DNA variants in *CACNA1C* modify Parkinson disease risk only when vitamin D level is deficient

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ABSTRACT

Objective: To evaluate the association between the genetic variants in *CACNA1C*, which encodes the $\alpha 1$ subunit of the L-type voltage-sensitive calcium channel (LVSCC) and Parkinson disease (PD) while accounting for interactions with vitamin D concentration.

Methods: Two independent case-control data sets (478 cases and 431 controls; 482 cases and 412 controls) were used. Joint effects of single nucleotide polymorphisms (SNPs) and SNP-vitamin D interaction were analyzed by comparing models containing vitamin D deficiency, SNP genotypes, SNP-vitamin D interaction, and covariates to a restricted model with only vitamin D deficiency and covariates. Meta-analysis was used to combine the joint effects in the 2 data sets. Analysis was stratified by vitamin D deficiency to demonstrate the pattern of SNP-vitamin D interaction.

Results: Vitamin D deficiency was associated with PD in both data sets (odds ratio [OR] = 1.9–2.7, $p \leq 0.009$). SNP rs34621387 demonstrated a significant joint effect (meta-analysis, $p = 7.5 \times 10^{-5}$; Bonferroni corrected, $p = 0.02$). The G allele at rs34621387 is associated with PD in vitamin D-deficient individuals in both data sets (OR = 2.0–2.1, confidence interval = 1.3–3.5, $p = 0.002$) but is not associated with PD in vitamin D-nondeficient individuals ($p > 0.8$ in both data sets).

Conclusions: Previous studies suggest that vitamin D deficiency is associated with PD and sustained opening of LVSCC contributes to the selective vulnerability of dopaminergic neurons in PD. Our data demonstrate that the association between genetic variations in *CACNA1C* and PD depends on vitamin D deficiency, providing one potential mechanism underlying the association between vitamin D deficiency and PD. *Neurol Genet* 2016;2:e72; doi: 10.1212/NXG.0000000000000072

GLOSSARY

25(OH)D = 25-hydroxyvitamin D; **DHP** = dihydropyridine; **GWAS** = genome-wide association studies; **LD** = linkage disequilibrium; **LVSCC** = L-type voltage-sensitive calcium channel; **NGRC** = NeuroGenetics Research Consortium; **PD** = Parkinson disease; **SN** = substantia nigra; **UDALL** = Morris K Udall Parkinson Disease Research Center of Excellence; **VDR** = vitamin D receptor.

Parkinson disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra (SN). Sustained opening of L-type voltage-sensitive calcium channels (LVSCC) in adult dopaminergic neurons in the SN has been suggested to cause excessive Ca^{2+} entry, leading to excessive mitochondrial stress and production of reactive oxygen species, rendering SN neurons vulnerable to cell death.¹ In the brain, $\text{Ca}_v1.2$, encoded by *CACNA1C*, accounts for approximately 90% of LVSCC activity, with $\text{Ca}_v1.3$, encoded by *CACNA1D*, accounting for the remaining activity.² $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ are antagonized by dihydropyridine (DHP) derivatives.

Supplemental data
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Both animal and retrospective human studies support a protective role of DHP derivatives in PD development.^{3–9} Nonetheless, genetic variations in *CACNA1C* or *CACNA1D* have not been studied with PD risk.

As a complex disease, PD has both genetic and environmental risk factors that act individually or through complex interactions to influence disease pathogenesis.^{10–18} Recently, several studies, including ours,¹⁹ have reported that lower circulating vitamin D concentration is associated with the risk of PD.^{20–22} Vitamin D is known to exert its biological actions by regulating the expression of many genes, providing a plausible molecular mechanism for gene-environment interactions. Previous studies have shown that vitamin D treatment decreases *CACNA1C* messenger RNA expression through the vitamin D receptor (VDR) in primary neuronal cultures.^{23,24} We hypothesize that *CACNA1C* and *CACNA1D* would be good candidate genes for PD and that the association of SNPs in these genes with PD might be modulated by circulating vitamin D concentrations.

