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Genetic Analysis of the *ZNF512B*, *SLC41A1,* and *ALDH2* Polymorphisms in Parkinson's Disease in the Iranian Population

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Aims: Parkinson's disease (PD) is one of the most common neurodegenerative disorders; its etiology includes both genetic and environmental factors and their interactions. The *ZNF512B*, *SLC41A1*, and *ALDH2* genes have recently been identified as contributing to PD. In this study we investigated the association of alleles of these genes with PD in the Iranian population. *Methods:* In a case–control study, rs2275294, rs11240569, and rs4767944, three single nucleotide polymorphisms in *ZNF512B*, *SLC41A1*, and *ALDH2* genes, respectively, were genotyped in 490 PD patients and 490 controls. The genotype and allele frequencies were compared between the two groups using chi-square and logistic regression tests. *Results:* A significant association between the rs11240569 polymorphism and a reduced risk of PD was found ($p = 0.014$, OR = 0.76, 95% CI: 0.60–0.94 for allele frequencies). We did not find any associations between PD and the rs2275294 and rs4767944 polymorphisms. *Conclusion:* The association of rs11240569 polymorphism in *SLC41A1* gene with reduced risk of PD was replicated in our population.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder following Alzheimer's disease affecting 1–2% of people over the age of 65 (de Lau and Breteler, 2006; Darvish *et al.*, 2013). Its main clinical characteristics include tremor, bradykinesia, muscle rigidity, and difficulties in movements (Emamalizadeh *et al.*, 2014; Pagano *et al.*, 2016). These manifestations are supposed to be due to degeneration of dopaminergic neurons in *substantia nigra* region of the brain (Jamshidi *et al.*, 2014). The patients with PD divide into three groups containing sporadic, familial, and monogenic cases with frequency of ~85%, 10–15%, and 5% of all cases, respectively (Goudarzian *et al.*, 2015). Etiologically, PD is influenced by both genetic and environmental factors; however, its exact etiology is not still completely clear (Schapira and Jenner, 2011; Haghnejad *et al.*, 2015).

There are a number of genes found to be direct causative factors for PD, including *SNCA*, *PINK1*, and *PARKIN* (Klein and Westenberger, 2012). Several other genes are also indicated to be associated with PD in an indirect manner according to studies performed on their polymorphisms and associations found between them in different populations. *ZNF512B*, *SLC41A1*, and *ALDH2* are three genes shown to be involved in PD etiology and their polymorphisms have been studied in PD and other similar diseases such as amyotrophic lateral sclerosis (ALS) due to overlaps in features and involved pathways of two disorders (Iida *et al.*, 2011; Wang *et al.*, 2015; Yang *et al.*, 2015; Zhang *et al.*, 2015).

ZNF512B is an activating factor for TGF-β signaling pathway, which has been proved to be an important survival promotion factor of dopaminergic neurons and thus is a protective agent against neurodegeneration (Schober *et al.*, 2007). *SLC41A1* is one of the several genes located in *PARK16* locus, a well-established susceptibility locus for PD

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Table 1. Age and Sex Distribution in Study Groups

Age (mean ± SD)			Gender				
			Case		Control		
Case	Control	p	Male	Female	Male	Female	p
59.32 ± 12.52	58.72 ± 12.22	0.44	262	228	258	232	0.84

SD, standard deviation.

(Satake *et al.*, 2009; Simon-Sanchez *et al.*, 2009). *SLC41A1* encodes a cytoplasmic integral protein involved in regulation of intracellular magnesium (Kolisek *et al.*, 2008; Mandt *et al.*, 2011; Kolisek *et al.*, 2012). Finally, *ALDH2* is a member of ALDH super family and encodes the aldehyde dehydrogenase 2 protein, an important enzyme for oxidation of aldehyde in brain, which its metabolites have been found to be involved in PD pathogenesis (Marchitti *et al.*, 2008; Nasstrom *et al.*, 2011). Three polymorphisms, rs2275294, an intronic polymorphism in *ZNF512B* gene, rs11240569, a synonymous coding variant in *SLC41A1*, and rs4767944, an intronic polymorphism in *ALDH2*, have been previously shown to be associated with PD (Wang *et al.*, 2015; Yang *et al.*, 2015; Zhang *et al.*, 2015), and on this study, we performed a replication study on association of these polymorphisms with PD in Iranian population.

