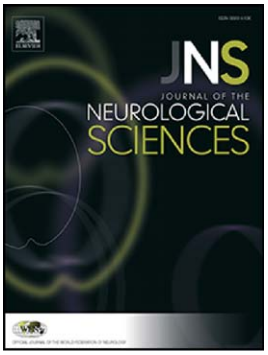


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# Vitamin D receptor gene polymorphisms and Parkinson's disease in a Population with High Ultraviolet Radiation Exposure

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**HIGHLIGHTS**

- The study population had high lifetime ultraviolet radiation (UVR) exposure.
- Increasing UVR exposure was not inversely associated with Parkinson's disease risk.
- Homozygotes for rs731236 (TaqI) TT (major allele) genotype had 31% lower PD risk.
- rs7975232 (ApaI) GG (minor allele) genotype was associated with 27% lower PD risk.
- Vitamin D receptor gene polymorphisms may modulate risk under high UVR conditions.

**ABSTRACT**

Introduction: A high prevalence of vitamin D deficiency has been reported in Parkinson's disease (PD). Epidemiologic studies examining variability in genes involved in vitamin D metabolism have not taken into account level of exposure to ultraviolet radiation (UVR). We examined whether exposure to UVR (as a surrogate for vitamin D levels) and variations in the vitamin D receptor gene (*VDR)* are associated with PD. Methods: Within a geographical information system (GIS) we linked participants' geocoded residential address data to ground level UV data to estimate historical exposure to UVR. Six SNPs in *VDR* were genotyped in non-Hispanic Caucasian subjects. Results: Average lifetime UVR exposure levels were >5000 $Wh/m^2$, which was higher than levels for populations in previous studies, and UVR exposure did not differ between cases and controls. Homozygotes for the rs731236 TT (major allele) genotype had a 31% lower risk of PD risk (OR = 0.69; 95% CI = 0.49, 0.98; p=0.04 for TT vs. TC + CC). The rs7975232 GG (minor allele) genotype was also associated with decreased risk of PD (OR = 0.63; 95% CI = 0.42, 0.93; p=0.02 for GG vs. TG + TT). The association between PD risk and a third locus, rs1544410 (BsmI), was not statistically significant after adjustment for covariates, although there was a trend for lower risk with the GG genotype. Conclusions: This study provides initial evidence that *VDR* polymorphisms may modulate risk of PD in a population highly exposed to UVR throughout lifetime.

## 1. INTRODUCTION

Parkinson's disease (PD) is a debilitating neurodegenerative disorder that is increasingly recognized as having a complex multi-factorial etiology. Environmental exposures and genetic factors influencing the response or susceptibility to environmental factors (i.e., gene-environment interactions) are thought to contribute to PD risk in populations[1]. Vitamin D has been investigated as a protective factor in neurological diseases including Alzheimer's disease and multiple sclerosis[2]. Researchers hypothesize that inadequate levels of circulating vitamin D could lead to dysfunction in the substantia nigra pars compacta, an area of the brain in which the characteristic dopaminergic degeneration occurs in parkinsonian disorders, and where the vitamin D receptor (VDR) and 1α-hydroxylase, the enzyme responsible for the formation of the bioactive form of vitamin D, are both highly expressed[3]. A high prevalence of vitamin D deficiency has been reported in PD patients[4]; PD has been associated with decreased bone mineral density[5], which itself may be related to deficiencies in vitamin D. A cross-sectional study of 137 Japanese PD patients found that levels of serum vitamin D were lower with increased disease severity[6], and a small randomized trial of vitamin D supplementation demonstrated it to be effective in preventing deterioration of the Hoehn and Yahr stage in PD [7]. In a Finnish population-based cohort study, the relative risk of incident PD after 29 years of follow-up was 0.33 comparing the highest to lowest quartile of serum vitamin D measured at baseline[8].

