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ORIGINAL ARTICLE

Vitamin D receptor gene variants in Parkinson's disease patients

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Parkinson's disease

Abstract *Background:* Vitamin D plays an important role in neurodegenerative disorders as a crucial neuro-immunomodulator. Accumulating data provide evidences that vitamin D receptor (VDR) gene is a candidate gene for susceptibility to Parkinson's disease (PD).

Aim: To find out whether the risk of the development of sporadic PD might be influenced by VDR gene polymorphisms in an Iranian population or not.

Subjects and methods: A genetic study was conducted to investigate the relationship between VDR gene polymorphisms and the severity of PD. Fifty-nine PD patients and 53 matched-healthy controls were genotyped using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis. For this purpose, four single nucleotide polymorphisms (SNPs) in VDR gene including *Fok*I T > C (rs 10735810), *Bsm*I A > G (rs 1544410), *Apa*I A > C (rs 7975232), and *Taq*I C > T (rs 731236) have been evaluated.

Results: Our genotyping studies revealed that holding *Apa*I a allele and *Fok*I f allele could significantly increase the risk of developing Parkinson's disease 1.85 and 2.46 times, respectively ($p = 0.023$ and 0.008). Moreover, Aa heterozygous of *ApaI* also shows a significantly elevated risk of developing PD when compared to AA homozygous (OR = 7.44, $p = 0.005$). For *Bsm*I and *Taq*I polymorphisms, no significant difference in genotype or allele distribution was found between PD patients and the controls. Moreover, in this study, no significant association was found between different genotypes and Hoehn & Yahr staging and Unified Parkinson Disease Rating Stage (UPDRS) rating scale.

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Conclusion: This study demonstrates a possible association between the VDR *Fok*I and *Apa*I polymorphism and PD, indicating that VDR polymorphisms may change genetic susceptibility to sporadic PD in the Iranian population.

1. Introduction

Parkinson's Disease (PD) is known as the most common neurodegenerative movement disorder in elderly people [1]. Recently, vitamin D3 has been suggested as an environmentally modifiable factor, effective in the pathogenesis of PD [2]. A significantly lower 25-hydroxyvitamin (25OH) D levels were observed both in Caucasian [3] and Japanese patients with more severe PD [4,2]. On the other hand, expression of vitamin D receptor (VDR) is tremendously high in neurons of the substantia nigra, where dopaminergic neurons are selectively lost in PD [5]. In previous studies, besides the neuroprotective effects of vitamin D3 on cells of the nervous system [6], there were some epidemiological evidences confirming the potential value of vitamin D in PD prevention [7,2].

However, multiple genetic studies have revealed an association between the risk of PD with polymorphism in the VDR gene [2,8,9], although the results are conflicting [10]. Based on genome-wide association studies, the correlations between VDR polymorphisms and both risk and age at the onset of PD have been investigated in a Caucasian population [2,9]. Moreover, overexpression of the *BsmI* A > G (rs 1544410) polymorphism was reported among Korean PD patients [8]. Recent studies have shown a significant correlation between VDR *FokI* T > C (rs 10735810) genotype and PD in the Japanese and Chinese Han populations [2,11]. In contrast to these results, Petersen et al. found no difference in genotype (*ApaI* A > C (rs 7975232), *BsmI*, *TaqI* C > T (rs 731236)) frequencies between PD cases and controls in the VDR polymorphisms in Faroe Island [12].

Previous data have shown that genetic variation in VDR gene could induce serious defects of receptor activation by altering the affinity of the receptor to vitamin D [13]. However, there has been no report on the severity of PD and vitamin D gene variants in the Iranian population. Hence, a genetic study was conducted to investigate the relationship between VDR gene polymorphisms and both risk and severity of PD.

2. Subjects and methods

2.1. Patient selection for genetic analysis

The case–control study of the Iranian PD patients was conducted during September to November 2011, as it has been previously described [14,15]. The people gave an informed consent and agreed to participate in the current study. The ethics committee of the Isfahan University of Medical Sciences approved the protocol for this study. It was according to Declaration of Helsinki.

All PD cases were examined clinically by the same experienced neurologist according to the United Kingdom Parkinson's Disease Brain Bank criteria [16]. Then, the Hoehn and Yahr scale (H&Y) was used for clinical staging according to four stages of 1–1.5, 2–2.5, 3, and 4–5 [17] as well as the motor part of the Unified Parkinson's Disease Rating Scale III (UPDRS III) [18]. Exclusion criteria included having a history of taking vitamin D or being diagnosed to have familial background or early onset of PD (< 40 years old) and lack of voluntary participation for DNA extraction. Finally, 59 PD patients and 53 age-sex matched controls were chosen for genetic analysis.

After receiving consent from patients and controls, a blood sample was taken and DNA was isolated using a standard salting out procedure. The presence of the VDR *Fok*I T > C (rs 10735810), *Bsm*I A > G (rs 1544410), *Apa*I A > C (rs 7975232), and *Taq*I C > T (rs 731236) SNPs were identified by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) according to the manufacturer's instructions. The primer information including their annealing temperature, PCR product length before and after the digestion and the exact position of amplification is listed in Table 1.

DNA fragments were separated in 2% agarose gels and visualized by Gel Red staining. The presence of the *Fok*I T > C (rs 2228570), *Bsm*I G > A (rs 1544410), *Apa*I C > A (rs 7975232), and *Taq*I T > C (rs 731236) polymorphisms was also confirmed by repeated PCR–RFLP analysis. Allelic nomenclature of dominant (FBAT) alleles is based on endonuclease success over its restriction sites *Fok*I, *Bsm*I, *Apa*I and *Taq*I. In view of that, recessive (fbat) alleles were used when the above restriction endonucleases fail to cut their corresponding DNA molecules.

2.2. Statistical analysis

Statistical analysis was carried out using online SISA software available at: http://www.quantitativeskills.com/sisa/index.htm. p-values less than 0.05 were considered statistically significant and odds ratios (OR) with 95% confidence intervals (95% CI) were calculated to judge the correlations between specific factors and the risk of developing PD.

3. Results

Allelic comparison of four polymorphic sites of VDR illustrated that *Apa-I* (*a*) and *Fok-I* (f) recessive alleles were very significantly related to the risk of developing PD (Table 2). As indicated in Table 2 and 51% of PD patients carry *a* allele, while only 36% of control individuals hold that allele. The same results were also recorded for *Fok-I* as 28.8% of Parkinson's disease patients carry *f* allele and only 14.1% of healthy individuals carry the same allele. As depicted in Table 2, holding *a* allele and *f* allele could significantly increase the risk of developing PD by 1.85 and 2.46, respectively (p value = 0.023 and 0.008).

Based on the significant allelic relationship, further analysis on genotypes was encouraged. As expected from allelic