METHODS Subjects. Subjects from 2 genome-wide association studies (GWAS) were included in the study. The first GWAS was conducted by the Morris K Udall Parkinson Disease Research Center of Excellence (UDALL) at the University of Miami,²⁵ and the second GWAS was conducted as part of the NeuroGenetics Research Consortium (NGRC).²⁶ Participant enrollment and clinical assessment were described previously in detail.^{25,26} In brief, cases were ascertained by neurology clinics associated with UDALL (from 1997 to 2003) or NGRC (from 2003 to 2009). Controls were ascertained from community outreach efforts or were spouses of individuals with PD or Alzheimer disease (M.A.P.-V., Principal Investigator). All participants with stored plasma samples available for vitamin D assessment were included in the study. Participants in this study are non-Hispanic whites by self-report and confirmed by principal component analysis of population structure in the GWAS.

Standard protocol approvals, registrations, and patient consents. All participants gave informed consent prior to these studies. All ascertainment protocols were approved by each contributing center's institutional review board.

Genetic markers. The UDALL data set was genotyped using Illumina 610-quad BeadChip (Illumina, San Diego, CA).²⁵ The NGRC data set was genotyped using Illumina HumanOmni1-Quad_v1-0_B BeadChip (Illumina).²⁶ Final marker sets after quality control (described in detail in these previous publications) were used for the imputation of untyped SNPs separately in each of the 2 data sets. Imputation allowed us to obtain genetic information on additional variants through the genome for a more thorough investigation. Both data sets were

imputed up to 38 million SNPs using IMPUTE2 and the 1000 Genomes reference panel (phase 1, March 2012).²⁷ During quality control, SNPs that had low imputation quality (info score <0.4) and low minor allele frequency (less than 1%) were removed from further analysis. SNPs within the 5-kb flanking regions of the start and end of *CACNA1C* and *CACNA1D* were examined for interaction with vitamin D status and association with PD.

Plasma vitamin D measurement. Stored plasma samples were analyzed using liquid chromatography tandem mass spectrometry at the Emory Clinical Translational Research Laboratory as described previously.¹⁹ In brief, the samples were analyzed using the methods outlined before.²⁸ Three quality control samples (approximately 6, 21, and 62 ng/mL) were included in duplicate at the beginning and end of each run. The assay was linear up to 200 ng/mL and had a limit of detection of 1 ng/mL. Total imprecision ranged from 10.8% to 17.1%. The assay was validated using the NIST 972a standards. The laboratory participates in the Vitamin D External Quality Assessment Scheme proficiency scheme. For both the discovery and replication data sets, the samples were organized into multiple batches for vitamin D measurement: each batch included ~220 samples with a balanced number of cases and controls, analyzed in random order with respect to affection status. Laboratory technicians were blinded to affection status.

Statistical analysis. Logistic regression analysis was used to evaluate the association between vitamin D status and PD, adjusting for age at sampling, sex, and sampling season in each data set separately using the R software package. Vitamin D deficiency was defined as having total 25-hydroxyvitamin D (25[OH]D) <20 ng/mL, and vitamin D insufficiency was defined as having total 25(OH)D <30 ng/mL. A joint test of SNP main effect and SNP-vitamin D interaction effect was conducted using a 2 degrees-of-freedom likelihood-ratio test.²⁹ This joint test compares a full model containing vitamin D deficiency, SNP dosage (additive genetic model), an SNP-vitamin D deficiency interaction term, and covariates (age at sampling, sex, and sampling season) to a restricted model with only vitamin D deficiency and covariates. Such an analysis will detect loci missed by main-effects analysis conducted in previous GWAS if the combined effect of the SNP effect and environmental interaction is stronger than the main effect of the SNP alone.