Vitamin D is produced in the body through the action of ultraviolet radiation (UVR) from sunlight on the skin. It is absorbed from the diet to a lesser extent with few natural food sources containing significant amounts of vitamin D. Fortified foods and supplements may also provide dietary vitamin D, yet most Caucasians can meet their vitamin D needs through adequate sunlight exposure[9, 10]. We recently conducted a registry-linkage study in Denmark that included 6,623 PD patients and 33,378 age-matched population controls and found that outdoor

work (based on trade codes and job titles), likely leading to higher year-round sunlight exposure, was inversely associated with PD risk[11].

The bioactive metabolite and circulating form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), is enzymatically generated through a number of steps known as the vitamin D metabolic pathway. Signaling of bioactive vitamin D occurs through its binding with the vitamin D receptor (VDR), and a number of polymorphisms with no known functionality in the *VDR* (gene that encodes the VDR) have been identified[12]. Genes in the vitamin D metabolic pathway have been implicated in animal models of PD[13]. Several epidemiologic studies of PD investigating genetic variability in the *VDR* have largely focused on four common *VDR* polymorphisms, and findings for these and other *VDR* polymorphisms have been generally inconsistent. A small study of Korean PD patients found that the *Bsm*I bb genotype was significantly more common in patients than controls, a result that has not been confirmed by three subsequent case-control studies[14-16]. Genotypes containing the major allele of *Fok*I, a known functional *VDR* polymorphism, have been associated with a greater than two-fold increase in PD[14, 16]. However, in a genome-wide association study (GWAS), none of eighteen *VDR* SNPs examined were associated with PD risk[17]. Three recent meta-analyses of studies of the most commonly investigated *VDR* polymorphisms generally suggest associations between the *Bsm*I and *Fok*I polymorphisms and PD susceptibility, but that may vary with the population studied [18] [19, 20].

Prostate cancer studies suggested that the contribution of *VDR* genotypes to risk may depend on the geographic location of the population studied, and the level of UVR or sunlight exposure[21, 22]. Environmental UVR conditions may also influence the association between VDR genotypes and risk of type 1 diabetes [23]. Here we examine associations between PD, UVR and *VDR* polymorphisms in a population with consistently high lifetime UVR exposure.

## 2. MATERIALS AND METHODS

### 2.1. Study population

The Parkinson Environment Gene (PEG) study is a population-based case-control study of PD in the Central Valley (CV) of California[24]. Briefly, study subjects were recruited between January 2001 and January 2007, resided in Fresno, Tulare, and Kern Counties, and had to have lived in California for at least 5 years prior to diagnosis or interview. Cases were recruited within 3 years of diagnosis and were confirmed as having clinically probable or possible PD by a UCLA movement disorder specialist. Altogether, 28 (90%) of the 31 practicing local neurologists who provided care for PD patients assisted in recruiting cases for this study. We solicited collaboration from Kaiser Permanente, Kern and Visalia Medical Centers and the Veteran's Administration, Parkinson's disease support groups, local newspapers and local radio stations that broadcast public service announcements. Of 379 confirmed probable or possible PD cases in PEG, 367 were considered idiopathic and provided information needed for the current analysis of UVR. During 2001-2007, we identified 755 eligible population controls, of which 346 (46%) were enrolled in PEG. During 2009-2011, a second wave of population control recruitment was initiated [the Center for Gene-Environment Research in Parkinson's Disease (CGEP) study] and an additional 607 controls were enrolled. For PEG and CGEP combined, 658 controls completed residential history questionnaires which provided data needed for the analysis of UVR.

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

### 2.2. Historical UVR Exposure Assessment

Historical UVR exposure was estimated at participants' lifetime residential addresses reported on questionnaires which were automatically geocoded using our proprietary geocoder (found at webgis.usc.edu), and then manually resolved in a multi-step process previously

described[25]. UVR exposure for the continental US was estimated using a previously-validated[26] GIS-based UVR exposure surface based on data from the National Solar Radiation Database (NSRAD) produced under the Department of Energy's Resource Assessment Program and interpolated using the ANUSPLIN procedure[27]. Estimates were made at the point-level of 30-year AVerage daily total GLObal solar radiation (AVGLO) defined as the total amount of direct and diffuse solar radiation in watt-hours per meter squared ($Wh/m^2$) received on a horizontal surface. To estimate UVR exposure for foreign addresses, the Global 30 Arc-Second Elevation Data Set (GTOPO30), a digital elevation model (DEM) from the United States Geological Survey was used. Using the point solar radiation tool in ArcGIS 10.0, annual global solar radiation was estimated for each location based on topography and surface features provided. Briefly, this tool calculates the global solar radiation as a function of direct and diffuse radiation.

