectively). The same trend was observed in PD patients with familial background or sporadic PD patients with age of onset >50 (Table 4). Minor allele carriers for rs7041 of GC gene in PD patients with age of onset >50 displayed mild disease stage (1–2.5), no freezing of gait and no falls compared to major allele carriers (Table 4). When the data set was stratified for PD duration and the disease stage simultaneously, the minor allele for GC gene rs7041 SNP was more frequent in all PD patients, sporadic PD patients with age of onset >50 or PD patients with familial background whose PD duration was <7 years compared with the patients with PD duration ≥7 years. These significant differences did not depend on the disease stage.

Minor allele carriers for *Fok*I of VDR gene were more frequent in all PD patients, sporadic PD patients or PD patients with age of onset >50 with PD duration ≥7 years compared with the patients with PD duration <7 years (Table 4). This significant difference was originated from the individuals whose disease stage was ≥3 and PD duration ≥7 years ($p = 0.016$, OR 2.3, 95 % CI 1.2–4.5; $p = 0.04$, OR 2.2, 95 % CI 1.0–4.6; $p = 0.03$, OR 2.8, 95 % CI 1.1–7.4, respectively). Minor allele carriers for *Apa*I of VDR gene in PD patients with age of onset <50 displayed no freezing of gait compared to major allele carriers. Detailed comparisons in subgroups were given in Table 4.

LEDD Comparisons Depending on the Serum 25OHD Levels

There was no significant correlation between 25OHD levels and LEDD in any patient groups. Data were not given.

Comparisons of Clinical Features Depending on the Serum 25OHD Levels

The 25OHD levels of PD patients without FoG were nearly significantly higher than patients with FoG. PD patients with age of onset <50 who displayed tremor-dominant initial symptom had lower level of serum 25OHD than patients who displayed akinetic-rigid initial symptom or both symptoms (Table 5).

VDR mRNA Expression Levels

The subjects in the *VDR profile group* were investigated to determine their VDR genotypes, their 25OHD levels and their VDR mRNA levels. The VDR mRNA levels were only determined for the *VDR profile group* in order to investigate the possible relationship between VDR mRNA, VDR genotypes and 25OHD. This group included only young healthy people in order to avoid the effects of the natural aging process on metabolism, and it included only educated people in order to control for quality of life.

The relative VDR mRNA expression level of *VDR profile group* was 3.9 ± 1.2 (Min: 1.8; Max: 6.7). There was no correlation between the blood VDR mRNA levels and serum 25OHD levels of *VDR profile group* ($p = 0.85$). There were also no correlation between *Apa*I, *Taq*I, *Tru*9I, *Bsm*I, *Fok*I genotypes and VDR mRNA levels in the same group (Supplementary Table 5). Minor allele carriers for *Bsm*I and "TT" genotype carriers for *Taq*I of VDR gene in *VDR profile group* had higher levels of serum 25OHD. Detailed comparisons were given in Supplementary Table 5.

Discussion

Vitamin D and PD

Vitamin D deficiency is common at all ages, but it is particularly prevalent in elderly people (Pludowski et al.

