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ORIGINAL ARTICLE

Association of VEGF gene polymorphisms with sporadic Parkinson's disease in Chinese Han population

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Abstract Recent evidence indicates that vascular endothelial growth factor (VEGF) is capable of protecting dopaminergic (DA) neurons. Parkinson's disease (PD) is a progressive neurodegenerative disease caused by the degeneration of nigrostriatal dopaminergic neurons. To evaluate the role of VEGF single nucleotide polymorphisms (SNPs) and haplotypes in PD, we performed a case–control study including 400 PD patients and 400 healthy-matched controls. Polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) analysis and DNA sequencing were used to detect the rs699947, rs2010963 and rs3025039 polymorphisms of the VEGF gene in cases and controls. Our study revealed that T allelic frequency of rs3025039 polymorphism was significantly higher in PD subjects (OR 1.497, 95 % CI 1.099–2.040, $P = 0.013$) than that in controls. Significant association for rs3025039 could be found in additive model (TT vs. CT vs. CC: OR 1.489, 95 % CI 1.018–2.177, $P = 0.040$) and dominant model (TT + CT vs. CC: OR 1.538, 95 % CI 1.068–2.216, $P = 0.021$). Subgroup analyses performed by gender suggested that this association could be found in male, but not in female. Moreover, it also demonstrated a significant association in the subgroup of late-onset PD (LOPD). However, for rs699947 and rs2010963 polymorphisms, genotype or allele frequencies did not differ between groups. No significant association could be found between rs699947 and rs2010963 polymorphism and PD risk. None of the observed haplotypes showed significant association with PD. Therefore, these results suggested that the VEGF gene might be associated with risk of developing sporadic PD in Han Chinese and the rs3025039 polymorphism may be a risk factor for sporadic PD.

Keywords Vascular endothelial growth factor (VEGF) · Parkinson's disease (PD) · Single-nucleotide polymorphisms (SNPs) · Haplotypes

Introduction

Parkinson's disease (PD) is the second most common age-related progressive neurodegenerative disorder after Alzheimer's disease and characterized by motor deficits including rigidity, akinesia, postural instability and resting tremor [1]. The gradual loss of dopaminergic neurons in the substantia nigra (SN) and the subsequent depletion of striatal dopamine are responsible for the development of PD-associated symptoms [2].

Vascular endothelial growth factor (VEGF) is well known as a cellular mitogen, and a vascular growth factor and permeability regulator [3]. Recently it has been reported to play a role in neuroprotection, neuronal survival, regeneration, growth, axonal outgrowth and differentiation [4]. VEGF exerts neuroprotective actions through multiple mechanisms such as the inhibition of programmed cell death, stimulation of neurogenesis and angiogenesis, enhancing blood brain barrier permeability for glucose, antioxidants activation [5–8]. VEGF has been revealed to

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display neuroprotective effects on dopaminergic (DA) neurons, also the neurorescue effects of VEGF on 6-hydroxydopamine (6-OHDA)-treated DA neurons in vitro and in vivo via direct and indirect vascular and neuronal mechanisms has been reported [9–13].

The VEGF gene is located at chromosome 6p21.3 and at least 30 single nucleotide polymorphisms (SNPs) in VEGF gene have been described in previous studies included the three common SNPs (rs2010963 in the 5-untranslated region, rs699947 in the promoter region and rs3025039 in the 3-untranslated region) which are associated to VEGF protein production [14–16]. These three common single nucleotide polymorphisms (SNPs) are functional polymorphisms, which are known as regulators of gene expression and can play a role in determining susceptibility to a disease [17]. Some functional polymorphisms of the VEGF gene have been reported to be related with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Alzheimer disease (AD) [18, 19]. Besides, a previous study reported rs3025039, rs699947 and rs2010963 were not associated with the risk of PD which was conducted in the Turkish population [20]. However, for the relatively small sample size and ethnic difference, the association between the three SNPs and the risk of PD in Asian population is unclear. Therefore, we conducted this study to demonstrate whether there is an association between the three common VEGF polymorphisms (rs3025039, rs699947 and rs2010963) and sporadic PD in Han Chinese population.

Materials and methods

In this case–control study, a total of 400 patients, who were diagnosed sporadic PD according to the UK Parkinson's Disease Brain Bank criteria, were recruited from the Department of Neurology of the Affiliated Hospital of Qingdao University and 400 matched healthy controls without any known history of neurodegenerative disorders were randomly selected from Health Examination Center of the hospital from April 2010 to July 2014. The patients with family history of Parkinsonism were excluded. A total of 400 Han Chinese patients with PD (59.0 ± 10.32 years) and 400 healthy controls (65.9 ± 7.45 years) were included in the study. The PD and control groups were well matched by age and sex. This study was approved by the Institute Ethical Committee and all participants provided written informed consent for our study. In our study, the PD and healthy control groups were matching in terms of ethnicity, age and gender.

Using a genomic DNA extraction kit (Roche, CA, USA), we extracted genomic DNA from peripheral blood lymphocytes. The rs3025039, rs699947 and rs2010963 polymorphisms for VEGF were genotyped with polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) method using the genomic DNA as a template. Primers designed, as previously reported or designed by Genetool. The primer sequences, the length of PCR-amplified fragments and restriction fragments are shown in Table 1. Furthermore, to determine the accuracy of the results, 10 % of PCR-amplified DNA samples were examined by DNA sequencing.

