Neurobiology of Aging 35 (2014) 1212.e11–1212.e13

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Negative results

Vitamin D receptor genetic variants and Parkinson's disease in a Taiwanese population

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ARTICLE INFO

Article history:
Received 6 July 2013
Received in revised form 3 October 2013
Accepted 26 October 2013
Available online 29 October 2013

Keywords:
Parkinson's disease
Vitamin D
Vitamin D receptor gene
Taiwan

ABSTRACT

Patients with Parkinson's disease (PD) have hypovitaminosis D status and genetic variants of vitamin D receptor (VDR) gene are recently shown to be associated with PD in a large-scale genome-wide association study in a Caucasian population. Few studies examined *VDR* genetic variants in large-scale Asian patients with PD. We therefore genotyped 6 *VDR* genetic variants in a total of 1492 Taiwanese subjects, including 700 patients with PD and 792 age and/or gender matched control subjects. We did not observe any significant associations between the studied genetic variants of *VDR* and the risk of PD. Our data suggest that genetic variations of the *VDR* gene did not play a major role in a Taiwanese PD population. Further studies of *VDR* and its interaction with serum vitamin D levels are warranted to clarify the potential role of vitamin D in PD pathogenesis.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder arising from the complex interaction of genetic and environmental factors (Lin et al., 2011b; Ross and Smith, 2007). Vitamin D, which is now considered a hormone rather than a vitamin, regulates numerous processes related to neuronal survival (Holick, 2007) and is recently shown as an environmentally modifiable factor in the pathogenesis of PD (Evatt et al., 2011; Newmark and Newmark, 2007). The vitamin D receptor (VDR) is the primary mediator of vitamin D's biological actions. The VDR is widely expressed in both the central and peripheral nervous systems. The highest expression level is found in the hypothalamus and in the dopaminergic neurons of the substantia nigra (Eyles et al., 2005), which expression pattern supports the notion that VDRs may play a role in the pathogenesis of PD.

Recently, the effects of genetic variants in the *VDR* gene have gained attention in neurodegenerative disorders (Beecham et al., 2009; Evatt et al., 2008; Gezen-Ak et al., 2007; Smolders et al., 2009). A genome-wide association study of late-onset Alzheimer's disease has found a strong association between single nucleotide polymorphisms (SNPs) near the 5′ end of *VDR* and risk of Alzheimer's disease in a Caucasian population (Beecham et al., 2009). Furthermore, 1 *VDR* genetic polymorphism, the BsmI major b allele (rs1544410), was reported to be associated with the incidence of PD in a Korean population (Kim et al., 2005). Another *VDR* variant, the FokI major C allele (rs10735810), was also found to be associated with PD in a Chinese population (Han et al., 2012). However, while 1 large-scale genome-wide association study found no association between these 2 polymorphisms and the risk of PD, it demonstrated that several *VDR* genetic variants in the 5′ end region of *VDR* are associated with risk of PD in a Caucasian population (Butler et al., 2011). Notably, recent studies have identified several PD candidate genes that contain putative VDR binding sites, including the *GAK* and *LRRK2* genes (Bai et al., 2010; Ramonet et al., 2011). These findings reinforce the possibility that *VDR* genetic variations may play a role in the pathogenic mechanism of PD.

We have previously performed comprehensive mutation analysis of multiple candidate genes in a cohort of patients with PD from Taiwan (Lee et al., 2009; Lin et al., 2008a, 2008b, 2011a; Wu et al., 2005). However, the major genetic causes in most patients with PD, especially early-onset ones, in our population are still unclear. Given the recent evidence that VDR genetic variants are involved in PD, we conducted a large-scale case-control study to investigate multiple VDR polymorphisms in a large Taiwanese cohort.

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0197-4580/$ – see front matter © 2014 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.10.094

2. Methods

A total of 1492 study participants, including 700 patients with PD and 792 age and/or gender matched control subjects, were included in this study. Among PD group, 527 were sporadic late-onset patients with PD, and 173 were early-onset patients with PD (age of onset <50 years). None had a family history of PD or were from consanguineous families. Mutations in the *a-synuclein, Parkin*, *PINK1*, *DJ-1*, *LRRK2, SCA2, SCA3*, *ATP13A2,* and *HTRA2* genes were excluded in all early-onset patients with PD. The diagnosis of PD was based on the UK PD Society Brain Bank clinical diagnostic criteria (Hughes et al., 1992). Unrelated adult volunteers without neurologic disease were recruited as control subjects from the community and from our hospital. Informed consent was taken from all the study participants, and the study was approved by the institutional ethics board committees.

