scientific reports

OPEN

A case–control study on vitamin D receptor gene polymorphisms in patients with Parkinson's disease in Bangladesh

Narayan Chandra Kundu[1✉], Anindya Kundu[2], Md. Ibrahim Khalil[1], K. M. Nazmul Islam Joy[1], Moushumi Sen[3], Zahid Hasan[4], Md. Sahabuddin[5], Md. Abdur Rafi[6] & Mohammad Jahid Hasan[7✉]

Parkinson's disease (PD) is a neurodegenerative disorder with a multifactorial etiology. This study aimed to investigate the association between vitamin D receptor (VDR) gene polymorphisms (ApaI, BsmI, FokI and TaqI) and the risk of PD in a Bangladeshi population. A case-control study was conducted with 100 PD patients and 100 age- and sex-matched healthy controls. Serum vitamin D levels were measured using a chemiluminescent immunoassay, and VDR gene polymorphisms were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Genetic models (allele, dominant, recessive and additive models) were used to assess the association between each polymorphism and PD risk. The mean age of the patients with PD was 63 years, with 65% being male, while the control group had a mean age of 54.5 years and 60% were male. In genetic models, the T allele of the ApaI gene demonstrated a significant association with PD (OR 1.92, 95% CI 1.20–3.13, p-value 0.007). This significant association persisted across both recessive and additive models (for recessive model: OR 2.17, 95% CI 1.10–4.55, p-value 0.027 and for additive model: OR 2.78, 95% CI 1.22–6.67, p-value 0.015). Similarly, the T allele of the FokI gene was found to be significantly associated with PD (OR 2.27, 95% CI 1.43–3.57, p-value 0.001). This association was also evident in both dominant and additive models (for dominant model: OR 2.56, 95% CI 1.45–4.55, p-value 0.001 and for additive model: OR 3.03, 95% CI 1.67–5.56, p-value 0.001). Conversely, no significant associations were observed for the genetic polymorphisms of the BsmI and TaqI genes across any of the genetic models examined. The findings suggest that specific VDR gene polymorphisms, particularly ApaI and FokI, are significantly associated with the risk of PD in the Bangladeshi population.

Keywords Parkinson's disease, VDR gene polymorphisms, Vitamin D, Neurodegenerative disorders, Bangladesh

Abbreviations

PD	Parkinson's disease
VDR	vitamin D receptor
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
UPDRS	Unified Parkinson Disease Rating Scale
MAF	Minor allele frequency
SNP	single nucleotide polymorphisms
HWE	Hardy-Weinberg Equilibrium

As the global life expectancy continues to rise, aging-related diseases, like neurodegenerative disorders, are emerging as major causes of disability. Parkinson's disease (PD) is one of the common disorders among these with

[1]Department of Neurology, Shaheed Suhrawardy Medical College, Dhaka 1207, Bangladesh. [2]University of Western Australia, Perth, WA 6009, Australia. [3]Department of Biochemistry, Anwer Khan Modern Medical College, Dhaka 1205, Bangladesh. [4]Bangladesh University of Health Science, Dhaka 1216, Bangladesh. [5]Bangladesh Specialized Hospital, Dhaka 1207, Bangladesh. [6]Pi Research & Development Center, Dhaka 1100, Bangladesh. [7]Tropical Disease and Health Research Center, Dhaka 1100, Bangladesh. ✉email: kunduneuro@yahoo.com; dr.jahid61@gmail.com

an annual number of new cases being more than six million worldwide[1,2]. This incidence is disproportionately increasing in low- and middle-income countries compared to the higher income countries like Bangladesh[3]. Although population-level prevalence data on PD in Bangladesh are limited, the estimated burden is unlikely to be negligible. The prevalence of PD in the Southeast Asia region is reported to be 94 per 100,000 population[4], while the neighboring country, India, has an estimated prevalence of 80 per 100,000 population[5]. Given the increasing life expectancy in Bangladesh, the incidence of PD is expected to rise in the coming years.

PD is a chronic, progressive neurodegenerative disorder, predominantly affecting the middle-aged and elderly individuals[1]. It primarily results from the degeneration of dopaminergic neurons in the substantia nigra of the midbrain and the accumulation of Lewy bodies—neuronal inclusions mainly composed of α-synuclein protein aggregates[6]. Clinically, PD manifests as a syndrome principally marked by motor symptoms such as bradykinesia, postural instability, rigidity, and resting tremor[7]. Though, the exact etiology of PD remains unclear, it is widely evident to be multifactorial, involving complex interactions between genetic and environmental factors, oxidative stress, mitochondrial dysfunction, inflammation, and immune dysregulation[1].

Among the genetic factors, VDR genes, which encodes the vitamin D receptor (VDR)—a nuclear receptor that mediates the action of vitamin D—may play a role in the development of PD[8,9]. Single nucleotide polymorphisms (SNPs) in the VDR gene, which can impact its function and expression, have been suggested to be associated with PD. Several SNPs in the VDR gene, such as ApaI (rs7975232), BsmI (rs1544410), TaqI (rs731236), and FokI (rs2228570), have been studied for their possible role in PD risk. For instance, a study on Korean population identified the BsmI polymorphism as a possible allele contributing to PD[10]. Studies from Hungary, Japan, and China have found that the FokI polymorphism is significantly linked to PD[11–13]. Additionally, a case-control study in the USA showed that FokI was associated with cognitive decline in PD patients[14]. On the other hand, some studies suggest that the TaqI TT genotype and ApaI GG genotype might offer protection against PD[15]. These SNPs can affect VDR in several ways, such as changing protein length, modifying binding site affinity, and altering receptor expression, which in turn may affect vitamin D function and lead to neuron degeneration and development of PD[16].