Table 4 Comparisons of genotype distributions according to clinical features

SNP ID	Genotypes	Initial symptom (frequency)		*p*	PD duration (frequency)		*p*	Disease stage (frequency)		*p*	FoG (frequency)		*p*	Falls (frequency)		*p*
		TD	AR or both		<7 years	≥7 years		<3	≥3		No	Yes		No	Yes	
ALL PD (independent from age of onset and familial background)																
VDR-*Taq*I	TT	0.42	0.39	0.58	0.40	0.41	0.88	0.42	0.41	0.94	0.41	0.42	0.90	0.40	0.42	0.72
	Tt+tt	0.58	0.61		0.60	0.59		0.58	0.59		0.59	0.58		0.60	0.58	
VDR-*Apa*I	AA	0.33	0.35	0.74	0.36	0.32	0.50	0.34	0.35	0.90	0.32	0.40	0.16	0.31	0.37	0.23
	Aa+aa	0.66	0.65		0.64	0.68		0.66	0.65		0.68	0.60		0.69	0.63	
VDR-*Tru*9I	UU	0.65	0.63	0.62	0.65	0.62	0.48	0.64	0.63	0.89	0.65	0.60	0.36	0.66	0.62	0.48
	Uu+uu	0.35	0.37		0.35	0.38		0.36	0.37		0.35	0.40		0.34	0.38	
VDR-*Bsm*I	BB+Bb	0.63	0.66	0.14	0.64	0.65	0.93	0.62	0.66	0.45	0.62	0.68	0.31	0.63	0.65	0.67
	bb	0.37	0.34		0.36	0.35		0.38	0.35		0.38	0.32		0.37	0.35	
VDR-*Fok*I	FF	0.46	0.50	0.45	0.52	0.39	0.01 OR 1.7	0.51	0.41	0.07	0.48	0.43	0.38	0.48	0.45	0.65
	Ff+ff	0.54	0.50		0.48	0.61		0.49	0.59		0.52	0.58		0.52	0.55	
GC-rs2282679	CC	0.58	0.55	0.53	0.53	0.65	0.03 OR 1.6	0.56	0.60	0.39	0.57	0.61	0.53	0.55	0.61	0.24
	CA+AA	0.42	0.45		0.47	0.35		0.44	0.40		0.43	0.39		0.45	0.39	
GC-rs3755967	GG	0.57	0.55	0.64	0.53	0.64	0.03 OR 1.6	0.55	0.60	0.30	0.56	0.61	0.45	0.54	0.61	0.17
	GA+AA	0.43	0.45		0.47	0.36		0.45	0.40		0.44	0.39		0.46	0.39	
GC-rs2298850	CC	0.57	0.56	0.74	0.53	0.64	0.04 OR 1.6	0.55	0.60	0.35	0.56	0.62	0.35	0.54	0.61	0.21
	CG+GG	0.43	0.44		0.47	0.36		0.45	0.40		0.44	0.38		0.46	0.39	
GC-rs7041	GG	0.32	0.34	0.58	0.29	0.41	0.01 OR 1.7	0.28	0.38	0.05 OR 1.6	0.30	0.38	0.13	0.29	0.36	0.12
	GT+TT	0.68	0.66		0.71	0.59		0.72	0.62		0.70	0.62		0.71	0.64	
PD sporadic																
No familial background																
VDR-*Taq*I	TT	0.42	0.37	0.39	0.41	0.38	0.58	0.43	0.40	0.73	0.42	0.40	0.72	0.41	0.42	0.91
	Tt+tt	0.58	0.63		0.59	0.62		0.57	0.60		0.58	0.60		0.59	0.58	
VDR-*Apa*I	AA	0.31	0.34	0.56	0.32	0.33	0.93	0.32	0.33	0.83	0.30	0.38	0.17	0.29	0.35	0.25
	Aa+aa	0.69	0.66		0.68	0.67		0.68	0.67		0.70	0.62		0.71	0.65	
VDR-*Tru*9I	UU	0.66	0.65	0.88	0.65	0.64	0.81	0.66	0.63	0.64	0.66	0.60	0.37	0.67	0.62	0.42
	Uu+uu	0.34	0.35		0.35	0.36		0.34	0.37		0.34	0.40		0.33	0.38	
VDR-*Bsm*I	BB+Bb	0.61	0.69	0.21	0.63	0.66	0.59	0.60	0.66	0.38	0.60	0.69	0.22	0.61	0.65	0.57
	bb	0.39	0.31		0.37	0.34		0.40	0.34		0.40	0.31		0.39	0.35	