Average lifetime UVR exposure [in watt-hours per meter-squared ($Wh/m^2$)] was calculated as the average of the sum of UVR exposure at each residence weighted for years at that residence. The continuous exposure variable was scaled to standard deviation (SD) units. Quartile categories of exposure were created based on the distribution of the UVR exposure variables in control subjects.

### 2.3. Selection of SNPs in *VDR*

Candidate SNPs in *VDR* were selected based on 1) findings from previous studies assessing vitamin D genes in PD, 2) previous genetic studies of vitamin D metabolism-related disorders, 3) potential biological functional relevance of a SNP [rs2228570 (Fok1)[28]], and/or 4) variants in the coding region of the gene leading to an amino acid change in the protein. We used Build 129 of the NCBI dbSNP, the NIEHS-sponsored GeneSNPs web portal (University of Utah Genome Center, 2007) and the NCBI OMIM database to identify candidate SNPs for genotyping. SNPs were required to have a predicted Caucasian population frequency >5%.

### 2.4. Biospecimen Collection, DNA Isolation and Genotyping

Biospecimens (either blood or saliva) provided by study participants were used to obtain DNA. For blood samples, a total of 20mL EDTA preserved blood was collected by venipuncture. Saliva samples were collected using an Oragene kit. DNA extraction from blood or saliva samples was performed by the UCLA Human Genetics Core Facility using Autopure LSTM nucleic acid purification instrument from Gentra Systems (gentra.com) or the best suitable Qiagen kit (i.e. DNeasy). DNA quality, purity, and concentration were measured using a UV spectrophotometer to determine the A260/A280 ratio (range of usually 1.7-2.0). DNA was quantitated using OD 260/280 and diluted for storage to 1:20 100 µl using $ddH_2O$. Genotyping of SNPs was performed at University of Washington using ABI TaqMan MGB chemistry with an ABI 7900 instrument in 384 well format. Call rates were > 90%. Genotyping was done on non-Hispanic Caucasian participants only; 283 cases and 423 controls were included in analyses of *VDR* polymorphisms. The non-Hispanic Caucasian participants who were genotyped had somewhat higher levels of education compared to the total study population which was 2.3% Black, 15.4% Latino/Hispanic, 2.3% Asian and 2.4% Native American, otherwise there were no substantive differences in demographic factors.

**2.5. Statistical Analyses**

We used unconditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (95% CI) of associations between UVR exposure and PD risk. For the quantitative UVR exposure variable, the OR was interpreted as the increase or decrease in PD risk per SD of exposure in $Wh/m^2$. For the categorical UVR exposure variable, the lowest quartile exposure assigned as the reference. *VDR* polymorphisms were assessed for Hardy-Weinberg Equilibrium in controls using a chi-square test. Analyses of *VDR* SNPs used genetic models to be consistent with previous studies whenever possible (Table 1). Multivariable models were adjusted for age at diagnosis (cases) or interview (controls) (continuous), sex, education (<12 years, 12 years, >12 years), smoking (never, or ever which included former and current smokers) and family history of PD (yes, no). Analyses of UVR exposure in all subjects

also included race (white, non-white). We assessed whether additional adjustment for UVR exposure (modeled as a categorical variable) changed estimated ORs for *VDR* SNPs. All analyses used SAS version 9.2 (SAS Institute Inc., Cary, NC, USA.).