Despite the extensive research conducted on VDR gene polymorphisms and their association with PD across different populations, studies focusing on the Bangladeshi population remain scarce. This population may exhibit distinct genetic characteristics in the VDR gene polymorphisms compared to populations from other regions of the world. Genetic diversity varies between populations due to differences in ancestry, environmental factors, and evolutionary pressures. South Asians generally have a higher prevalence of vitamin D deficiency compared to other populations[17], which might be linked to genetic factors such as VDR polymorphisms. For example, studies on Indian populations have found different frequencies of VDR gene SNPs (like FokI, BsmI, and TaqI) compared to European or East Asian populations[18], suggesting that Bangladeshi individuals may also exhibit unique variations. While studies in different populations have identified significant associations between certain VDR gene polymorphisms—such as BsmI, FokI, ApaI, and TaqI—and PD, there is limited research on the Bangladeshi population. Therefore, the study of these polymorphisms in the Bangladeshi population is crucial for understanding whether these genetic variations contribute similarly to PD susceptibility or offer new insights. Identifying population-specific associations could help tailor preventative and therapeutic strategies to the unique genetic makeup of Bangladeshis. Hence, the present study aimed to investigate the association between PD and polymorphisms in VDR genes, specifically ApaI (rs7975232), BsmI (rs1544410), FokI (rs10735810), and TaqI (rs731236) in Bangladesh.

Methods
Study design and setting
This case-control study was conducted in the Department of Neurology at Shaheed Suhrawardy Medical College and Hospital, a tertiary care facility located in Dhaka, the capital city of Bangladesh, from January 2023 to December 2023. Being a tertiary referral center in a densely populated metropolitan area, the hospital serves a diverse population from various regions of the country. Although the study primarily included participants who sought care at this hospital, the wide catchment area likely provided a heterogeneous sample reflective of the broader Bangladeshi population.

Participants
A total of 200 individuals were included in the present study (100 individuals diagnosed with PD as cases and 100 individuals without diagnosis of PD as controls). Sample size was calculated from the assumption of prevalence of *FokI* genotype TT, which is a significant risk factor for PD at 16.6% in the control group as reported by Hu et al. (2020)[9], for detecting an odds' ratio (OR) of 2.5, with 80% power and 95% level of significance. From these assumptions, the calculated sample size was 107 in each group. However, after exclusion of incomplete data for gene analysis, a total of 200 individuals (100 cases and 100 controls) were included in the final analysis.

A purposive sampling technique according to the inclusion and exclusion criteria was used to include the participants.

The inclusion criteria for cases were adult individuals aged between 18 and 80 years who were clinically diagnosed as PD according to the Movement Disorder Society Clinical Diagnostic Criteria[19]. While PD predominantly affects middle-aged and elderly individuals, we considered the younger group also in our study, particularly the age group of (18 to 30) as a more generalist approach, to account for potential cases of early-onset PD, a recognized subtype of PD that can present in younger adults. However, no individuals from this age group could be recruited in the present study. Patients with severe disease requiring hospital admission and comorbid with other neurological or psychiatric diseases were excluded from the study. On the other hand, individuals without PD matched for age (+/-5 years) were included as controls.

Ethical consideration

The study protocol was reviewed and approved by Institutional review board of Shaheed Suhrawardy Medical College and Hospital, Dhaka, Bangladesh (**ShSMC/Ethical/2023/063**). The all authors declare no human subjects were harmed and the procedures followed were in accordance with the ethical standards and regulations established by the Helsinki Declaration of the World Medical Association. Informed written consent was obtained from all the participants involved in the study.

Data collection

After inclusion in the study as cases or controls according to the eligibility criteria, an informed written consent was obtained from the participants. After that, a face-to-face interview was conducted by the attending neurologist using a structured questionnaire. The questionnaire included sociodemographic information such as age, sex, socioeconomic status, comorbidities, vitamin D supplementation, and exposure to sunlight related information. A sun exposure index[20] was utilized to estimate the amount and duration of sunlight exposure. This data was collected by asking subjects to illustrate their typical skin exposure on a diagram. The rule of nines was then applied to calculate sunlight exposure, assigning 18% to the front and back torso and each leg, 9% to the arms and head, and 5% to the face alone[21]. This figure was subsequently multiplied by the reported average sun exposure per week without sunscreen to determine the sun exposure index for each subject[20]. For the patients with PD, Hoehn and Yahr stage of PD[22] and the Unified Parkinson Disease Rating Scale (UPDRS) was also used to assess the level of disability[23].

Laboratory procedure

After the interview, a 5 ml blood sample was collected using standard procedures. Samples were centrifuged promptly to separate serum from cellular components. Serum samples intended for the analysis of vitamin D and calcium levels were stored at -4 °C temporarily and were assayed within 24 h of collection to minimize the risk of analyte degradation. For VDR gene polymorphism analysis, the remaining serum was stored at an optimum temperature of -80 °C for approximately four weeks to preserve its integrity for genetic analysis.