Table 4 continued

SNP ID	Genotypes	Initial symptom (frequency)		*p*	PD duration (frequency)		*p*	Disease stage (frequency)		*p*	FoG (frequency)		*p*	Falls (frequency)		*p*
		TD	AR or both		<7 years	≥7 years		<3	≥3		No	Yes		No	Yes	
VDR-*Fok*I	FF	0.43	0.51	0.18	0.50	0.38	0.05	0.51	0.38	0.03	0.47	0.40	0.29	0.48	0.43	0.42
							OR 1.6			OR 1.7						
	Ff+ff	0.57	0.49		0.50	0.62		0.49	0.62		0.53	0.60		0.52	0.57	
GC-rs2282679	CC	0.60	0.53	0.26	0.55	0.63	0.16	0.55	0.61	0.28	0.56	0.63	0.28	0.53	0.62	0.14
	CA+AA	0.40	0.47		0.45	0.37		0.45	0.39		0.44	0.37		0.47	0.38	
GC-rs3755967	GG	0.58	0.53	0.35	0.54	0.62	0.19	0.53	0.61	0.19	0.55	0.63	0.22	0.52	0.62	0.08
	GA+AA	0.42	0.47		0.46	0.38		0.47	0.39		0.45	0.37		0.48	0.38	
GC-rs2298850	CC	0.58	0.54	0.44	0.55	0.62	0.21	0.54	0.61	0.24	0.55	0.64	0.15	0.53	0.62	0.11
	CG+GG	0.42	0.46		0.45	0.38		0.46	0.39		0.45	0.36		0.47	0.38	
GC-rs7041	GG	0.33	0.36	0.54	0.31	0.41	0.08	0.29	0.39	0.09	0.31	0.40	0.16	0.30	0.37	0.19
	GT+TT	0.67	0.64		0.69	0.59		0.71	0.61		0.69	0.60		0.70	0.63	
PD sporadic																
Age onset >50																
VDR-*Taq*I	TT	0.45	0.37	0.25	0.41	0.43	0.79	0.41	0.45	0.65	0.41	0.49	0.36	0.41	0.45	0.59
	Tt+tt	0.55	0.63		0.59	0.57		0.59	0.55		0.59	0.51		0.59	0.55	
VDR-*Apa*I	AA	0.32	0.34	0.80	0.34	0.33	0.91	0.33	0.32	0.80	0.33	0.30	0.70	0.30	0.35	0.45
	Aa+aa	0.68	0.66		0.66	0.67		0.67	0.68		0.67	0.70		0.70	0.65	
VDR-*Tru*9I	UU	0.64	0.70	0.37	0.67	0.64	0.65	0.68	0.63	0.53	0.68	0.58	0.22	0.70	0.61	0.21
	Uu+uu	0.36	0.30		0.33	0.36		0.32	0.37		0.32	0.42		0.30	0.39	
VDR-*Bsm*I	BB+Bb	0.59	0.72	0.06	0.64	0.64	0.99	0.61	0.65	0.65	0.62	0.65	0.69	0.61	0.65	0.62
	bb	0.41	0.28		0.36	0.36		0.39	0.35		0.38	0.35		0.39	0.35	
VDR-*Fok*I	FF	0.41	0.56	0.04	0.52	0.34	0.02	0.50	0.40	0.17	0.45	0.47	0.87	0.49	0.42	0.39
				OR 1.8			OR 2.1									
	Ff+ff	0.59	0.44		0.48	0.66		0.50	0.60		0.55	0.53		0.51	0.58	
GC-rs2282679	CC	0.59	0.55	0.59	0.53	0.67	0.07	0.55	0.63	0.26	0.56	0.67	0.16	0.52	0.65	0.09
	CA+AA	0.41	0.45		0.47	0.33		0.45	0.37		0.44	0.33		0.48	0.35	
GC-rs3755967	GG	0.58	0.55	0.67	0.53	0.67	0.05	0.54	0.63	0.21	0.55	0.67	0.14	0.52	0.65	0.06
							OR 1.8									
	GA+AA	0.42	0.45		0.47	0.33		0.46	0.37		0.45	0.33		0.48	0.35	
GC-rs2298850	CC	0.58	0.55	0.67	0.53	0.66	0.11	0.55	0.62	0.34	0.55	0.67	0.14	0.52	0.64	0.12
	CG+GG	0.42	0.45		0.47	0.34		0.45	0.38		0.45	0.33		0.48	0.36	
GC-rs7041	GG	0.32	0.38	0.39	0.29	0.48	0.008	0.27	0.43	0.02	0.29	0.49	0.02	0.27	0.41	0.04
							OR 2.3			OR 2.0			OR 2.3			OR 1.9
	GT+TT	0.68	0.62		0.71	0.52		0.73	0.57		0.71	0.51		0.73	0.59	