Besides a different genotyping platform, the 2 GWAS data sets are also different in distribution of vitamin D concentration, age, sex, and sampling season covariates (table 1). Therefore, joint tests were conducted in each data set separately. To achieve better statistical power while accommodating the heterogeneity between the 2 data sets, the joint test results in the 2 data sets were then combined using a meta-analysis approach. This meta-analysis used the joint meta-analysis extension of METAL.³⁰ To adjust for multiple testing, simpleM was used to estimate the effective number of independent tests taking into account linkage disequilibrium (LD) between SNPs³¹; this estimate of independent tests was used to determine a significant threshold for each gene, adjusting for the number of independent tests. To unravel the nature of the interactions between SNP dosage and vitamin D deficiency, stratification analysis was performed to evaluate the associations between SNPs and PD risk in vitamin D-deficient and vitamin D-nondeficient strata separately. The use of prevalent cases could lead to potential survival bias. Sensitivity analysis was carried out by duration of disease to test the effect of considering disease duration in the case definition on the identified association, i.e., cases with shorter duration ("near-incident" cases with less than 3 years of duration) or longer duration (3 years or longer).

Table 1 Clinical characteristics of subjects from the 2 independent data sets

	UDALL			NGRC		
	Patients with PD (N = 478)	Controls (N = 431)	p Value	Patients with PD (N = 484)	Controls (N = 412)	p Value
Sex, female, %	37	65	<0.0001	30	56	<0.0001
Age at sampling, mean $\pm$ SD, y	65 $\pm$ 17	70 $\pm$ 13	<0.0001	68 $\pm$ 11	65 $\pm$ 13	0.009
Age at onset, mean $\pm$ SD, y	56 $\pm$ 13	N/A	N/A	60 $\pm$ 12	N/A	N/A
Symptom duration, mean $\pm$ SD	15.3 $\pm$ 9.5	N/A	N/A	8.0 $\pm$ 6.2	N/A	N/A
Sampling season, July–December, %	48	49	0.8496	55	47	0.027
Plasma 25(OH)D total, mean $\pm$ SE	25.59 $\pm$ 0.56	30.14 $\pm$ 0.56	<0.0001	27.82 $\pm$ 0.52	29.94 $\pm$ 0.59	0.007

Abbreviations: NGRC = NeuroGenetics Research Consortium; PD = Parkinson disease; SE = standard error; UDALL = Morris K Udall Parkinson Disease Research Center of Excellence.

Additional sensitivity analyses were performed by age at onset and sex to test whether the identified association is restricted to early or later age at onset of PD and whether it is sex specific.

To investigate the potential biological basis for the gene-vitamin D interaction at the most significant SNP, 1000 Genomes phase 3 data were used to identify all SNPs that are in LD within a 1 million base pair region surrounding the significant SNP. In addition, publicly available data from CHIP-Seq experiments with VDR^{32,33} were also used to evaluate the functional relevance of SNPs.

RESULTS Characteristics of subjects in 2 data sets.

Table 1 displays the characteristics of participants from the 2 data sets. In both data sets, the percentage of women is lower in the PD cases than controls, consistent with prior observations of increased prevalence of PD in men. In the UDALL data set, the controls have older age at sampling than cases because of the intentional recruitment of controls at older ages to reduce the possibility of including controls who would develop PD after ascertainment. In the NGRC data set, the controls have younger age at sampling than cases. There is no difference in sampling season between cases and controls in UDALL; however, in NGRC, more PD cases were sampled between July and December (when higher vitamin D levels are usually observed for the same individual) than in controls. Owing to these differences in clinical characteristics and genotyping

platforms between the 2 data sets, we elected to perform individual analyses in each data set separately.

Lower vitamin D concentrations are associated with PD in both data sets. Total plasma 25(OH)D concentrations are significantly lower in PD cases than in controls in both data sets (table 1). Total 25(OH)D was also analyzed categorically, using the established clinical criteria for vitamin D deficiency (25[OH]D < 20 ng/mL) and vitamin D insufficiency (25[OH]D < 30 ng/mL). Cases with PD are significantly more likely than controls having vitamin D deficiency (25[OH]D < 20 ng/mL) and vitamin D insufficiency (25[OH]D < 30 ng/mL) in both data sets (table 2). In addition, a dosage effect was consistently observed in both data sets, with vitamin D deficiency more strongly associated with PD than was vitamin D insufficiency (odds ratio [OR] = 2.63 vs 2.13 in UDALL, OR = 1.56 vs 1.34 in NGRC).