Subjects and Methods

Study population and single nucleotide polymorphism genotyping

We designed a case–control study, including 490 PD patients and 490 unrelated healthy sex- and age-matched controls (Table 1). The inclusion criteria were the presence of late onset PD in sporadic form and absence of any other neurologic disease in cases. There were also no familial relationships between the cases. Both case and controls were ethnically Iranian. DNA was extracted from peripheral blood of all participants using a standard salting out method. Rs2275294 in *ZNF512B*, rs11240569 in *SLC41A1*, and rs4767944 in *ALDH2* gene were genotyped in all subjects using polymerase chain reaction (PCR)-RFLP method with *Bse*YI, *Btg*I, and *Psi*I restriction enzymes, respectively. The details of primer sequences, PCR conditions, and restriction enzymes are presented in Table 2. All PCR reactions were performed in 25 μL of reaction mixture and PCR programs also contained an initial 95°C and a final step of 72°C for 5 min. The digested fragments were observed on 2–3% agarose gels using ethidium bromide staining under UV light.

Statistical analyses

Deviation from the Hardy–Weinberg equilibrium was tested using Fisher's exact test in all three studied single nucleotide polymorphisms (SNPs) in the subject population. Pearson's χ^2-tests were applied to test for significance in differences of genotype and allele frequencies between groups. A p-value less than 0.05 (two tailed) was considered to be statistically significant. The distribution of genotype frequencies was also analyzed under three different genetic models (log-additive, recessive, and dominant) using SNPassoc package of R version 3.2.0 (Gonzalez *et al.*, 2007). All other statistical analyses were also performed using R version 3.2.0.

Results

The population was in Hardy–Weinberg equilibrium for all studied polymorphisms. Analysis of genotype and allele frequency distribution revealed significant differences between case and control groups for rs11240569 in SLC41A1 gene ($p = 0.015$, OR [95% CI] = 0.76 [0.60–0.65]), so that the minor allele G was associated with reduced risk of the disease. However, the difference was not significant for any of rs2275294 and rs4767944 polymorphisms (Table 3). There was also no association between PD and rs2275294 and rs4767944 under any of the genetic models. The association was significant for rs11240569 under log-additive and recessive models (Table 4).

Table 2. The Primer Sequences and PCR and Digestion Conditions for Studied Polymorphisms

Polymorphisms	Primer sequences (5→3)	PCR conditions (°C/s)			Restriction enzyme digestion	Alleles	DNA fragment size (bp)
		Denature	Annealing	Extension			
rs2275294	F: AGGCATGGAGGCTAGAGTG R: GTGTTCCGTGTGCCTTGG	95/30	58.7/30	72/30	*Bse*YI at 37°C for 1 hour	A G	250 154 + 96
rs11240569	F: ACAGTATTCTAGGAAGCAGCAG R: GGAGAACGGGAGCCAGAG	95/30	59.4/30	72/30	*Btg*I at 37°C for 1 hour	A G	242 + 50 150 + 92 + 50
rs4767944	F: AGGCACCATACAGAAATGTTCA R: TGGTGTAGAGTGCTGGACAT	95/30	56.7/30	72/30	*Psi*I at 37°C for 1 hour	C T	225 143 + 82

PCR, polymerase chain reaction.

Table 3. Genotype Distribution and Allele Frequencies in Case and Control Groups

		Genotype frequencies (%)				*Allele frequencies (%)*			
Gene (SNP)	*Subjects*	*A/A*	*A/G*	*G/G*	p	*A*	*G*	*OR (95% CI)*	p
ZNF512B	Case ($N=490$)	11 (2)	137 (28)	342 (70)	0.89	159 (16)	821 (84)	0.97 (0.76–1.24)	0.81
(rs2275294)	Control ($N=490$)	10 (42)	143 (46)	337 (12)		163 (17)	817 (83)		
SLC41A1	Case ($N=490$)	345 (70)	133 (27)	12 (3)	0.023[a]	823 (84)	157 (16)	0.76 (0.60–0.94)	0.014[a]
(rs11240569)	Control ($N=490$)	318 (65)	145 (30)	27 (5)		781 (80)	199 (20)		
		C/C	*C/T*	*T/T*		*C*	*T*		
ALDH2	Case ($N=490$)	344 (70)	133 (27)	13 (3)	0.58	160 (16)	820 (84)	1.01 (0.79–1.29)	0.95
(rs4767944)	Control ($N=490$)	339 (41)	143 (46)	9 (13)		159 (16)	821 (84)		

[a]Considered as significant.
SNP, single nucleotide polymorphism.