## 3. RESULTS

Average lifetime UVR exposure of the study population was 5030 $Wh/m^2$, which was higher, as expected, than levels for populations included in other studies of *VDR* polymorphisms and PD (Table 1). As results for the total study population were similar to those limited to participants who had genotyping done, Table 2 and 3 are simplified to present the latter. Cases were slightly older than controls, somewhat less educated, more likely to have a family history of PD, and to have never smoked cigarettes (Table 2). Cases and controls did not differ in their average lifetime UVR exposure, or their exposure 10, 20 or 30 years prior to diagnosis (cases) or study entry (controls). There was no association between PD risk and increasing UV exposure, or comparing participants with higher to the lowest quartile of exposure (Table 3).

**Table 1. Summary of Epidemiologic Studies of Parkinson's Disease Risk and Vitamin D Receptor Polymorphisms, by City or Region and 2014 Estimated Annual Ultraviolet Radiation Exposure**

| Author, year | City or region, Country | Latitude (degrees) | 2014 Estimated Annual UV exposure (Watts-hours/meter$^2$) | Study Population | rs731236 (TaqI) | rs7975232 (ApaI) | rs1544410(BsmI) | rs2228570 (FokI) | rs4334089 | rs11568820(Cdx2) |
|---|---|---|---|---|---|---|---|---|---|---|
| Kim et al., 2005 | Seoul, Korea | 37 N | 3863 | 85 sporadic cases<br>231 hospital controls, frequency matched on age and gender | -- | -- | Homozygous minor allele (*bb)* genotype significantly more common in patients than controls | -- | -- | -- |
| Butler et al., 2011 | United States, multiple locations | Durham: 36 N<br>Miami: 26 N | Durham, NC: 4406<br>Miami, FL: 4834 | 1,086 cases<br>1,286 unaffected relatives (family study) | Not associated with risk in family study. | -- | Not associated with risk in GWAS. | -- | Associated with risk in early onset subset; but not in late onset subset in family study. Not associated with risk in GWAS. | -- |
| Han et al., 2012 | Beijing & Shandong, China | 39 N (Beijing)<br>36 N (Shandong) | 3863 | 260 sporadic cases<br>282 hospital controls | -- | -- | No differences in allele or genotype frequency between cases and controls. | Tagged by rs10735810<br>Genotypes with major allele C: OR = 2.16 (95% CI = 1.26, 3.70) for CC+CT vs. TT; OR = 1.14 (95% CI = 0.88, 1.33) for CC vs. CT+TT | -- | -- |
| Lin et al., 2013 | Taipei, Taiwan | 25 N | 4894 | 700 sporadic cases<br>792 age and/or gender-matched hospital or community controls | -- | -- | -- | -- | No differences in allele or genotype frequency between cases and controls. | -- |
| Lv et al., 2013 | Hunan, China | 27 N | 4894 | 483 cases<br>498 age- and sex-matched controls | No differences in allele or genotype frequency between cases and controls. | -- | -- | -- | No differences in allele or genotype frequency between cases and controls. | -- |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Petersen et al., 2013 | Faroe Islands | 62 N | 1773.0 | 121 cases identified from pharmacy, hospital system and neurology specialty 235 population controls | No differences in allele or genotype frequency between cases and controls. | No differences in allele or genotype frequency between cases and controls. | No differences in allele or genotype frequency between cases and controls. | -- | -- | -- |
| Torok et al., 2013 | Szeged, Hungary | 46 N | 3060 | 100 sporadic cases 109 hospital controls | No differences in allele or genotype frequency between cases and controls. | No differences in allele or genotype frequency between cases and controls. | No differences in allele or genotype frequency between cases and controls. | Tagged by rs10735810 Genotypes with major allele C: OR = 2.68 (95% CI = 1.21, 5.91) for CC+CT vs. TT | -- | -- |
| Current study | Central Valley, California, USA | 37 N | 5200 | 283 sporadic cases 423 age-sex-race-matched population controls | Genotypes homozygous for major allele T: OR = 0.67 (95% CI = 0.47, 0.95) for TT vs TC + CC | Genotypes homozygous for minor allele G: OR = 0.63 (95% CI = 0.42, 0.94) for GG vs. TT + TG | Genotypes homozygous for major allele G: OR = 0.71 (95% CI = 0.50, 1.01) for GG vs. GA+AA | No differences in allele or genotype frequency between cases and controls. | No differences in allele or genotype frequency between cases and controls. | No differences in allele or genotype frequency between cases and controls. |