DNA was extracted from venous blood using standard protocols (Lin et al., 2008a). We genotyped 6 SNPs of the *VDR* gene, including rs2853559, rs4334089, rs7299460, rs7968585, rs7976091, and rs10083198 using TaqMan genotyping assays on the StepOne Plus real-time polymerase chain reaction machine (Applied Biosystems, Foster City, CA, USA). These SNPs are all located in the 5′ untranslated region (UTR) of the *VDR* gene and are associated with either the risk or early-onset age of PD (Butler et al., 2011). The sequences of the primers used in this study are listed in supplementary Table 1.

The Hardy-Weinberg equilibrium for genotype frequency was determined in cases and control subjects. The χ^2 test was used to compare the frequencies of the alleles and genotypes in cases and control subjects. Logistic regression was used to test for associations between genotype and PD using an additive model, and the group that was homozygous for the more common allele was used as the baseline risk group. p values were generated using a Wald test. A 2-tailed p value <0.05 was considered significant. Power calculations were completed using the CaTs Genetic Power Calculator (Skol et al., 2006) with settings for a multiplicative genetic model. The prevalence of PD in Taiwan is estimated to be 130 cases per 100,000 individuals (Chen et al., 2001), and the odds ratios for each risk allele of the tested genetic variants was approximately 1.3 as estimated in previous studies (Han et al., 2012). Statistical analysis was performed using STATA version 8.0.

3. Results

The mean age of the patients with PD at symptom onset was 57.6 ± 11.8 years (range, 38–72 years), and the mean age of the patients with PD at study enrollment was 68.7 ± 11.2 years (range, 42–80 years). The patient with PD cohort included 395 men and 305 women (male: female ratio, 1.3:1). We did not detect any deviations from the Hardy-Weinberg equilibrium in the genotype frequencies of PD patients or control subjects. The frequencies of the genotypes and alleles of 6 *VDR* genetic variants are shown in Table 1. We did not observe any significant associations between the studied 6 genetic variants of *VDR* and the risk of PD in our population. The statistical power of the tested SNPs was 93% in our sample.

Table 1
Distribution of genotype polymorphisms and estimated odds ratio (OR) in relation to Parkinson's disease (PD) risk

	Patients with PD N = 700	Control subjects N = 792	OR (95% CI)	p
rs2853559 (chromosome location: 48282805)				
GG	305 (43.5)	314 (39.6)	1.00	
GA	312 (44.6)	383 (48.4)	0.84 (0.67–1.06)	0.15
AA	83 (11.9)	95 (12.0)	0.90 (0.63–1.29)	0.58
AA + GA versus GG			0.85 (0.68–1.06)	0.16
rs4334089 (chromosome location: 48286015)				
GG	223 (31.9)	245 (30.9)	1.00	
GA	361 (51.5)	409 (51.7)	0.93 (0.74–1.17)	0.55
AA	116 (16.6)	138 (17.4)	0.82 (0.60–1.12)	0.21
AA + GA versus GG			1.06 (0.86–1.31)	0.57
rs7299460 (chromosome location: 48296268)				
TT	239 (34.1)	275 (34.7)	1.00	
CT	349 (49.8)	393 (49.6)	1.08 (0.85–1.38)	0.53
CC	112 (16.1)	124 (15.7)	1.25 (0.90–1.73)	0.18
TT + CT versus CC			1.12 (0.89–1.42)	0.33
rs7968585 (chromosome location: 48232093)				
CC	369 (52.7)	421 (53.2)	1.00	
CT	270 (38.6)	298 (37.6)	1.03 (0.83–1.28)	0.76
TT	61 (8.7)	73 (9.2)	0.95 (0.66–1.38)	0.80
CC + CT versus TT			1.02 (0.83–1.25)	0.86
rs7976091 (chromosome location: 32517508)				
CC	234 (33.4)	250 (31.6)	1.00	
CT	361 (51.6)	401 (50.6)	0.96 (0.75–1.23)	0.77
TT	105 (15.0)	141 (17.8)	0.79 (0.57–1.11)	0.18
TT + CT versus CC			0.92 (0.73–1.16)	0.49
rs10083198 (chromosome location:46582232)				
CC	213 (30.4)	259 (32.7)	1.00	
CT	343 (49.0)	389 (49.1)	1.07 (0.85–1.35)	0.57
TT	144 (20.6)	144 (18.2)	1.06 (0.78–1.43)	0.71
CT + TT versus CC			1.07 (0.86–1.33)	0.57