Table 4 continued

SNP ID	Genotypes	Initial symptom (frequency)		*p*	PD duration (frequency)		*p*	Disease stage (frequency)		*p*	FoG (frequency)		*p*	Falls (frequency)		*p*
		TD	AR or both		<7 years	≥7 years		<3	≥3		No	Yes		No	Yes	
PD sporadic																
Age onset <50																
VDR-*Taq*I	TT	0.34	0.38	0.72	0.42	0.31	0.27	0.46	0.33	0.23	0.46	0.27	0.09	0.42	0.36	0.59
	Tt+tt	0.66	0.62		0.58	0.69		0.54	0.67		0.54	0.73		0.58	0.64	
VDR-*Apa*I	AA	0.28	0.35	0.46	0.29	0.33	0.65	0.26	0.35	0.38	0.19	0.50	0.004	0.25	0.36	0.31
													OR 4.3			
	Aa+aa	0.72	0.65		0.71	0.67		0.74	0.65		*0.81	0.50		0.75	0.64	
VDR-*Tru*9I	UU	0.70	0.54	0.13	0.60	0.64	0.68	0.60	0.63	0.80	0.60	0.63	0.79	0.58	0.64	0.59
	Uu+uu	0.30	0.46		0.40	0.36		0.40	0.37		0.40	0.37		0.42	0.36	
VDR-*Bsm*I	BB+Bb	0.68	0.62	0.57	0.60	0.69	0.37	0.57	0.67	0.35	0.56	0.73	0.13	0.61	0.64	0.77
	bb	0.32	0.38		0.40	0.31		0.43	0.33		0.44	0.27		0.39	0.36	
VDR-*Fok*I	FF	0.47	0.41	0.57	0.42	0.43	0.95	0.54	0.35	0.08	0.52	0.30	0.05	0.44	0.43	0.89
													OR 2.5			
	Ff+ff	0.53	0.59		0.58	0.57		0.46	0.65		0.48	0.70*		0.56	0.57	
GC-rs2282679	CC	0.62	0.49	0.23	0.58	0.57	0.95	0.54	0.58	0.73	0.56	0.57	0.97	0.56	0.57	0.89
	CA+AA	0.38	0.51		0.42	0.43		0.46	0.42		0.44	0.43		0.44	0.43	
GC-rs3755967	GG	0.60	0.49	0.32	0.58	0.55	0.77	0.51	0.58	0.55	0.54	0.57	0.83	0.53	0.57	0.70
	GA+AA	0.40	0.51		0.42	0.45		0.49	0.42		0.46	0.43		0.47	0.43	
GC-rs2298850	CC	0.60	0.51	0.45	0.58	0.57	0.95	0.51	0.61	0.42	0.54	0.60	0.61	0.53	0.60	0.55
	CG+GG	0.40	0.49		0.42	0.43		0.49	0.39		0.46	0.40		0.47	0.40	
GC-rs7041	GG	0.34	0.32	0.88	0.38	0.31	0.50	0.34	0.30	0.70	0.35	0.27	0.42	0.36	0.29	0.48
	GT+TT	0.66	0.68		0.62	0.69		0.66	0.70		0.65	0.73		0.64	0.71	
PD with familial background																
VDR-*Taq*I	TT	0.39	0.44	0.68	0.35	0.50	0.18	0.40	0.46	0.60	0.38	0.50	0.35	0.39	0.45	0.60
	Tt+tt	0.61	0.56		0.65	0.50		0.60	0.54		0.62	0.50		0.61	0.55	
VDR-*Apa*I	AA	0.41	0.38	0.74	0.45	0.29	0.14	0.40	0.42	0.84	0.38	0.45	0.58	0.37	0.45	0.49
	Aa+aa	0.59	0.62		0.55	0.71		0.60	0.58		0.62	0.55		0.63	0.55	
VDR-*Tru*9I	UU	0.64	0.56	0.46	0.65	0.54	0.30	0.60	0.65	0.64	0.64	0.60	0.78	0.63	0.62	0.94
	Uu+uu	0.36	0.44		0.35	0.46		0.40	0.35		0.36	0.40		0.37	0.38	
VDR-*Bsm*I	BB+Bb	0.70	0.58	0.28	0.70	0.61	0.41	0.65	0.65	0.96	0.66	0.65	0.93	0.66	0.65	0.96
	bb	0.30	0.42		0.30	0.39		0.35	0.35		0.34	0.35		0.34	0.35	
VDR-*Fok*I	FF	0.56	0.47	0.40	0.60	0.41	0.09	0.51	0.56	0.69	0.51	0.52	0.90	0.48	0.57	0.45
	Ff+ff	0.44	0.53		0.40	0.59		0.49	0.44		0.49	0.48		0.52	0.43	