**Table 2. Characteristics of non-Hispanic Caucasian Parkinson's disease cases and unaffected controls included in analyses of VDR gene polymorphisms, PEG & CGEP Studies**

| Characteristic: Mean ± standard deviation or n(%) | Cases (n=283) | Controls (n=423) | OR (95%CI) |
|---|---|---|---|
| **Age at diagnosis (cases) or entry (controls), years** | 70.9 ± 10.5 | 67.6 ± 12.0 | 1.03 (1.01, 1.04) |
| | | | |
| **Sex** | | | |
| Male | 158 (55.8) | 212 (50.1) | *1.00 (Ref)* |
| Female | 125 (44.2) | 211 (49.9) | 0.79 (0.59, 1.08) |
| **Education** | | | |
| Some high school or less | 27 (9.5) | 24 (5.7) | *1.00 (Ref)* |
| High school graduate | 86 (30.4) | 85 (20.1) | 0.90 (0.48, 1.68) |
| More than high school | 170 (60.1) | 314 (74.2) | 0.48 (0.27, 0.86) |
| | | | |
| **Smoking status** | | | |
| Never | 156 (55.1) | 195 (46.1) | *1.00 (Ref)* |
| Ever | 127 (44.9) | 228 (53.9) | 0.70 (0.52, 0.94) |
| | | | |
| **Family History of PD** | | | |
| No | 241 (85.2) | 386 (91.3) | *1.00 (Ref)* |
| Yes | 42 (14.8) | 37 (8.8) | 1.82 (1.14, 2.91) |

**Table 3. Adjusted odds ratios (95% CI) from logistic regression models for associations between ultraviolet radiation and PD risk, genotyped subjects only**

| UVR exposure, watts-hours/meter$^2$, mean ± standard deviation or number (%) | Cases | Controls | Adjusted[a,b] OR (95% CI) |
|---|---|---|---|
| Average lifetime exposure | 5007 ± 219 | 4992 ± 243 | 1.0 (0.87, 1.16) |
| Q1: <4930 [mean: 4717 median: 4768] | 78 (27.9) | 116 (31.7) | *1.00 (Referent)* |
| Q2: 4930-5103 [mean: 5031 median: 5038] | 83 (29.6) | 90 (24.6) | 1.06 (0.71, 1.58) |
| Q3: 5104-5178 [mean: 5146 median: 5146] | 67 (23.9) | 90 (24.6) | 1.02 (0.68, 1.53) |
| Q4: ≥5179 [mean: 5224 median: 5212] | 52 (18.6) | 70 (19.1) | 0.93 (0.60, 1.45) |
| | | | |
| Below the mean | 161 (57.5) | 206 (56.3) | *1.00 (Referent)* |
| At or above the mean | 119 (42.5) | 160 (43.7) | 0.95 (0.71, 1.28) |

[a]ORs adjusted for age at diagnosis or entry (continuous), sex, education (less than high school, high school, more than high school), smoking (ever, never), family history of PD (yes, no)

[b]ORs for continuous UVR exposure are expressed per standard deviation

Allelic and genotypic frequencies did not differ between non-Hispanic Caucasian cases and controls for most of six polymorphisms examined (Supplemental Table 1). For *VDR* SNPs rs731236 (defining the TaqI locus) and rs7975232 (defining the ApaI locus), frequencies of the minor alleles were more common in cases than controls.

