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HLA-DRA is associated with Parkinson's disease in Iranian population

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Summary

The rs3129882, a noncoding variant in *HLA-DR*, was found to be associated with Parkinson's disease (PD) using several genome-wide association studies. The aim of this replication study was to explore the relationship between this variant and PD in Iranian population. Genomic DNA was extracted from peripheral blood samples, and the rs3129882 SNP was genotyped using a PCR-RFLP method in 520 PD patients and 520 healthy Iranian controls. Significant differences were found in allele frequencies between patients and controls (χ^2 = 4.64, P = 0.031). Under additive and dominant models, the association of the SNP with PD risk is significant, where the A allele was observed to be protective. The results suggest that rs3129882 polymorphism may be a risk factor for PD in Iranian. This is the first study reporting such an association in this population. More replication studies are needed to confirm this data.

Introduction

Parkinson's disease (PD; OMIM 168600) is a progressive neurodegenerative disorder, which impairs the patient's movement abilities. PD is estimated to affect about 1–2% of the population over the age of 65 years (Darvish *et al.*, 2013a,b; Emamalizadeh *et al.*, 2014). Based on existent knowledge, PD is caused by numerous environmental and genetic factors, which underlie the loss of nigral dopaminergic neurons (Schapira & Jenner, 2011; Welzel & Walsh, 2011). To date, at least 13 single genes contributing to mendelian forms of PD have been detected (Lesage & Brice, 2009). However, susceptibility variants are mainly involved in sporadic form of PD, and several risk factors have been identified via genome-wide association studies to be associated with the disease Pihlstrom *et al.* (2013). Single nucleotide polymorphisms (SNPs) such as rs823128, rs11248051 and rs3129882 has identified as risk factors for the disease (Simon-Sanchez *et al.*, 2009; Hamza *et al.*, 2010; Chang *et al.*, 2011). The latest one, which is an SNP within Human Leucocyte Antigens (HLA) region, was subject of various association studies among different populations. A recent publication was a study performed on Caucasian populations. In this investigation rs3129882 (A/G), a noncoding variant located in intron 1 of *HLA-DRA*, was shown to be strongly associated with sporadic PD (Hamza *et al.*, 2010).

In this study, we carried out a replication study on Iranian patients and controls to determine the association between this variant and sporadic PD.

Materials and methods

Subjects

This study was performed on a total of 1040 subjects comprising 520 unrelated Iranian patients and 520 healthy controls. The mean age of patients was 59.5 ± 14.5 years and was composed of 278 men and 242 women (male:female ratio, 1.14:1). The two groups had no significant differences in distributions of age and gender. Besides, two studied groups were adjusted for the ethnic origin and area of residences. The diagnostic criteria for PD were based upon the UK Parkinson's disease Society Brain Bank Clinical Diagnostic Criteria, and the disease was diagnosed by

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a neurologist. Controls were individuals free of PD or a related movement disorder at the time of examination. Informed consent was obtained from all patients and family members who participated in the study. The study was approved by the ethic committee at Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Genetic analysis

Genomic DNA was extracted from peripheral blood samples following a standard salting out protocol (Miller *et al.*, 1988). The rs3129882 variant in intron 1 of *HLA-DRA* was genotyped through a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as previously described (Chiang *et al.*, 2012). Primers sequence and the enzyme recognition site were as follows: 5′AGATAAAAGGT GGAATTGCCAAGGT3′ (forward primer), 5′CACTG TTTACATTTTAGAGTCAGAAGCTTTT3′ (reverse primer) and GAANNNNTTC (*Pdm*I). The underlines in the reverse primer sequence and *Pdm*I recognition site indicate the mismatch nucleotide and polymorphic site, respectively. The PCR was performed in a reaction containing 150 ng genomic DNA, 0.3 mM of each primer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$ and 0.6 U Taq polymerase. The PCR profile consisted of 95°C for 5 min followed by 35 cycles of 95°C for 45 s, 60°C for 45 s and 72°C for 40 s and a final extension step of 72°C for 5 min. The Products were digested with the *Pdm*I (Thermo Scientific, Vilnius, Lithuania) and separated on a 3.0% agarose gel (allele A, 210-bp fragment; allele G, 183- and 27-bp fragments). The accuracy of the genotyping method was confirmed by the Sanger sequencing of number of PCR fragments (15 AA, 15 AG and 15 GG).

Statistical analysis

Fisher's exact test was used to confirm the Hardy–Weinberg equilibrium (HWE) of rs3129882 in both cases and controls. Pearson's χ^2-tests were applied to test for significance indifferences of genotype and allele frequencies between two groups. We also examined the association of rs3129882 (A/G) with the risk of PD, under three models (additive (G/G = 0, G/A = 1 and A/A = 2), dominant (A/A and G/A vs. G/G) and recessive (G/G and G/A vs. A/A)) using SNPassoc package of R version 3.0.1 (http://www.Rproject.org) Gonzalez *et al.* (2007). All other data were also analysed using R version 3.0.1. Odds ratio together with 95% confidence interval (CI) was estimated, and a $P < 0.05$ was considered as the statistically significant degree.

Results

The rs3129882 (A/G) polymorphism was examined in 520 sporadic PD patients and 520 healthy subjects. Distributions of the rs3129882 (A/G) polymorphism in both PD and healthy groups showed no evidence of deviation from Hardy–Weinberg's equilibrium. There was no significant difference in gender and age distributions between two groups ($P > 0.05$).

The allele and genotype frequencies of SNP rs3129882 (A/G) in study groups are shown in Table 1. Significant differences were found in allele ($P = 0.031$) and genotype frequencies ($P = 0.005$) between patients and controls. The association of the SNP with PD risk was also significant under additive and dominant models, where the A allele was observed to be protective. The data are shown in Table 2.