Analysis of serum vitamin D level

To assess the serum vitamin D levels in cases and controls, a competitive immunoassay technique was employed, which involved the release of 25-OH Vitamin D from the binding protein in the sample using a low pH denaturant. Anti-vitamin D was bound to the wells, and unbound materials were removed through washing. The bound HRP conjugate was then measured by a luminescent reaction[24]. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) along with an electron transfer agent was added to the wells. The HRP in the bound conjugate catalyzed the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) enhanced the light signal and prolonged its emission. The light signals were read by the system, with the amount of HRP conjugate bound being inversely proportional to the concentration of 25-OH D present. Vitamin D levels were categorized as deficient (< 20 ng/ml), insufficient (20–<30 ng/ml), and sufficient (> 30 ng/ml)[25].

Detection of VDR gene SNPs

A number of four functional SNPs from regulatory regions of the VDR genes was selected based on their reported association with Parkinson's disease and their location in the gene and minor allele frequency (MAF). These VDR SNPs were ApaI (rs7975232) [G > T], TaqI (rs731236) [T > C], BsmI (rs1544410) [A > G] and FokI (rs2228570) [C > T].

For the analysis of VDR gene SNPs, genomic DNA was first extracted from the collected whole blood samples using the FavorPrep Blood Genomic Extraction Mini Kit (Favorgen Biotech Corp., Taiwan)[26]. The concentration and purity of the extracted DNA were assessed via agarose gel electrophoresis, confirming its suitability for subsequent PCR amplification.

PCR was performed to amplify the regions of the VDR gene containing the polymorphic sites of interest: ApaI (rs7975232, G > T), TaqI (rs731236, T > C), BsmI (rs1544410, A > G), and FokI (rs2228570, C > T). Each reaction was carried out in a 15 µl volume containing 4.0 µl of DNA (10–50 ng/ml), 1.5 µl of 10x buffer, 0.12 µl of 200 µmol dNTPs, 0.7 µl of forward and reverse primers (10 µmol each), 0.105 µl of Hot Start Taq DNA polymerase (5 U/µl), and 7.875 µl of ddH2O. The specific primers used were as follows: ApaI (Forward: 5'-CAG AGC ATG GAC AGG GAG CAA G-3', Reverse: 5'-GCA ACT CCT CAT GGC TGA GGT CTC A-3'), TaqI (Forward: 5'-CAG AGC ATG GAC AGG GAG CAA G-3', Reverse: 5'-GCA ACT CCT CAT GGC TGA GGT CTC A-3'), BsmI (Forward: 5'-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3', Reverse: 5'-AAC CAG CGG GAA GAG GTC AAG GG-3'), and FokI (Forward: 5'-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3', Reverse: 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3')[27,28].

PCR cycling conditions included an initial denaturation at 94 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 45 s, and elongation at 72 °C for 45 s. A final extension step at 72 °C for 10 min was included to ensure complete amplification. The PCR products were verified by electrophoresis on a 1.5% agarose gel, where successful amplification was confirmed by comparing the product sizes to a 100 bp DNA ladder.

Following PCR amplification, RFLP analysis was performed to detect the specific polymorphisms. The amplified DNA fragments were digested with the appropriate restriction enzymes: ApaI (GGGCC^C) for the G > T polymorphism, TaqI (T^CGA) for the T > C polymorphism, BsmI (A^GCT) for the A > G polymorphism, and FokI (GGATG^C) for the C > T polymorphism. Each digestion was carried out in a 15 µl reaction mixture containing 4.0 µl of the PCR product, 1.5 µl of 10x buffer, 0.16 µl of 20 U/µl restriction enzyme, 0.2 µl of 10 µg/µl BSA, 0.187 µl of spermidine, and 8.952 µl of H2O. The digestion conditions were specific for each enzyme:

ApaI digestion at 25 °C, TaqI at 65 °C, BsmI at 37 °C, and FokI at 37 °C, all performed for 2 h in a water bath. To confirm the accuracy of the PCR-RFLP results, a 10% of the samples were randomly selected and reanalyzed. The results of the repeated analyses were consistent with the initial findings, ensuring the reliability and accuracy of the genotyping method.

The digested products were then resolved on a 3% agarose gel, stained with ethidium bromide, and visualized under UV light. The presence of polymorphic alleles was confirmed by the specific band patterns observed on the gel, which corresponded to the expected digestion fragments for each SNP. This analysis enabled the identification of each polymorphism based on the characteristic restriction patterns generated by the respective enzymes.

Statistical analysis

All statistical analyses were carried out using R version 4.2.3. Differences in demographic variables and vitamin D levels between the patients with PD and controls were evaluated with Chi-square test (for categorical variables) or student's t-test (for continuous variables). Besides, Chi-square test was used to verify whether the cases and controls were in Hardy-Weinberg Equilibrium (HWE)[29]. Four genetic models were used to evaluate the association between frequencies of the alleles and genotypes of VDR genes and the risk of PD. These were (i) allele model (A allele vs. a allele, where A is dominant allele and a is recessive allele); (ii) dominant model (AA + Aa vs. aa); (iii) recessive mode (AA vs. Aa + aa); and (iv) additive model (AA vs. aa and Aa vs. aa)[30]. A p-value of < 0.05 were considered as statistical significance.