Chromosome position is based on National Center for Biotechnology Information genome build 36.3.
Key: CI, confidence interval; OR, odds ratio.

4. Discussion

We conducted a case-control association analysis of multiple genetic variants of the *VDR* gene in a Taiwanese population. We did not observe the genetic variants in the 5′ UTR of *VDR* associated with the risk of PD.

The previously reported risk SNPs of *VDR* are located in introns between the alternatively spliced first exons and generate alternative *VDR* transcripts with different 5′ UTR regions (Butler et al., 2011 and literature data are summarized in Table 2). Although the functions of these alternative transcripts of *VDR* in the brain have not been studied extensively, SNP-mediated allelic-specific alternative splicing may underlie the intronic SNP association with PD in

Table 2
Summary of published data for genetic variants of vitamin D receptor (VDR) gene and risk of Parkinson's disease (PD)

Study group	Ethnicity	Case number	Genetic variants	MAF (PD case)	MAF (control)	OR (95% CI)	p
Han et al., 2012	Chinese	260 PD; 282 controls	rs1544410	0.08	0.07	1.15 (0.73–1.81)	0.57
			rs10735810	0.35	0.39	1.34 (1.04–1.72) for common C allele	0.02
Suzuki et al., 2012	Japanese	137 PD patients	rs1544410	0.12	N.A.	N.A.	
			rs10735810	0.41	N.A.	N.A., but wild type CC genotype had a milder form of PD (0.32; 0.16–0.66)	0.002
			rs11568820	0.42	N.A.	N.A.	
			rs7976091	0.24	N.A.	N.A.	
			rs731236	0.11	N.A.	N.A.	
Kim et al., 2005	Koreans	85 PD; 231 controls	rs1544410	0.09	0.14	N.A.	0.04

Key: CI, confidence interval; MAF, minor allele frequency; N.A, not available; OR, odds ratio; PD, Parkinson's disease.

previous studies. Our results are not supportive with these findings that genetic variants in the 5′ UTR of *VDR* increase the risk of PD (Butler et al., 2011). In addition to possible ethnic differences, we speculate one of the reasons for the result difference is the serum vitamin D status, which we did not measure in our study, may influence the effect size of a *VDR* polymorphism and therefore influence the findings of association.

There are some limitations in our study. We lack serum vitamin D levels in individual subjects and information of sunlight exposure, which could influence the serum level of active vitamin D. It has been found that vitamin D status modulated the association between *VDR* polymorphisms and incidence of type I diabetes mellitus with the odds ratio of the risk allele increasing with higher vitamin D levels in the studied population (Ponsonby et al., 2008). It has been speculated that the *VDR* polymorphisms only manifest phenotypic variations in the presence of certain vitamin D level. This hypothesis is supported by a recent report that a positive association between *VDR* genetic variants and multiple sclerosis has only been found in studies conducted in regions at lower latitudes but not in regions at higher latitudes, which correlates with duration of sun exposure and therefore with active vitamin D levels (Smolders et al., 2009). Therefore, it is important to include assessment of vitamin D levels in future studies to fully characterize the genetic effects of VDR polymorphisms in PD.

In conclusion, our data suggest that genetic variations in the 5′ UTR of the *VDR* gene did not play a major role in a Taiwanese PD population. Further studies of *VDR* and its interaction with serum vitamin D levels in PD are warranted to clarify the potential role of vitamin D in PD pathogenesis.

Disclosure statement

The authors report no actual or potential conflicts of interest.

Acknowledgements

The authors thank the participants of the study and are grateful to the National Taiwan University Hospital for financial support of this work (grant NTUH#102-S2072). The authors thank the staff of the Second Core Laboratory, Department of Medical Research, and National Taiwan University Hospital for providing expert technical support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.10.094.

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