Table 4 continued

SNP ID	Genotypes	Initial symptom (frequency)		*p*	PD duration (frequency)		*p*	Disease stage (frequency)		*p*	FoG (frequency)		*p*	Falls (frequency)		*p*
		TD	AR or both		<7 years	≥7 years		<3	≥3		No	Yes		No	Yes	
GC-rs2282679	CC	0.54	0.63	0.46	0.50	0.72	0.04 OR 2.6	0.58	0.56	0.82	0.60	0.52	0.52	0.59	0.57	0.82
	CA+AA	0.46	0.37		0.50	0.28		0.42	0.44		0.40	0.48		0.41	0.43	
GC-rs3755967	GG	0.54	0.63	0.46	0.50	0.72	0.04 OR 2.6	0.58	0.56	0.82	0.60	0.52	0.52	0.59	0.57	0.82
	GA+AA	0.46	0.37		0.50	0.28		0.42	0.44		0.40	0.48		0.41	0.43	
GC-rs2298850	CC	0.54	0.63	0.46	0.50	0.72	0.04 OR 2.6	0.58	0.56	0.82	0.60	0.52	0.52	0.59	0.57	0.82
	CG+GG	0.46	0.37		0.50	0.28		0.42	0.44		0.40	0.48		0.41	0.43	
GC-rs7041	GG	0.28	0.28	0.99	0.22	0.41	0.05 OR 2.5	0.26	0.33	0.45	0.27	0.33	0.58	0.26	0.33	0.47
	GT+TT	0.72	0.72		0.78	0.59		0.74	0.67		0.73	0.67		0.74	0.67	

Chi-square; *df*: 1; significance 2-sided; significance also confirmed with Cramer's V/Kendall's tau-c

2014). Sato et al. showed that Japanese women with PD had very low 25OHD levels (Sato et al. 1997) and suggested that low 25OHD levels and high hip fracture incidence were common in PD patients (Sato et al. 2001). Evatt et al. (2008) showed similar results in a European Caucasian population. An association between high serum 25OHD levels and a reduced risk of PD has been demonstrated in a longitudinal study of the Finnish population (Knekt et al. 2010). Negative correlations between serum 25OHD levels and PD severity were also reported (Sato et al. 1997, 2005; Meamar et al. 2015). Moghaddasi et al. (2013) suggested a reverse association between the duration of PD and 25OHD levels. Although the biological relevance is not clear, vitamin D was suggested to alter cholinergic, dopaminergic and noradrenergic neurotransmitter systems in central nervous system (CNS) (Eyles et al. 2013). This effect is maintained by the regulation of choline acetyltransferase enzyme (Sonnenberg et al. 1986), cholinergic receptors (Kumar et al. 2011), dopamine 1 (DA1) and DA2 receptors (Peeyush et al. 2010; Peeyush Kumar et al. 2011), tyrosine hydroxylase enzyme (Smith et al. 2006; Cass et al. 2012) via VDR (Gezen-Ak et al. 2014).

Our results indicated a strong relationship between low serum 25OHD levels and PD, independent from age and sex. These results support previous studies that have reported significantly lower levels of 25OHD in individuals suffering from PD, AD, mood disorders and cognitive decline (Annweiler et al. 2010; Cherniack et al. 2008; Evatt et al. 2008; Llewellyn et al. 2009, 2010; McCann and Ames 2008; Wilkins et al. 2006; Knekt et al. 2010; Sato et al. 1997, 2001, 2005; Meamar et al. 2015).