Homozygotes for the TaqI TT (major allele) genotype had a 31% lower risk of PD risk (OR = 0.69; 95% CI = 0.49, 0.98; p=0.04 for TT vs. TC+CC, unadjusted for multiple testing) (Table 4). Homozygotes for the ApaI GG (minor allele) genotype had a 37% decreased risk of PD (OR = 0.63; 95% CI = 0.42, 0.93; p=0.02 for GG vs. TG+TT, unadjusted for multiple testing). The association between PD risk and the BsmI locus was not statistically significant after adjustment for covariates in regression models (OR = 0.73; 95% CI = 0.52, 1.03; p=0.07 for major allele homozygous GG genotype vs. GA+AA). The SNPs rs2228570 (tagging the FokI site), rs11568820 (tagging the Cdx2 site) and rs4334089 were not associated with PD risk. Additional adjustment for average lifetime exposure to UVR minimally strengthened the protective effect of TaqI and BsmI major allele homozygous genotypes (by ~2%); otherwise it did not change OR estimates for *VDR* SNPs (Table 4), nor did we find any association of UVR exposure level on PD risk once adjusted for any of the six *VDR* polymorphisms.

**Table 4. Adjusted odds ratios (95% CI) from logistic regression models for associations between VDR polymorphisms and PD risk**

| *VDR* SNP | Cases (n=283) | Controls (n=423) | Adjusted OR (95% CI)[a] | p-value | Adjusted OR (95% CI)[b] | p-value |
|---|---|---|---|---|---|---|
| **rs731236 (TaqI)** | | | | | | |
| TT | 77 (27.3) | 153 (36.3) | **0.69 (0.49, 0.98)** | ***0.04*** | **0.67 (0.47, 0.95)** | ***0.03*** |
| TC + CC | 205 (72.7) | 268 (63.4) | *1.00 (Referent)* | | *1.00 (Referent)* | |
| **rs7975232 (ApaI)** | | | | | | |
| GG | 46 (16.3) | 104 (24.8) | **0.63 (0.42, 0.93)** | ***0.02*** | **0.63 (0.42, 0.94)** | ***0.02*** |
| TG + TT | 236 (83.7) | 315 (75.2) | *1.00 (Referent)* | | *1.00 (Referent)* | |
| **rs1544410 (BsmI)** | | | | | | |
| GG | 79 (28.6) | 151 (36.3) | **0.73 (0.52, 1.03)** | **0.07** | **0.71 (0.50, 1.01)** | **0.06** |
| GA + AA | 197 (71.4) | 265 (63.7) | *1.00 (Referent)* | | *1.00 (Referent)* | |
| **rs2228570 (FokI)** | | | | | | |
| CC | 109 (38.5) | 153 (36.3) | 1.12 (0.81, 1.55) | 0.49 | 1.18 (0.85, 1.65) | 0.32 |
| CT + TT | 174 (61.5) | 269 (63.7) | *1.00 (Referent)* | | *1.00 (Referent)* | |
| **rs4334089 (Vance)** | | | | | | |
| CC | 152 (53.7) | 228 (53.9) | 0.98 (0.71, 1.34) | 0.89 | 0.98 (0.71, 1.36) | 0.91 |
| CT + TT | 131 (46.3) | 195 (46.1) | *1.00 (Referent)* | | *1.00 (Referent)* | |
| **rs11568820(Cdx2)** | | | | | | |
| GG | 183 (64.9) | 266 (62.9) | 1.05 (0.75, 1.45) | 0.79 | 1.07 (0.76, 1.50) | 0.71 |
| GA + AA | 99 (35.1) | 157 (37.1) | *1.00 (Referent)* | | *1.00 (Referent)* | |

[a]adjusted for age (continuous; at diagnosis for cases, at interview for controls), sex, education (<12 years, 12 years, >12 years), smoking (ever, never), family history of PD in a primary relative (yes, no)

[b]adjusted for covariates in (a) and average lifetime UV exposure (quartiles)