Analysis of interaction between vitamin D deficiency and SNPs in *CACNA1C* and *CACNA1D*. Since the strongest association was found between vitamin D deficiency and PD, the initial gene-environment interaction analysis was focused on analyzing the association of PD with the joint effects of SNP dosage, and its interaction with vitamin D deficiency. In total, 1,244 SNPs in *CACNA1C* and 504 SNPs in *CACNA1D* were available for analysis. The effective number of independent tests after adjusting for LD is 274 and 175 in *CACNA1C* and *CACNA1D*, respectively. To maximize statistical power, we performed a meta-analysis across both data sets. The strongest evidence for a joint effect was found at SNP rs34621387 (meta-joint test $p = 7.5 \times 10^{-5}$, Bonferroni-adjusted $p = 0.02$) in the third intron of *CACNA1C* (figure, A). Within *CACNA1D*, the lowest meta-joint test p value is 0.02, which would not remain significant after adjusting for multiple testing (figure, B). To further evaluate the nature of the interaction, associations between rs34621387 and PD were evaluated in individuals who were vitamin D

Table 2 Association between vitamin D status and PD risk

	Prevalence, %		OR	95% CI	p Value
	Case	Control			
UDALL					
Vitamin D deficiency	36	17	2.64	1.88-3.71	<0.0001
Vitamin D insufficiency	71	54	2.13	1.58-2.89	<0.0001
NGRC					
Vitamin D deficiency	26	19	1.56	1.12-2.18	0.009
Vitamin D insufficiency	61	55	1.34	1.01-1.77	0.042

Abbreviations: CI = confidence interval; NGRC, NeuroGenetics Research Consortium; OR = odds ratio; PD = Parkinson disease; UDALL = Morris K Udall Parkinson Disease Research Center of Excellence.

Each point represents a common variant (minor allele frequency >5%) in *CACNA1C* (A) and *CACNA1D* (B). In total, 1,244 SNPs in *CACNA1C* and 504 SNPs in *CACNA1D* were analyzed. A joint test of SNP main effect and SNP-vitamin D interaction effect was conducted using a 2 degrees-of-freedom likelihood-ratio test that compares a full model containing vitamin D deficiency, SNP dosage, an SNP-vitamin D deficiency interaction term, and covariates to a restricted model with only vitamin D deficiency and covariates. Each circle represents the p value of the meta-analysis of the joint tests using both UDALL and NGRC data sets. The most significant SNP in each gene is displayed as a purple diamond. The color of the circles indicates LD (r^2) between each SNP and the top SNP. The blue line displays the recombination rates estimated from phase 2 HapMap. NGRC = NeuroGenetics Research Consortium; SNP = single nucleotide polymorphism; UDALL = Morris K Udall Parkinson Disease Research Center of Excellence.

deficient or vitamin D nondeficient separately in each data set. This analysis revealed that the association of rs34621387 and PD depends on vitamin D concentration: allele G is associated with PD in the vitamin D-deficient stratum in both data sets (OR = 2.0, $p = 0.002$ and OR = 2.1, $p = 0.002$) but is not associated with PD in vitamin D-nondeficient stratum in both data sets ($p > 0.8$) (table 3).

Sensitivity analysis suggested that this pattern of association (restricted to vitamin D-deficient individuals) is not affected by age at onset, disease duration, or sex (table e-1 at Neurology.org/ng).

Functional relevance of the intronic SNP rs34621387 in *CACNA1C*. No SNPs in substantial LD ($r^2 > 0.2$) with rs34621387 were found to be located in a

Table 3 Association between rs34621387 and PD in the presence or absence of vitamin D deficiency

	SNP-PD Association			p Value for SNP-vitamin D deficiency interaction	Minor allele (G) frequency	
	OR	95% CI	p Value		Case	Control
UDALL				0.007		
Vitamin D-deficient stratum (case = 172; control = 75)	2.02	1.31-3.17	0.002		0.50	0.36
Vitamin D-nondeficit stratum (case = 305; control = 355)	1.02	0.80-1.31	0.871		0.48	0.48
NGRC				0.007		
Vitamin D-deficient stratum (case = 127; control 79)	2.12	1.33-3.45	0.002		0.52	0.36
Vitamin D-nondeficit stratum (case = 355, control = 333)	0.99	0.79-1.25	0.95		0.45	0.45