Vitamin D Binding Protein (GC) and PD

GC is responsible for binding, solubilization and transport of vitamin D and its metabolites (Delanghe et al. 2015; Bhan 2014). GC rs7041 SNP was reported to be associated with serum GC levels and 25OHD affinity (Bhan 2014). A GWA study indicated that GC rs2282679 was associated with serum 25OHD levels in individuals of European descent of 15 cohorts (Wang et al. 2010). Given that we investigated GC SNPs in order to understand whether low levels of 25OHD that were seen in PD are associated with any SNPs in GC or not? Our results indicated that "**TT**" genotype of rs7041 SNP in GC gene was more frequent in all PD patients. The subgroups pointed out that there was no association between this genotype and PD patients with familial background or sporadic PD patients with age of onset younger than 50. This situation might be due to the low number of patients included in these groups. Besides, there was no association between any GC haplotype and PD. To our knowledge, there is no other study investigating GC SNPs in PD patients.

Table 5 Comparisons of serum 25OHD levels according to the clinical features of the disease

	Initial symptom (frequency)		*p*	PD duration (frequency)		*p*	Disease stage (frequency)		*p*
	TD	AR or both		<7 years	≥7 years		<3	≥3	
ALL PD (independent from age of onset and familial background) 25OHD Mean ± SD	14.7 ± 13.0 (0.63)	14.6 ± 8.3 (0.37)	0.96	14.8 ± 11.9 (0.66)	14.5 ± 10.3 (0.34)	0.85	15.3 ± 13.1 (0.58)	14.4 ± 9.7 (0.42)	0.45
PD sporadic No familial background 25OHD Mean ± SD	14.7 ± 14.1 (0.62)	14.4 ± 7.7 (0.38)	0.81	14.8 ± 12.7 (0.66)	14.3 ± 10.6 (0.34)	0.75	15.7 ± 14.3 (0.55)	13.6 ± 9.7 (0.45)	0.17
PD sporadic Age onset >50 25OHD Mean ± SD	15.8 ± 15.9 (0.65)	14.5 ± 8.8 (0.35)	0.54	15.5 ± 14.2 (0.72)	15.2 ± 13.1 (0.28)	0.89	16.7 ± 16.0 (0.59)	14.0 ± 11.5 (0.41)	0.21
PD sporadic Age onset <50 25OHD Mean ± SD	*11.8 ± 5.2 (0.56)	14.1 ± 5.1 (0.44)	0.05 (after Welch 0.05)	12.7 ± 5.2 (0.52)	13.2 ± 5.5 (0.48)	0.65	12.4 ± 5.7 (0.45)	12.8 ± 5.0 (0.55)	0.73
PD with familial background 25OHD Mean ± SD	14.6 ± 9.0 (0.64)	15.5 ± 10.1 (0.36)	0.67	14.6 ± 9.3 (0.67)	15.2 ± 9.5 (0.33)	0.78	14.4 ± 9.2 (0.67)	17.8 ± 9.4 (0.33)	0.13

	FoG (frequency)		*p*	Falls (frequency)		*p*
	No	Yes		No	Yes	
ALL PD (independent from age of onset and familial background) 25OHD Mean ± SD	15.5 ± 12.9 (0.73)	13.4 ± 7.5 (0.27)	0.14 (after Welch 0.06)	14.4 ± 9.1 (0.55)	15.5 ± 14.3 (0.45)	0.37
PD sporadic No familial background 25OHD Mean ± SD	15.4 ± 13.9 (0.72)	12.9 ± 7.3 (0.28)	0.15 (after Welch 0.06)	14.5 ± 9.2 (0.52)	15.0 ± 15.3 (0.48)	0.73
PD sporadic Age onset >50 25OHD Mean ± SD	16.4 ± 15.6 (0.77)	13.1 ± 8.8 (0.23)	0.19 (after Welch 0.08)	15.1 ± 10.0 (0.55)	16.2 ± 18.4 (0.45)	0.59
PD sporadic Age onset <50 25OHD Mean ± SD	12.6 ± 5.9 (0.62)	12.8 ± 4.4 (0.38)	0.90	12.7 ± 6.3 (0.46)	12.6 ± 4.4 (0.54)	0.92
PD with familial background 25OHD Mean ± SD	15.6 ± 9.6 (0.75)	14.8 ± 8.4 (0.25)	0.72	14.2 ± 9.1 (0.64)	17.7 ± 9.3 (0.36)	0.10