Results

A total of 100 patients with PD and 100 controls were included in the present study. Patients with PD were older (mean age 63 years, SD 9.22) compared to the control group (mean age 57 years, SD 12.44) with a similar male-female ratio. A larger proportion of cases lived in urban areas (74% vs. 56%) and had higher level of education (42% vs. 14%). Besides, overweight and obesity were more prevalent in patients (48% vs. 32%). Type 2 diabetes mellites and hypertension were most common comorbidities in both groups. Sunlight exposure index was lower in case group compared to control group (8.11 vs. 10.24). A significantly higher proportion of patients with PD were on vitamin D supplementation compared to the controls (73% vs. 16%) (Table 1).

Serum vitamin D level was comparatively higher in patients with PD than in controls (27 ng/ml vs. 20.4 ng/ml, p-value < 0.05). Similarly, vitamin D deficiency was less prevalent in patient group (24% in cases vs. 50% in controls). Serum calcium level was comparatively lower in case group (mean 8.71, SD 1.35 mg/dl) compared to control group (mean 9.42, SD 1.75 mg/dl), though statistically not significant (Table 1).

Average age of diagnosis of PD of the patients was 59.3 (SD 9.3) years, and average duration of the disease was 4.1 (SD 3.0) years. Majority of the patients were in second or third stages of disease according to the Hoehn and Yahr staging, and their average UPDRS score was 72.3 (Table 2).

In the analysis of the ApaI gene, the T allele was observed to be more prevalent among patients with Parkinson's disease (PD) compared to controls, with frequencies of 59% and 47%, respectively. Additionally, the GT and TT genotypes were more commonly found in the PD group. Regarding the BsmI gene, although the G allele was more frequently present in patients with PD, this finding did not reach statistical significance. The distribution of AA, AG, and GG genotypes for the BsmI gene was comparable across both groups. In case of the FokI gene, a higher prevalence of the T allele and the TT genotype among patients with PD. Conversely, the distribution of T and C alleles for the TaqI gene did not differ significantly between the two groups; however, the TC genotype was more frequently observed in the PD cases, although this difference was also statistically insignificant (Table 3).

In genetic models, the T allele of the ApaI gene demonstrated a significant association with PD (OR 1.92, 95% CI 1.20–3.13, p-value 0.007). This significant association persisted across both recessive and additive models (for recessive model: OR 2.17, 95% CI 1.10–4.55, p-value 0.027 and for additive model: OR 2.78, 95% CI 1.22–6.67, p-value 0.015). Similarly, the T allele of the FokI gene was found to be significantly associated with PD (OR 2.27, 95% CI 1.43–3.57, p-value 0.001). This association was also evident in both dominant and additive models (for dominant model: OR 2.56, 95% CI 1.45–4.55, p-value 0.001 and for additive model: OR 3.03, 95% CI 1.67–5.56, p-value 0.001). Conversely, no significant associations were observed for the genetic polymorphisms of the BsmI and TaqI genes across any of the genetic models examined (Table 4).

Discussion

Our study found significant associations between several VDR gene polymorphisms and the risk of PD. We examined ApaI (rs7975232), BsmI (rs1544410), FokI (rs10735810), and TaqI (rs731236) VDR genes for association with PD. Among these, the FokI (rs2228570) T allele (in reference to C allele) and ApaI (rs7975232) T allele (in reference to G allele) were significantly associated with higher risk of PD.

In our investigation of the FokI (rs2228570) gene, we observed a significant association between the T allele and an increased risk of PD, a finding consistent across both dominant and additive models. This aligns with existing literature that consistently demonstrates a correlation between the T allele of the FokI gene and PD risk in both Asian populations[31,32] and Caucasian populations[13,33]. Recent meta-analyses have further confirmed the association of the FokI polymorphism with PD, particularly within Asian population[8,34]. Conversely, some studies, including that of Kang et al., did not report a significant link between the FokI gene and PD[7]. These contrasting results might be attributable to the differences in population ancestry, lifestyle, environmental conditions, or other confounding factors. Our study population from Bangladesh represents a South Asian genetic and environmental background, whereas studies like Kang et al. reflect a Northeast Asian population. These geographic and genetic differences may influence the interaction of VDR polymorphisms with vitamin

Characteristic	Cases, $n = 100$	Controls, $n = 100$	p-value
Age (years), mean ± SD	62.98 ± 9.22	56.72 ± 12.40	0.061
Sex			0.131
Male	63 (63.00)	73 (73.00)	
Female	37 (37.00)	27 (27.00)	
Residence			0.008
Rural	26 (26.00)	44 (44.00)	
Urban	74 (74.00)	56 (56.00)	
Education			< 0.001
No formal education	7 (7.00)	30 (30.00)	
Up to primary	16 (16.00)	19 (19.00)	
Secondary	29 (29.00)	22 (22.00)	
Higher secondary	6 (6.00)	15 (15.00)	
University graduate	42 (42.00)	14 (14.00)	
Family income			< 0.001
Low	39 (39.00)	21 (21.00)	
Middle	34 (34.00)	10 (10.00)	
High	27 (27.00)	69 (69.00)	
Comorbidities			
Type 2 diabetes mellitus	50 (50.00)	39 (39.00)	0.118
Hypertension	66 (66.00)	56 (56.00)	0.147
Other comorbidities	3 (3.00)	3 (3.00)	0.121
Smoking			0.084
Never smoked	69 (69.00)	59 (59.00)	
Previous smoker	24 (24.00)	24 (24.00)	
Current smoker	7 (7.00)	17 (17.00)	
BMI (kg/m^2), mean ± SD	25.07 ± 3.56	23.45 ± 3.26	0.001
BMI category			0.038
Underweight	2 (2.00)	6 (6.00)	
Normal weight	50 (50.00)	62 (62.00)	
Overweight	48 (48.00)	32 (32.00)	
Family history of PD	12 (12.00)	0 (0.00)	< 0.001
Sun exposure index, mean ± SD	8.11 ± 2.83	10.24 ± 3.83	< 0.001
Vitamin D supplementation	73 (73.00)	16 (16.00)	< 0.001
Serum vitamin D level (ng/ml), mean ± SD	27.04 ± 12.38	20.39 ± 9.77	< 0.001
Serum Ca level (mg/dl), mean ± SD	8.71 ± 1.35	9.42 ± 1.75	0.263