Abbreviations: CI = confidence interval; NGRC, NeuroGenetics Research Consortium; OR = odds ratio; PD = Parkinson disease; SNP = single nucleotide polymorphism; UDALL = Morris K Udall Parkinson Disease Research Center of Excellence.

known VDR binding site, as delimited by reported Chip-Seq experiments.^{32,33} We used RegulomeDB³⁴ and HaploReg³⁵ to determine whether rs34621387 or its proxies are located within any other regulatory elements. This analysis found that rs34992881 ($r^2 = 0.76$ with rs34621387) is located within a region displaying epigenomic signatures for chromatin enhancer, which include histone H3 lysine 4 trimethylation (H3K4me3), H3K27 acetylation (H3K27ac), and H3K9ac. In addition, the enhancer signatures are only detected in some of the tissues or cell types surveyed by the Roadmap Epigenome project, suggesting the possibility of tissue- or cell-specific regulation of gene expression of *CACNA1C*.

DISCUSSION It is well established that vitamin D exerts its main biological actions by regulating the expression of a large array of genes and one of them is *CACNA1C*.^{23,24,32,33} Using 2 case-control data sets where vitamin D deficiency is significantly associated with PD, we found that vitamin D status significantly modified the association between SNPs in *CACNA1C* and PD: genetic effects of *CACNA1C* only manifest in individuals with vitamin D deficiency, but not in individuals who are not vitamin D deficient. As such, the effect of *CACNA1C* in PD would have become difficult to detect in a sample where a substantial portion of individuals have sufficient vitamin D levels, if only SNP main effects were examined. Our study demonstrates the importance of incorporating environmental exposure to understand the genetic underpinning for interindividual phenotypic variance in PD.

Using existing databases, no definitive evidence for regulatory potential at rs34621387 or its proxies was observed. Therefore, the biological mechanism underlying the association of rs34621387 and PD in the

presence of vitamin D deficiency is currently not understood. Interestingly, SNP rs1006737, in the same intron (intron 3) as rs34621387 in *CACNA1C*, has been widely replicated in association with schizophrenia and other neuropsychiatric conditions, making it one of the most robust findings in psychiatric genetics.³⁶⁻³⁸ Similar to rs34621387, no compelling evidence for regulatory function was initially found for rs1006737 or proxies, using existing databases. Subsequent studies revealed that SNPs in LD with rs1006737 interact with the proximal promoter region and direct allelic-specific expression of *CACNA1C* in human induced pluripotent stem cell-derived neurons, providing a molecular basis for the SNP-phenotype association.^{39,40} SNP rs1006737 and rs34621387 are not in strong LD with each other ($r^2 < 0.1$). Whether rs34621387 confers genetic risk to PD by directing allelic-specific expression of *CACNA1C*, in a similar fashion as rs1006737 in schizophrenia, will require further, more in-depth functional studies.

Previous studies have shown that vitamin D inhibits the expression of *CACNA1C*.^{23,24} One possible mechanism underlying the observed interaction is that high vitamin D levels effectively suppress LVSCC expression and thus reduce the activity of LVSCC in the dopaminergic neurons in SN, masking the effect of rs34621387.

It has been proposed that Ca_v1.3, encoded by *CACNA1D*, might be particularly responsible for the selective death of dopaminergic neurons in the SN based on the observation that Ca_v1.3 channels open at relatively hyperpolarized membrane potentials, which makes them more suitable for pacemaking.¹ We did not find any evidence for association between genetic variants in *CACNA1D* and PD, even after taking into account vitamin D status, which is consistent with the lack of evidence suggesting that *CACNA1D* is regulated by vitamin D.^{23,24} Our study,

however, does not exclude the possibility that genetic variations in *CACNA1D* are involved in PD pathogenesis as our study is limited by a modest sample size, a focus on common variants, and interactions with vitamin D concentration. In addition, we used an ethnically homogeneous population (i.e., non-Hispanic whites) to minimize confounding by population substructure. Additional studies are needed to assess generalizability to other populations.