The patient with initial predominant symptom akinetic rigid was combined with the patients who have both symptoms due to the low number of patients with both symptoms. The 25OHD comparision depending on the course of the predominant symptoms (TT, TB and BB) in groups did not differ, and the comparisons were repeated as TT+TB versus BB and as TT versus TB+BB. The 25OHD level of TT+TB was significantly lower than BB patients only in PD sporadic age of onset <50 group ($p = 0.04$ after Welch); data were not given

TD, tremor dominant; AR, akinetic rigid; both, TD+AR; TT, tremor-dominant onset with ongoing tremor-dominant symptoms; TB, tremor-dominant onset with ongoing bradykinesia symptoms; BB, bradykinesia-dominant onset with ongoing bradykinesia-dominant symptoms

Although only rs7041 of GC was associated with the PD risk, the minor allele carriers for rs2282679, rs3755967 and rs2298850 of GC gene in sporadic PD patients with age of onset >50 had significantly lower levels of serum 25OHD compared to homozygous major allele carriers. Besides, the homozygous major allele for GC gene rs2282679, rs3755967, rs2298850 SNPs were associated with higher serum 25OHD levels and the same genotypes were associated with PD especially in the patients with age of onset >50 whose disease stage was less than 3 and PD duration ≥7 years. Thus, these genotypes might be responsible for slower progression of the disease by enabling higher levels of serum 25OHD.

Although "TT" genotype of rs7041 of GC gene might be a risk factor, the minor allele carriers (GT+TT) in PD patients with age of onset >50 displayed mild disease stage (<3), no freezing of gait and no falls. The situation is consistent regarding the clinical features of mild stage. When the data set stratified for PD duration and the disease stage simultaneously, the minor allele was seen more frequent in patients with PD duration <7 years, but the association did not depend on the disease stage. This indicated no relation of rs7041 SNP with the disease progression. We assume that the risk of having GC rs7041 genotype and low levels of vitamin D due to remaining GC SNPs might be two different mechanisms in PD.

Vitamin D Receptor (VDR) and PD

The first evidence for genetic risk factors for AD and VDR *Apa*I polymorphism was provided by our studies (Gezen-Ak et al. 2012, 2007). Beecham et al. (2009) and Butler et al. (2011) reported supporting results. Besides, the *Fok*I C allele (or "F") was reported to be significantly more common in PD patients in the Hungarian (Torok et al. 2013) and Chinese Han populations (Han et al. 2012). Suzuki et al. suggested that higher 25OHD levels and the VDR *Fok*I CC (FF) genotype might be independently associated with milder forms of PD (Suzuki et al. 2012) and that vitamin D_3 supplementation might stabilize PD for a short period in patients with *Fok*I TT (ff) or CT (Ff) genotypes (Suzuki et al. 2013). Liu et al. (2013) showed that the *Taq*I "T" allele of VDR gene was associated with male PD patients. Kim et al. (2005) showed that the "b" allele of the *Bsm*I SNP was significantly more common in Korean PD patients. Negative results were also observed in some other populations (Lv et al. 2013).

Our study indicated that having an *Apa*I (rs7975232) "a" allele significantly increases the risk of developing PD in all PD groups irrespective of age, age of onset or familial background. Yet subgroups indicated that this association was originated from sporadic PD patients with age of onset older than 50. There was no association between clinical features of the disease and *Apa*I genotypes in these groups. On the other hand, minor allele carriers for *Fok*I of VDR gene were more frequent in all PD patients, sporadic PD patients or PD patients with age of onset >50 whose disease stage was ≥3 and PD duration ≥7 years. These results support Suzuki et al. studies (Suzuki et al. 2012, 2013). We should note that the only SNP that changes the protein structure of VDR is known to produce an elongated form of VDR and is *Fok*I site in exon 2 of the VDR gene (Uitterlinden et al. 2004).

The possible functional contribution of VDR genotypes to PD was observed in drug doses. Minor allele carriers for *Taq*I of VDR gene used significantly lower LEDD, whereas "aa" carriers for *Apa*I SNP of VDR gene used significantly higher LEDD. This statement was viable only for PD patients with familial background in our study. Besides, there was no relation between 25OHD levels and LEDD in any patient groups.