Table 1. Baseline characteristics of the participants ($n = 200$).

D metabolism and other environmental exposures. Additionally, the study had a relatively larger sample size and incorporated adjustments for vitamin D levels, which may have influenced their findings. Such variations highlight the importance of considering genetic, environmental, and lifestyle factors in understanding the role of VDR polymorphisms in PD pathogenesis.

The VDR FokI gene is recognized as a functionally significant polymorphism, distinguished from other VDR single nucleotide polymorphisms (SNPs) because it does not correlate with other genes and directly impacts the translation initiation site of the VDR protein. The T allele results in a longer variant of the VDR protein, whereas the C allele produces a shorter variant that is more active and more effectively regulates key neurotropic factors[35–37]. Moreover, the FokI T allele is associated with lower intestinal calcium absorption compared to the C allele[35], potentially resulting in decreased vitamin D levels that could influence susceptibility to PD. Lower vitamin D levels, in turn, may impair the neuroprotective and anti-inflammatory roles of the VDR protein, leading to increased neuronal vulnerability and degeneration in dopaminergic pathways, which are hallmarks of PD pathophysiology. This suggests that individuals with the FokI T allele may have a compromised ability to counteract neuroinflammation and oxidative stress, both of which are implicated in PD progression. These observations may elucidate the adverse impact of the VDR FokI T allele on the risk of developing PD.

Furthermore, our study identified a significant association between the T allele of the ApaI gene and PD within recessive and additive models. The current literature presents limited evidence regarding the association of the ApaI polymorphism with PD. Most previous studies did not report significant relationships between ApaI polymorphisms and PD[13,31–33,38]. Nonetheless, a recent meta-analysis indicated a significant association between ApaI polymorphism and Alzheimer's disease, another neurodegenerative disorder, suggesting a potential link between ApaI polymorphism and neurodegenerative diseases. ApaI polymorphisms, though classified as silent

Characteristics	n (%) or Mean (SD)
Age at diagnosis of PD (years), mean (SD)	59.32 ± 9.38
Duration of PD (years), mean (SD)	4.13 (3.03)
HY stage, n (%)	
Stage 1	5 (5.00)
Stage 2	43 (43.00)
Stage 3	40 (40.00)
Stage 4	9 (9.00)
Stage 5	3 (3.00)
UPDRS score, mean (SD)	
Part-1	15.98 (5.78)
Part-2	16.36 (7.75)
Part-3	37.67 (16.06)
Part-4	2.30 (2.79)
Overall	72.31 (28.34)

Table 2. Disease related characteristics of the patients with PD ($n = 100$). n (%): number with percentage.

VDR gene	Genotype/allele	Case, $n = 100$	Control, $n = 100$	p-value
ApaI (rs7975232)	G allele	82 (41.0)	106 (53.0)	0.007
	T allele	118 (59.0)	94 (47.0)	
	GG	15 (15.0)	28 (28.0)	0.024
	GT	52 (52.0)	50 (50.0)	
	TT	33 (33.0)	22 (22.0)	
BsmI (rs1544410)	A allele	101 (50.5)	119 (59.5)	0.072
	G allele	99 (49.5)	81 (40.5)	
	AA	27 (27.0)	37 (37.0)	0.132
	AG	47 (47.0)	45 (45.0)	
	GG	26 (26.0)	18 (18.0)	
FokI (rs2228570)	C allele	52 (26.0)	72 (36.0)	0.001
	T allele	148 (74.0)	128 (64.0)	
	CC	11 (11.0)	8 (8.0)	0.036
	CT	30 (30.0)	56 (56.0)	
	TT	59 (59.0)	36 (36.0)	
TaqI (rs731236)	T allele	136 (68.0)	151 (75.5)	0.196
	C allele	64 (32.0)	49 (24.5)	
	TT	40 (40.0)	57 (57.0)	0.063
	TC	56 (56.0)	37 (37.0)	
	CC	4 (4.0)	6 (6.0)	

Table 3. Allele and genotype frequency of VDR genes of the participants ($n = 200$).

mutations, may influence the stability and translation efficiency of VDR mRNA. These effects could disrupt the expression of VDR protein and, consequently, its downstream signaling pathways, including those involved in calcium homeostasis and neuronal survival. Impaired VDR signaling may exacerbate the loss of dopaminergic neurons in PD by reducing cellular resistance to oxidative damage and inflammation. While the precise effects remain to be elucidated, ApaI polymorphisms may induce silent codon changes that do not alter the VDR protein but could influence the stability and translation efficiency of VDR mRNA[39]. Future studies are needed to explore this relationship within the Bangladeshi population.