## 4. DISCUSSION

Experimental studies implicate the vitamin D pathway in PD pathogenesis. Low doses (1-100nM) of 1,25-dihydroxyvitamin D protected cultured rat mesencephalic dopaminergic neurons from toxicity from exposure to L-buthionine sulfoximine and 1-methyl-4-phenylpyridium ions ($MPP^+$). Pre-treating cultures with this active vitamin D metabolite attenuated the generation of reactive oxygen species resulting from this chemically induced toxicity[30]. *In vivo* studies of rats pre-treated with 1,25-dihydroxyvitamin D and then lesioned with 6-hydroxydopamine (6-OHDA) showed better locomotor activity than untreated lesioned rats[31], and evidence of partial protection of dopaminergic neurons[32]. Vitamin D may act mechanistically to enhance production of glial cell line-derived neurotrophic factor, which has been shown to reduce 6-OHDA-induced damage to dopaminergic neurons. Interestingly, a recent report suggested that *VDR*-knockout mice have a behavioral phenotype similar to human PD, exhibiting muscular and motor impairments with preserved cognitive function[13].

We used measured UVR radiation data as a surrogate estimate of long-term vitamin D levels, employing linked geocoded historical residential addresses data for subjects. UVR exposure stimulates the physiological production of bioactive vitamin D in the body [33], and correlations have been reported between UVR exposure and measured serum vitamin D levels[34]. In sunny months, the physiologic vitamin D state is primarily governed by exposure to solar radiation with dietary vitamin D making a negligible contribution [35]. We did not have direct measurements of physiologic vitamin D levels from participants over the lifetime; it is also possible that estimated UVR does not reflect actual UVR exposure. Random error in UVR exposure would tend to bias the calculated effect estimates to the null. Furthermore, factors such as time spent indoors, skin pigmentation, coverage by clothing or protection by sunscreen, as well as dietary sources of vitamin D could modify our exposure estimates. We did not, however, find substantial differences in effect estimates for UVR between all subjects and those

of European ancestry only. It is possible that behavioral differences may exist between PD patients and their unaffected peers, such that after diagnosis, cases may spend less time outdoors. Reverse causation could explain previous findings of higher vitamin D deficiency in PD. However, in our study, cases were enrolled in early stages of disease. A strength of our approach to estimating UVR exposure is that we did not rely on recall of sun exposure by subjects to reconstruct historical exposure.

The VDR is a member of the steroid/thyroid hormone super family of transcription regulation factors[12]. The actions of the bioactive metabolite and circulating form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), are initiated when it binds to the VDR[36]. Rs731236 tags TaqI, a synonymous restriction fragment length polymorphism(RFLP) located in the coding region of the *VDR,* while SNP rs7975232 tags ApaI, a RFLP located in the non-coding region of the *VDR*[17]. The two variants are not in strong linkage disequilibrium(LD) with each other ($r^2$ for pair-wise LD = 0.597) (http://www.broadinstitute.org/mpg/snap/ldsearchpw.php) and are in weak LD with FokI*,* the only currently known functional polymorphism in the *VDR* that can result in short and long variants of the VDR protein[12]. The association between these SNPs and PD risk might be due to LD with an unknown locus or loci elsewhere in the *VDR*. The LD of the non-coding region of the *VDR*, extends into the 3' regulatory regions, which are known to be involved in regulation of expression, especially through regulation of mRNA stability[12].

While the *VDR* is expressed in neurons and glial cells distributed throughout the human brain, gene expression is most prominent in the substantia nigra, the area of the midbrain in which dopaminergic cells are depleted in PD, as well as the hypothalamus[3]. *VDR*-mediated transcription can be induced by dopamine, which might imply that gene expression normally regulated by *VDR* could be influenced by dopamine levels[37], suggesting a possible interrelationship between vitamin D and dysfunction in PD.