Discussion

Parkinson's disease is a complex neurodegenerative disorder classified as sporadic (90% cases) and familial (10% cases) types (Chang *et al.*, 2011; Dexter & Jenner, 2013). Association studies, mainly GWAS, have been widely used in an attempt to identify common genetic variations that carry an increased risk to develop the disease. They have discovered susceptibility loci for PD (Satake *et al.*, 2009; Simon-Sanchez *et al.*, 2009; Hamza *et al.*, 2010; Do *et al.*, 2011; Saad *et al.*, 2011; Lill *et al.*, 2012; Pankratz *et al.*, 2012). The association of Human Leucocyte Antigen (HLA) with PD was revealed in a GWAS carried out on Caucasian population by Hamza *et al.* (2010). They indicated a positive relationship between rs3129882 and the increased risk of PD. It is assumed the intronic variant plays a role as a cis-acting regulatory element, which correlates significantly with the overexpression of DR antigens in substantia nigra. The finding is not so far beyond expectation because of the previous evidences for involvement of neuroinflammation and adaptive immunity in PD pathogenesis (Tansey *et al.*, 2007; Gagne & Power, 2010). Our study, in consistent with Hamzah *et al.*, showed a significant association

Table 1. Genotype and alleles frequencies of SNP rs3129882 in Parkinson's disease (PD) and control groups

	Genotype frequencies (%)				Allele frequencies (%)		
Subjects	G/G	A/G	A/A	*P*-value (χ^2)	G	A	*P*-value (χ^2)
PD	172 (33.07)	238 (45.76)	110 (21.15)	0.005* (10.35)	582 (55.97)	458 (44.03)	0.031* (4.64)
Control	127 (24.42)	281 (54.03)	112 (21.73)		533 (51.25)	507 (48.75)	

*P value less than 0.05 considered as significant.

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Table 2. The association of rs3129882 with Parkinson's disease (PD) under three inheritance models

Model	Control (%)	PD (%)	OR (95% CI)	*P* value*
Additive				
G/G = 0	127 (24.4%)	172 (33.1%)	0.83 (0.70 0.99)	0.038*
A/G = 1	281 (54.0%)	238 (45.8%)		
A/A = 2	112 (21.5%)	110 (21.2%)		
Dominant				
G/G	127 (24.4%)	172 (33.1%)	0.65 (0.50, 0.86)	0.002*
A/G-A/A	393 (75.6%)	348 (66.9%)		
Recessive				
G/G-A/G	408 (78.5%)	410 (78.8%)	0.98 (0.73, 1.31)	0.879
A/A	112 (21.5%)	110 (21.2%)		

*P value less than 0.05 considered as significant.

between mentioned SNP and the risk of PD. Moreover, the higher frequency of A allele in controls suggesting a protective role for this allele. Similarly, it can be postulated that G allele plays as a susceptible factor for the disease. Statistical analysis of genotypes reflecting the GG genotype to be more frequent in patients compared with healthy individuals suggesting the susceptible nature of this genotype.

Our data are in parallel with several other studies on different populations. A cohort study on Chinese population showed a statistically significant relationship between this variant and late-onset sporadic form of PD (Guo *et al.*, 2011). Puschmann *et al.* fail to find any association; in a recessive model, they reported the GG genotype to have a protective role in their studied population (Puschmann *et al.*, 2011). Similarly, an original study followed by a meta-analysis of four GWAS data set showed a lack of association between rs3129882 and PD in French population, suggested rs660895 located on *HLA-DRB1* as a susceptibility factor (Ahmed *et al.*, 2012). This lack of association also was observed in two independent study on Dutch (Simon-Sanchez *et al.*, 2011), Scandinavian (Pihlstrom *et al.*, 2013; Ran *et al.*, 2013) and Taiwanese populations (Chiang *et al.*, 2012; Lin *et al.*, 2013). Such discrepancies may be because of genetic heterogeneity or sample size differences among various studies. Technical differences in genotyping assay can be another reason for such diversity. Our finding was also supported by Zhao *et al.* who reported the protective role of A allele (Zhao *et al.*, 2013). They showed that this allelic effect transmits via a dominant model (OR = 0.77, 95% CI = 0.62, 0.96, $P = 0.018$). More specifically, it has been shown that this polymorphism is in relation with the late onset of disease ($P = 2.4 \times 10^{-8}$) (Hamza *et al.*, 2010). Based on a hypothesis, that genetic architecture is different between sporadic and familial PD cases, a recent study reported that HLA rs3129882 was more strongly associated with sporadic PD than familial PD (Hill-Burns *et al.*, 2014).

Data from Meta-analysis studies provide additional source of comparison. Interestingly, it has been postulated that there may be more than one PD associated variant within the HLA region, so that haplotypes including this alleles increases the risk of the disease (Hill-Burns *et al.*, 2011). Wissemann *et al.* put a step forward suggesting that noncoding SNPs including rs3129882 located in HLA region can be related to the disease, regardless of HLA classes. They suggested these SNPs to be in a first line of association before the HLA associations, reflecting the involvement of regulatory elements (Wissemann *et al.*, 2013).

In conclusion, to the best of our knowledge, this is the first study designed to assess the role of the rs3129882 gene variants in a large replicated case–control of Iranian patients with PD. We detected a significant difference in both allelic and genotype frequency in the Parkinson's disease group compared with the control group. These results suggest the rs3129882 (A/G) polymorphism may be a risk factor for PD in Iranian population. However, these data still need to be validated in other populations.

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Conflict of interest

The authors declare no conflict of interest.

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