In contrast, we did not find significant associations between other VDR gene polymorphisms, such as BsmI and TaqI, and PD risk. The existing literature also presents limited evidence linking these polymorphisms to PD. For instance, Kim et al.[40] reported that the recessive model of the BsmI gene was associated with an increased risk of PD, while Liu et al.[41] noted a mild association between the TaqI dominant model and PD, but only among male patients. However, the majority of studies did not establish significant associations for these polymorphisms in either Asian or Caucasian populations[11,34].

Current evidence indicates that vitamin D may significantly influence the development of PD[42,43]. As a fat-soluble hormone, vitamin D can traverse the blood-brain barrier, implicating it in central nervous system processes. It plays a critical role in various physiological functions, including cell proliferation, differentiation,

VDR Gene	Model	Genotype	OR (95% CI)	p-value
ApaI (rs7975232)	Allelic	T vs. G Ref.	1.92 (1.20–3.13)	0.007
	Dominant	TT vs. GG + GT Ref.	1.72 (0.93–3.33)	0.085
	Recessive	GT + TT vs. GG Ref.	2.17 (1.10–4.55)	0.027
	Additive	TT vs. GG Ref.	2.78 (1.22–6.67)	0.015
		TT vs. GT Ref.	1.43 (0.74–2.86)	0.286
BsmI (rs1544410)	Allelic	G vs. A Ref.	1.43 (0.97–2.13)	0.072
	Dominant	GG vs. AA + AG Ref.	1.59 (0.81–3.23)	0.178
	Recessive	AG + GG vs. AA Ref.	1.59 (0.87–2.94)	0.134
	Additive	GG vs. AA Ref.	1.96 (0.90–4.35)	0.091
		GG vs. AG Ref.	1.37 (0.67–2.86)	0.392
FokI (rs2228570)	Allelic	T vs. C Ref.	2.27 (1.43–3.57)	0.001
	Dominant	TT vs. CC + CT Ref.	2.56 (1.45–4.55)	0.001
	Recessive	CC vs. CT + TT Ref.	1.41 (0.54–3.86)	0.483
	Additive	TT vs. CC Ref.	1.19 (0.42–3.23)	0.732
		TT vs. CT Ref.	3.03 (1.67–5.56)	0.001
TaqI (rs731236)	Allelic	C vs. T Ref.	1.33 (0.86–2.04)	0.196
	Dominant	CC vs. TT + TC Ref.	2.56 (0.72–9.09)	0.148
	Recessive	TT vs. TC + CC Ref.	1.25 (0.71–2.21)	0.437
	Additive	TC vs. TT Ref.	1.37 (0.79–2.38)	0.259
		TT vs. CC Ref.	1.43 (0.38–5.38)	0.588

Table 4. Genetic models of VDR genes and its association with PD. *OR* Odd's ratio.

neurotrophic regulation, neurotransmission, immune modulation, and neuroplasticity[44]. Furthermore, vitamin D exhibits neuroprotective properties, particularly in the context of dopaminergic neurons, which are primarily affected in PD, reinforcing the hypothesis of a relationship between vitamin D and PD[45].

While we examined the potential association of vitamin D levels with PD, our findings did not yield significant associations. Notably, our study's cross-sectional design posed limitations in investigating this relationship thoroughly. Interestingly, we observed higher vitamin D levels in PD cases, despite limited sunlight exposure, compared to controls. However, this observation may be misleading, as a substantially higher proportion of patients with PD were receiving vitamin D supplementation compared to controls, which could obscure the true scenario. Thus, the interpretation of this finding warrants caution.

The identification of these VDR polymorphisms, particularly in context of Bangladesh, could enable the early identification of individuals at higher genetic risk of developing PD, and targeted preventive measures. Additionally, these findings could guide research into therapeutic interventions, such as personalized vitamin D supplementation strategies, to modulate VDR activity and potentially reduce PD risk or slow disease progression. By understanding the genetic and environmental interactions influencing PD, this study paves the way for precision medicine approaches tailored to the unique genetic profile of the population of Bangladesh.

We intended to explore any association between serum calcium levels and PD; however, no significant correlation was found in our study. Nonetheless, a slightly lower calcium level was observed in patients with PD, which might be attributed to the progression of the disease. The underlying mechanism could involve impaired calcium homeostasis, as PD pathogenesis is linked to mitochondrial dysfunction, oxidative stress, and neuroinflammation, all of which can disrupt calcium regulation[46]. Further research is needed to elucidate this relationship and its potential clinical implications.

Our study had several limitations that should be considered. Firstly, while we measured Vitamin D levels, we did not exclude participants who were taking Vitamin D supplements, which might have influenced the results. A notable limitation of our study was the inability to perform subgroup analyses comparing vitamin D levels between non-supplemented cases and controls due to the small sample size of patients with PD who were not on vitamin D supplementation. Similarly, we could not assess the independent association of VDR gene polymorphisms with vitamin D and calcium levels, nor their interactions in relation to PD. These analyses would have provided additional insights into the natural relationship between vitamin D status, genetic variations, and PD risk. Future studies with larger sample sizes are warranted to explore these aspects in greater detail. Moreover, our sample size may have been insufficient to detect smaller effect sizes or complex interactions between genetic factors and the risk of PD. Besides, specific ethnic or ancestral subgroups might be underrepresented, which may limit the generalizability of the findings to all communities within Bangladesh. Finally, the case-control design was inherently susceptible to selection bias and limited our ability to establish causality between VDR polymorphisms and PD risk. Additionally, the absence of longitudinal data prevented us from assessing the long-term impact of Vitamin D levels and VDR polymorphisms on the progression of PD, limiting our understanding of the temporal relationship between these factors and disease development.