A small study of 85 Korean PD patients and 231 controls found the BsmI *bb* genotype (indicating the presence of a restriction site on both genes) more common in patients[38]. Haplotypes in the *VDR* that include the BsmI locus were associated with varying mRNA expression levels[12]. In Hungarian and Chinese case-control studies, CC+TC genotypes of FokI (SNP rs10735810 tagged the FokI restriction site) were more common than the TT genotype in PD patients[14, 16]. Other case-control studies of these RLFPs and other *VDR* polymorphisms including both smaller and larger numbers of cases did not find associations[15, 39, 40]. A recent two-stage GWAS study of an initial 770 non-Hispanic Caucasian PD families analyzed tag SNPs covering all common variants in and around *VDR* including four RFLPs we and others have examined. That study identified more than one SNP in the 5‘ end of the *VDR,* not including SNPs near TaqI, as being associated with PD. In the replication stage, 18 SNPs in and around the *VDR* (16 of which overlapped with those in analyses in the family dataset) were tested in 267 PD cases and 267 controls, and three were statistically significantly associated with age of onset but not risk[17].

In our population-based case-control study of PD in the CV of California where UVR levels were higher than in previous studies of *VDR* polymorphisms, we found associations with two vitamin D receptor SNPs, tagging the TaqI and ApaI loci. PD risk was reduced in subjects homozygous for the major allele TaqI TT genotype (by 31%), and in those homozygous for the minor allele ApaI GG genotype (by 37%). Adjustment for average lifetime UVR exposure did not change OR estimates for any polymorphic loci. The *VDR* tag SNPs rs2228570 (Fok1), rs11568820 (Cdx2) and rs4334089 were not associated with risk. However, we were statistically ‘underpowered’ to detect associations between these variants and PD risk given expected weak underlying genetic effects[29].

Previous studies have suggested that associations between *VDR* polymorphisms and other conditions may depend on the level of UVR exposure. In a population-based case-control

study of non-Hodgkin lymphoma (NHL), carriers of the TaqI CC genotype who reported < 7 hours a week of sun exposure had an increased risk of NHL compared to TT carriers with the same low level of sun exposure[41]. In prostate cancer, the TaqI T allele was more common in cases than controls in a southern European population[22]; associations between variants at FokI and Cdx2 sites were restricted to a high UVR-exposed subgroup in a British population[21]. In a meta-analysis of 16 studies from 19 regions of *VDR* polymorphisms and type 1 diabetes, under high UVR conditions the relative risk associated with major alleles reflecting the absence of a restriction site was > 1 at FokI and BsmI and < 1 for TaqI[23].

Because of study eligibility criteria, at enrollment, cases and controls had similar exposure to UVR at their residence, and all lived in a geographical region with significant solar radiation. For addresses in the three counties, mean radiation was 5179.5 $Wh/m^2$ with a narrow range of 4884-5517 $Wh/m^2$, indicating only an 11% difference in UV exposure between maximum and minimum. The measure of lifetime UVR exposure used in analyses took into account exposure at historical residential addresses located both in and outside of the CV. Even with an exposure range of 4200-5350 $Wh/m^2$ from all addresses, we did not have sufficient variability to test gene-environment interactions, as all participants were highly exposed, and we lacked a “low” UVR exposure sub-group. Participants with the lowest lifetime exposure to UVR in our study (i.e., 4200 $Wh/m^2$) were still exposed at levels higher than populations in several studies[14-16]. As such, observed gene effects reflect the relationship between *VDR* polymorphisms and PD in the context of lifelong high UV exposure. If the combination of genetic and environmental factors is important for the effect of the *VDR* in PD, our study provides data at the high end of UVR exposure. Differences in findings for *VDR* SNPs between studies could be due to background levels of UVR exposure in the population studied. While there was not complete overlap in the polymorphisms examined, generally existing studies that included populations with very low UVR exposure [15, 16, 38], did not detect PD associations in the *VDR*

SNPs in which we did identify associations. This could be further investigated by studying the impact of *VDR* genetic variation on PD in other very high or very low UVR exposed populations. Our analyses of UVR were performed in our study population that was ~78% non-Hispanic Caucasian, and analyses of *VDR* polymorphisms were restricted to non-Hispanic Caucasian participants, thus potentially limiting the generalizability of our findings to non-Hispanic Caucasian populations only.

In conclusion, our study suggests that *VDR* polymorphisms are associated with PD in a population with high UVR exposure. Additional investigations should continue to examine the vitamin D metabolic pathway, UVR and vitamin D in PD.

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