As in other case-control association studies of vitamin D concentration and PD, our results could reflect “reverse causation,” i.e., lower vitamin D concentrations in patients with PD are a consequence of the disease (lower levels of sun exposure due to reduced mobility and less time spent outdoors) rather than a cause. However, a previous longitudinal study demonstrated that lower vitamin D levels at baseline are associated with increased risk of PD during a 29-year follow-up²⁰; a second study showed that patients with early, nondisabling PD and relatively normal mobility have lower vitamin D levels²¹; and our own work demonstrated that lower vitamin D₂, a form of vitamin D that is obtained independently from sunlight exposure, is associated with PD, as is vitamin D₃ (the form of vitamin D that is mainly obtained from sunlight exposure).¹⁹

Our data demonstrate that genetic variations in *CACNA1C* and vitamin D deficiency act together to increase PD risk. This study also highlights the importance of incorporating environmental exposure to identify genetic factors influencing interindividual phenotypic variance in PD.

AUTHOR CONTRIBUTIONS

L.W. and W.K.S. conceived and designed the experiments. J.L.H., C.P.Z., H.P., M.A.P.-V., and J.M.V. were responsible for collecting biosamples and obtaining genotype data. M.L.E. and J.C.R. were responsible for measuring vitamin D concentrations. L.M., G.W.B., and E.R.M. imputed genotype data and performed statistical analyses. L.W., W.K.S., and J.M.V. interpreted the data. L.W. wrote the manuscript. W.K.S. and J.M.V. edited the manuscript. All authors read and approved the final manuscript.

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DISCLOSURE

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REFERENCES

1. Surmeier DJ, Schumacker PT. Calcium, bioenergetics, and neuronal vulnerability in Parkinson's disease. *J Biol Chem* 2013;288:10736–10741.
2. Sinnegger-Brauns MJ, Huber IG, Koschak A, et al. Expression and 1,4-dihydropyridine-binding properties of brain L-type calcium channel isoforms. *Mol Pharmacol* 2009;75:407–414.
3. Chan CS, Guzman JN, Ilijic E, et al. “Rejuvenation” protects neurons in mouse models of Parkinson's disease. *Nature* 2007;447:1081–1086.
4. Wang R, Ma Z, Wang J, Xie J. L-type Cav1.2 calcium channel is involved in 6-hydroxydopamine-induced neurotoxicity in rats. *Neurotox Res* 2012;21:266–270.
5. Kupsch A, Sautter J, Schwarz J, Riederer P, Gerlach M, Oertel WH. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in non-human primates is antagonized by pretreatment with nimodipine at the nigral, but not at the striatal level. *Brain Res* 1996;741:185–196.