Conclusions

In conclusion, our study highlights the association between the VDR gene polymorphisms and risk of PD in a Bangladeshi population. Specifically, presence of T allele in FokI and ApaI VDR genes were associated with an increased risk of PD.

Data availability

Patient-level data will be available on request from the corresponding author.

References

1. Bloem, B. R., Okun, M. S. & Klein, C. Parkinson's disease. *Lancet* **397**, 2284–2303 (2021).
2. Ou, Z. et al. Global trends in the incidence, prevalence, and years lived with disability of Parkinson's disease in 204 countries/territories from 1990 to 2019. *Front. Public. Heal.* **9.**, 9:776847. https://doi.org/10.3389/fpubh.2021.776847 (2021). Epub ahead of print.
3. Ben-Shlomo, Y., Darweesh, S., Llibre-Guerra, J., Marras, C., San Luciano, M. & Tanner, C. The epidemiology of Parkinson's disease. *Lancet.* **403**(10423), 283–292. https://doi.org/10.1016/S0140-6736(23)01419-8 (2024).
4. Pereira, G.M. et al. A systematic review and meta-analysis of the prevalence of Parkinson's disease in lower to upper-middle-income countries. *npj Parkinsons Dis.* **10**, 181. https://doi.org/10.1038/s41531-024-00779-y (2024).
5. Dhiman, V. et al. A Systematic Review and Meta-analysis of Prevalence of Epilepsy, Dementia, Headache, and Parkinson Disease in India. *Neurology India*, **69**(2), 294–301. https://doi.org/10.4103/0028-3886.314588 (2021).
6. Dexter, D. T. & Jenner, P. Parkinson disease: from pathology to molecular disease mechanisms. *Free Radic Biol. Med.* **62**, 132–144 (2013).
7. Rodriguez-Oroz, M. C. et al. Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. *Lancet Neurol.* **8**, 1128–1139 (2009).
8. Cui, X. et al. The vitamin D receptor in dopamine neurons; its presence in human substantia Nigra and its ontogenesis in rat midbrain. *Neuroscience* **236**, 77–87 (2013).
9. Lv, L. et al. The relationships of vitamin D, vitamin D receptor gene polymorphisms, and vitamin D supplementation with Parkinson's disease. *Transl Neurodegener.* **9**, 34 (2020).
10. Kang, S. Y. et al. Vitamin D receptor polymorphisms and Parkinson's disease in a Korean population: revisited. *Neurosci. Lett.* **628**, 230–235 (2016).
11. Gao, J. et al. Association between vitamin D receptor polymorphisms and susceptibility to Parkinson's disease: an updated meta-analysis. *Neurosci. Lett.* **720**, 134778 (2020).
12. Hu, W. et al. Vitamin D receptor rs2228570 polymorphism and Parkinson's disease risk in a Chinese population. *Neurosci. Lett.* **717**, 134722 (2020).
13. Török, R. et al. Association of vitamin D receptor gene polymorphisms and Parkinson's disease in Hungarians. *Neurosci. Lett.* **551**, 70–74 (2013).
14. Gatto, N. M. et al. Vitamin D receptor gene polymorphisms and cognitive decline in Parkinson's disease. *J. Neurol. Sci.* **370**, 100–106 (2016).
15. Gatto, N. M. et al. Vitamin D receptor gene polymorphisms and Parkinson's disease in a population with high ultraviolet radiation exposure. *J. Neurol. Sci.* **352**, 88–93 (2015).
16. Haussler, M. R. et al. Molecular mechanisms of vitamin D action. *Calcif Tissue Int.* **92**, 77–98 (2013).
17. Lips, P. Vitamin D status and nutrition in Europe and Asia. *J. Steroid Biochem. Mol. Biol.* **103**, 620–625 (2007).
18. Rao Vupputuri, M. et al. Prevalence and functional significance of 25-hydroxyvitamin D deficiency and vitamin D receptor gene polymorphisms in Asian Indians. *Am. J. Clin. Nutr.* **83**, 1411–1419 (2006).
19. Postuma, R. B. et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* **30**, 1591–1601 (2015).
20. Barger-Lux, M. J. & Heaney, R. P. Effects of above average summer sun exposure on serum 25-Hydroxyvitamin D and calcium absorption. *J. Clin. Endocrinol. Metab.* **87**, 4952–4956 (2002).
21. Knaysi, G. A., Crikelair, G. F. & Cosman, B. The role of nines: its history and accuracy. *Plast. Reconstr. Surg.* **41**, 560–563 (1968).
22. Modestino, E. J. Hoehn and Yahr staging of Parkinson Rsquo s disease in relation to neuropsychological measures. *Front. Biosci.* **23**, 4649 (2018).
23. Martinez-Martin, P. et al. Expanded and independent validation of the movement disorder Society–Unified Parkinson's disease rating scale (MDS-UPDRS). *J. Neurol.* **260**, 228–236 (2013).
24. Summers, M. et al. Luminogenic reagent using 3-chloro-4-hydroxy acetanilide to enhance peroxidase luminol chemiluminescence. In: *Clinical Chemistry*. Amer Assoc Clinical Chemistry 2101 L Street Nw, Suite 202, Washington, Dc … pp. S73–S73.
25. Holick, M. F. et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J. Clin. Endocrinol. Metab.* **96**, 1911–1930 (2011).
26. Price, C. W., Leslie, D. C. & Landers, J. P. Nucleic acid extraction techniques and application to the microchip. *Lab. Chip.* **9**, 2484 (2009).
27. Islam, M. K., Siddhika, A., Das, M., Akhter, S., Hassan, Z. & Ali, L. Association of vitamin D receptor gene Bsm1 (A>G) and Fok1 (C>T) polymorphism in the pathogenesis of impaired glucose tolerance in Bangladeshi subjects. *SMU Medical Journal*, **1**(1), 67–76 (2014).
28. Sahabuddin, M. et al. Association of vitamin D receptor gene Apa1 (G>T) and Taq1 (T>C) polymorphisms in the pathogenesis of impaired glucose tolerance in subjects of Bangladeshi origin. *International Journal of Green and Herbal Chemistry*, **4**(3), 357–369 (2015).
29. Rohlfs, R. V. & Weir, B. S. Distributions of Hardy–Weinberg equilibrium test statistics. *Genetics* **180**, 1609–1616 (2008).
30. Horita, N. & Kaneko, T. Genetic model selection for a case–control study and a meta-analysis. *Meta Gene.* **5**, 1–8 (2015).
31. Han, X. et al. Vitamin D receptor gene polymorphism and its association with Parkinson's disease in Chinese Han population. *Neurosci. Lett.* **525**, 29–33 (2012).
32. Tanaka, K. et al. Vitamin D receptor gene polymorphisms, smoking, and risk of sporadic Parkinson's disease in Japan. *Neurosci. Lett.* **643**, 97–102 (2017).
33. Gezen-Ak, D. et al. GC and VDR SNPs and vitamin D levels in Parkinson's disease: the relevance to clinical features. *NeuroMolecular Med.* **19**, 24–40 (2017).
34. Wang, X. et al. Vitamin D receptor polymorphisms and the susceptibility of Parkinson's disease. *Neurosci. Lett.* **699**, 206–211 (2019).
35. Arai, H. et al. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *J. Bone Min. Res.* **12**, 915–921 (1997).