Surprisingly, minor allele carriers for *Bsm*I of VDR gene both *in PD patients* and *healthy subjects* had significantly higher levels of serum 25OHD compared to homozygous major allele carriers. This might indicate the effect of VDR on vitamin D levels depending on the transcriptional regulation of the vitamin D metabolism-related enzymes. Additionally, *Taq*I minor allele carriers in healthy subjects also had significantly higher levels of serum 25OHD. These results also confirmed in an independent healthy group (*VDR profile group*) although the sample size is low.

VDR Haplotypes and PD

We found that the "**TaubF**" haplotype is a risk factor for AD in our previous study (Gezen-Ak et al. 2012). Our present study shows that the "**TaUbF**" haplotype was nearly significantly higher in all PD patients. The two haplotypes differ only for the *Tru*9I SNP (U/u). "**TaUb**" haplotype became significant risk factor for PD after we excluded the farthest SNP (*Fok*I). However, the results of both of our studies indicate that "a" is a risk factor. The differences between significant haplotypes of our AD and PD studies might be due to the differences in sample sizes. The "**TAUbF**" haplotype was a very significant protective haplotype in the present study. Although Gatto et al. (2015) showed a significant association for the *Apa*I SNP and PD in non-Hispanic Caucasians, they found that the "a" allele is a protective factor. This discrepancy might be due to the other genetic properties of the two different populations. The *1000 Genomes Browser* provides a data set of VDR SNPs in *Homo sapiens* GRCh37. p13. The data set shows a higher frequency of "a" and lower frequency of "A" alleles for the *Apa*I SNPs in eastern Asian populations, whereas it shows a higher frequency of "A" and lower frequency of "a" alleles or similar frequencies for both alleles in most of the other populations. The Turkish population has the "a" allele as a minor allele, like Utah residents with northern and western European ancestry, who had a similar allelic distribution on the 1000 Genomes Browser. (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/?chr=NC_000012.11&from=48238337&to=48239337&mk=48238837:48238837|rs7975232&gts=rs7975232).

In contrast to the other studies, the patient and control groups of the Turkish cohort did not differ for the other four SNPs of VDR (*Taq*I, *Bsm*I, *Fok*I, *Tru*9I). One explanation for these differing results came from the Alkan et al. (2014) study about the genetic background of the Turkish population. Alkan et al. showed that the genetic variation of the contemporary Turkish population is best described within the context of the Southern European/Mediterranean gene pool. They suggested that rare and private genetic variation in Turkey might have more functional impact than variation shared among populations (Alkan et al. 2014).

VDR SNPs and VDR mRNA Levels

Because we found very similar haplotypes in the PD and AD groups (Gezen-Ak et al. 2012), we tried to understand

the reason for the association. *Bsm*I, *Tru*9I and *Apa*I restriction enzymes recognize the SNPs located in intron 8, which have strong linkage disequilibrium with the polymorphisms in the 3′ untranslated region, which is thought to regulate VDR mRNA expression (Uitterlinden et al. 2004). Thus, VDR genotypes were compared with VDR mRNA levels in the VDR profile group but no relationship was observed.

Conclusion

Consequently, this is the first study demonstrating the association between GC rs7041 "TT" genotype and PD. We suggest that VDR *Apa*I allelic variations are related to both PD and AD, especially the "A"-containing VDR haplotypes, which are protective, and the "a"-containing haplotypes, which confer risk of neurodegenerative disorders in the Turkish population. The present study also shows that VDR or GC variations are related to serum 25OHD levels in PD or healthy subjects, and low levels of 25OHD are strongly associated with PD in the Turkish population. The relations we observed between the clinical features of PD patients and VDR or GC genotypes and low 25OHD levels might suggest that low 25OHD and risk genotypes have effect on PD independently.

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Compliance with Ethical Standards

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

Ethical approval Participants in the present study were treated according to the ethical principles for medical research involving human participants described in the World Medical Association's Declaration of Helsinki, and the study was approved by the Ethics Committee of Istanbul University.

Informed consent Signed informed consent was obtained from all study participants.

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