Discussion

Three polymorphisms, rs11240569, rs2275294, and rs4767944, were studied here in a case–control study, including 490 PD patients and 490 healthy controls, to investigate possible associations with PD. The C allele and CC genotype of the rs11240569 polymorphism were found to be significantly associated with decreased risk of PD, but neither of the other polymorphisms showed any significant differences in distributions of alleles and genotypes between cases and controls. The rs11240569 polymorphism was reported to be associated with PD in Chinese population and similar to our study, they found the association of the minor allele C and the CC genotype with reduced risk of PD (Wang *et al.*, 2015). However, two previous studies in European and Chinese populations had inconsistent results and found no associations (Tucci *et al.*, 2010; Yan *et al.*, 2011).

For the other two polymorphisms rs2275294 and rs4767944, our results were in contrast to previous studies, so that we observed no associations between these SNPs and PD, but in previous studies, the rs4767944 was investigated in one study in Han Chinese population and reported to be associated with PD (Zhang *et al.*, 2015), and rs2275294 polymorphism was also showed to be associated with the risk of ALS and PD in Han Chinese population (Yang *et al.*, 2015). rs2275294 SNP was also showed to be associated with ALS in two other studies both in Japanese population (Iida *et al.*, 2011; Tetsuka *et al.*, 2013). However, one large cohort study exists that shows the rs2275294 polymorphism is not associated with ALS in Chinese population (Ju *et al.*, 2015). These evident discrepant observations in different populations may be due to several factors, including different genetic contexts of populations, founder effects, and different sample sizes and methodologies, which cause different distributions of alleles and other markers in populations.

SLC41A1 functions as a regulator of magnesium homeostasis in cells, which is required for several cellular processes, including signal transduction, growth and proliferation, enzyme activities, and several metabolic pathways (Saris *et al.*, 2000). Cellular magnesium is found to be an important factor in preventing the pathology of neuron cells (Hashimoto *et al.*, 2008), so that deficiencies in magnesium have been found to lead to loss of dopaminergic neurons (Oyanagi *et al.*, 2006), and this may explain how this gene is related to PD etiology. Genetic polymorphisms exert their effects through several mechanisms. The rs11240569 polymorphism is a synonymous-coding variant, which causes p.Thr113Thr. This kind of polymorphisms, which does not cause any alterations in protein structure or sequence, may affect gene expression or function by the DNA sequence changes, by means of altering DNA binding factors or changing local chromatin structure, both affecting gene expression levels (Wang *et al.*, 2005). So, the rs11240569 synonymous polymorphism may affect gene expression or function by alteration of local chromatin structure or by influencing the promoter function by means of changing protein bindings in the region.

In conclusion, we found that the rs11240569 polymorphism of the *SLC41A1* gene is significantly associated with PD in Iranian population, but no significant relationships were observed between rs4767944 and rs2275294 polymorphism and PD. More studies in different populations and with larger sample sizes are required to confirm these findings and functional studies may also be helpful in finding the effect of these polymorphisms on gene function and PD pathology.

Table 4. Analysis of Genotype Distributions Under Three Genetic Models

	log-Additive (G/G = 0, A/G = 1, A/A = 2)		*Recessive (A/A vs. A/G + G/G)*		*Dominant (G/G vs. A/G + AA)*	
SNP	p	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*
rs2275294	0.80	0.97 (0.77–1.24)	0.82	1.10 (0.46–2.62)	0.72	0.95 (0.73–01.25)
rs11240569	0.02[a]	0.77 (0.60–0.95)	0.013[a]	0.43 (0.22–0.86)	0.06	0.78 (0.59–1.02)
	log-Additive (A/A = 0, A/C = 1, C/C = 2)		*Recessive (C/C vs. A/C + A/A)*		*Dominant (A/A vs. A/C + C/C)*	
rs4767944	0.95	1.01 (0.78–1.29)	0.38	0.69 (0.29–1.62)	0.72	1.05 (0.80–1.38)

[a]Considered as significant.

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Author Disclosure Statement

No competing financial interests exist.

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