36. Jurutka, P. W. et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. *Mol. Endocrinol.* **14**, 401–420 (2000).
37. Colin, E. M. et al. Consequences of vitamin D receptor gene polymorphisms for growth Inhibition of cultured human peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D 3. *Clin. Endocrinol. (Oxf).* **52**, 211–216 (2000).
38. Li, C. et al. Vitamin D receptor gene polymorphisms and the risk of Parkinson's disease. *Neurol. Sci.* **36**, 247–255 (2015).
39. Morrison, N. A. et al. Prediction of bone density from vitamin D receptor alleles. *Nature* **367**, 284–287 (1994).
40. Kim, J. S. et al. Association of vitamin D receptor gene polymorphism and Parkinson's disease in Koreans. *J. Korean Med. Sci.* **20**, 495 (2005).
41. Liu, Y. et al. Vitamin D: preventive and therapeutic potential in Parkinson's disease. *Curr. Drug Metab.* **14**, 989–993 (2013).
42. Fullard, M. E. & Duda, J. E. A review of the relationship between vitamin D and Parkinson disease symptoms. Front neurol; 11. Epub ahead of print 27 May 2020. https://doi.org/10.3389/fneur.2020.00454
43. Peterson, A. L. A review of vitamin D and Parkinson's disease. *Maturitas* **78**, 40–44 (2014).
44. Gáll, Z. & Székely, O. Role of vitamin D in cognitive dysfunction: new molecular concepts and discrepancies between animal and human findings. *Nutrients* **202**;13:3672 .
45. Hafiz, A. A. The neuroprotective effect of vitamin D in Parkinson's disease: association or causation. *Nutr. Neurosci.* **27**, 870–886 (2024).
46. Alimonti, A. et al. Serum chemical elements and oxidative status in Alzheimer's disease, Parkinson disease and multiple sclerosis. *Neurotoxicology*, **28**(3), 450–456. https://doi.org/10.1016/j.neuro.2006.12.001 (2007).

Acknowledgements

Author contributions

Conceptualization: NCK and AK; Formal analysis: NCK, AK, MIK, KMNIJ, MS, ZH, MAR and MJH; Investigation: NCK, AK, MIK, KMNIJ, MS, ZH, MAR and MJH; Methodology: NCK, AK, MIK, KMNIJ, MS, ZH, MAR and MJH; Resources: NCK and AK; Supervision: NCK, AK, MIK, KMNIJ, MS and ZH; Writing – original draft: NCK, AK, MAR and MJH; Writing – review & editing: NCK, AK, MIK, KMNIJ, MS, ZH, MAR and MJH; All authors have read and agreed to the published version of the manuscript.

Funding

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study protocol was reviewed and approved by Institutional review board of Shaheed Suhrawardy Medical College and Hospital, Dhaka, Bangladesh (ShSMC/Ethical/2023/063). The all authors declare no human subjects were harmed and the procedures followed were in accordance with the ethical standards and regulations established by the Helsinki Declaration of the World Medical Association. Informed written consent was obtained from all the participants involved in the study.

Additional information

Correspondence and requests for materials should be addressed to N.C.K. or M.J.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.