6. Ilijic E, Guzman JN, Surmeier DJ. The L-type channel antagonist isradipine is neuroprotective in a mouse model of Parkinson's disease. *Neurobiol Dis* 2011;43:364–371.
7. Mosharov EV, Larsen KE, Kanter E, et al. Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. *Neuron* 2009;62:218–229.
8. Pasternak B, Svanstrom H, Nielsen NM, Fugger L, Melbye M, Hviid A. Use of calcium channel blockers and Parkinson's disease. *Am J Epidemiol* 2012;175:627–635.
9. Ritz B, Rhodes SL, Qian L, Schernhammer E, Olsen JH, Friis S. L-type calcium channel blockers and Parkinson disease in Denmark. *Ann Neurol* 2010;67:600–606.
10. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum Mol Genet* 2009;18:R48–R59.
11. Nalls MA, Pankratz N, Lill CM, et al. Large scale meta analysis of genome-wide association data in Parkinson's disease reveals 6 novel risk loci. *Nat Genet* 2014;46:989–993.
12. Elbaz A, Clavel J, Rathouz PJ, et al. Professional exposure to pesticides and Parkinson disease. *Ann Neurol* 2009;66:494–504.
13. Chen H, Jacobs E, Schwarzschild MA, et al. Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease. *Ann Neurol* 2005;58:963–967.
14. Hernan MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann Neurol* 2002;52:276–284.
15. Scott WK, Zhang F, Stajich JM, Scott BL, Stacy MA, Vance JM. Family-based case-control study of cigarette smoking and Parkinson disease. *Neurology* 2005;64:442–447.
16. Hancock DB, Martin ER, Stajich JM, et al. Smoking, caffeine, and nonsteroidal anti-inflammatory drugs in families with Parkinson's disease. *Arch Neurol* 2007;64:576–580.
17. Hamza TH, Chen H, Hill-Burns EM, et al. Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. *PLoS Genet* 2011;7:e1002237.
18. Hancock DB, Martin ER, Fujiwara K, et al. NOS2A and the modulating effect of cigarette smoking in Parkinson's disease. *Ann Neurol* 2006;60:366–373.
19. Wang L, Evatt ML, Maldonado LG, et al. Vitamin D from different sources is inversely associated with Parkinson disease. *Mov Disord* 2015;30:560–566.
20. Knekt P, Kilkkinen A, Rissanen H, Marniemi J, Saaksjarvi K, Heliövaara M. Serum vitamin D and the risk of Parkinson disease. *Arch Neurol* 2010;67:808–811.
21. Evatt ML, Delong MR, Khazai N, Rosen A, Triche S, Tangpricha V. Prevalence of vitamin D insufficiency in patients with Parkinson disease and Alzheimer disease. *Arch Neurol* 2008;65:1348–1352.
22. Ding H, Dhima K, Lockhart KC, et al. Unrecognized vitamin D3 deficiency is common in Parkinson disease: Harvard biomarker study. *Neurology* 2013;81:1531–1537.
23. Brewer LD, Thibault V, Chen KC, Langub MC, Landfield PW, Porter NM. Vitamin D hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons. *J Neurosci* 2001;21:98–108.
24. Gezen-Ak D, Dursun E, Yilmazer S. The effects of vitamin D receptor silencing on the expression of LVSCC-A1C and LVSCC-A1D and the release of NGF in cortical neurons. *PLoS One* 2011;6:e17553.
25. Edwards TL, Scott WK, Almonte C, et al. Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. *Ann Hum Genet* 2010;74:97–109.
26. Hamza TH, Zabetian CP, Tenesa A, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet* 2010;42:781–785.
27. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;5:e1000529.
28. Lensmeyer G, Poquette M, Wiebe D, Binkley N. The C-3 epimer of 25-hydroxyvitamin D(3) is present in adult serum. *J Clin Endocrinol Metab* 2012;97:163–168.
29. Kraft P, Yen YC, Stram DO, Morrison J, Gauderman WJ. Exploiting gene-environment interaction to detect genetic associations. *Hum Hered* 2007;63:111–119.
30. Manning AK, LaValley M, Liu CT, et al. Meta-analysis of gene-environment interaction: joint estimation of SNP and SNP x environment regression coefficients. *Genet Epidemiol* 2011;35:11–18.
31. Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008;32:361–369.
32. Ramagopalan SV, Heger A, Berlanga AJ, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res* 2010;20:1352–1360.
33. Meyer MB, Benkusky NA, Lee CH, Pike JW. Genomic determinants of gene regulation by 1,25-dihydroxyvitamin D3 during osteoblast-lineage cell differentiation. *J Biol Chem* 2014;289:19539–19554.
34. Boyle AP, Hong EL, Hariharan M, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res* 2012;22:1790–1797.
35. Yeates TO. Determination of the correct reference frame from an atomic coordinate list. *Acta Crystallogr A* 1990;46:625–626.
36. Erk S, Meyer-Lindenberg A, Schnell K, et al. Brain function in carriers of a genome-wide supported bipolar disorder variant. *Arch Gen Psychiatry* 2010;67:803–811.
37. Ripke S, O'Dushlaine C, Chambert K, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet* 2013;45:1150–1159.
38. Ripke S, Sanders AR, Kendler KS, et al. Genome-wide association study identifies five new schizophrenia loci. *Nat Genet* 2011;43:969–976.
39. Roussos P, Mitchell AC, Voloudakis G, et al. A role for noncoding variation in schizophrenia. *Cell Rep* 2014;9:1417–1429.
40. Yoshimizu T, Pan JQ, Mungenast AE, et al. Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. *Mol Psychiatry* 2015;20:284.

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