The PCR reaction was carried out in a total volume of 10 μl containing 2 pmol of each primer, 37.5 ng genomic DNA, 0.25U Taq DNA polymerase, 200 μM of dNTP (2.5 Mm) and 1 μl 10× PCR buffer. The PCR conditions used for rs699947 were: heated at 95 °C for 5 min and 35 cycles were performed with denaturation for 45 s at 94 °C, hybridization for 45 s at 60 °C and extension for 40 s at 72 °C, followed by a final extension of 72 °C for 7 min. A DNA region of 325 bp size was amplified with PCR and the obtained products were genotyped by BglII enzyme. For the rs3025039 polymorphism, the PCR conditions were as follows: after denaturation at 95 °C for 5 min, 35 cycles were performed for 30 s at 94 °C, 68 °C annealing for 30 s, and 72 °C for 60 s, and a final elongation at 72 °C for

Table 1 Primary information on genotyping assays for three SNPs of VEGF gene

SNP	Primer sequence	Product size (bp)	Restriction enzyme	Restriction fragments of genotype (bp)
rs699947	F:GGATGGGGCTGACTAGGTAAGC	325	BglII	325 = CC
	R:AGCCCCCTTTTCCTCCAAC			325, 202, 123 = AC
				202, 123 = AA
rs3025039	F:AAGGAAGAGGAGACTCTGCGCAGAGC	208	NlaIII	208 = CC
	R:TAAATGTATGTATGTGGGTGGGTGTGTCTACAGG			208, 122, 86 = TC
				122, 86 = TT
rs2010963	F:ATTTATTTTTGCTTGCCATT	304	BsmFi	304 = CC
	R: GTCTGTCTGTCTGTCCGTCA			304, 193, 111 = CG
				193, 111 = GG

7 min. A DNA region of 208 bp was amplified from the genomic DNA, and PCR product was genotyped by NlaIII enzyme. Also For rs2010963, the conditions were: heated at 94 °C for 5 min, followed by 10 cycles of 94 °C for 30 s, 63 °C for 30 s, 72 °C for 30 s and 30 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, then followed by a final extension of 72 °C for 7 min. A DNA region of 304 bp was amplified and genotyping was carried out by cleaving the PCR product with BsmFi enzyme.

Statistical analyses

We use direct counting to estimate genotype and allele frequencies. Statistical analysis was performed with the SPSS Statistics 19.0 software. The difference in genotype frequencies and allele distributions was analyzed using the Pearson's Chi-square test. To avoid the assumption of genetic models, additive model (minor allele homozygotes versus heterozygotes versus major allele homozygotes), dominant model (heterozygotes plus minor allele homozygotes versus major allele homozygotes) and recessive model (minor allele homozygotes versus major allele homozygotes plus heterozygotes) were all analyzed. Hardy–Weinberg equilibrium (HWE) between expected and observed genotype distributions was also assessed by Chi-square test. The odds ratio (OR) and 95 % confidence intervals (CI) were calculated by logistic regression analysis to assess the relative risk. The linkage disequilibrium and the three-site haplotype frequencies were estimated using the HaploView software program. A P value of less than 0.05 was considered to indicate a statistically significant difference.

Results

The observed genotype frequencies for these three SNPs were consistent with (HWE) P value >0.05 in the control subjects (for rs699947, $P = 0.104$ and for rs3025039, $P = 0.058$ and for rs2010963, $P = 0.072$). The allele and genotype frequencies for these three polymorphisms in PD groups and controls are listed in Table 2. The results of association analysis under the three different genetic models (additive, dominant, and recessive) were summarized in Table 3.

For rs2010963 and rs699947 polymorphisms, there were no differences between PD patients and the controls ($P = 0.418$, $P = 0.507$, respectively, in Table 2). Furthermore, no significant statistical differences were observed in the genotype distributions and allele frequencies between the age and gender subgroups for both rs2010963 and rs699947. As for the two SNPs (rs2010963, rs699947), no significant differences were observed in all comparison models between cases and healthy controls in Table 3.

For rs3025039, we identified a significant difference in genotype distribution between PD and control in the total sample ($P = 0.035$). The PD patients showed a higher T allele frequency than the healthy-matched control (OR 1.497, 95 % CI 1.099–2.040, $P = 0.013$). Significant association with increased risk of sporadic PD in Han Chinese population was consistently observed for rs3025039 under the additive model (TT vs. CT vs. CC: OR 1.489, 95 % CI 1.018–2.177, $P = 0.040$) and dominant model (TT + CT vs. CC: OR 1.538, 95 % CI 1.068–2.216, $P = 0.021$). In subgroup analysis, the difference was

Table 2 Genotype and allele frequencies of VEGF gene in patients and controls

rs699947	Genotype					Allele		
	N	AA (%)	CA (%)	CC (%)	*P*	C (%)	A (%)	*P*
PD	400	10 (2.5)	134 (33.5)	256 (64.0)	0.507	646	154	0.264
ControL	400	13 (3.2)	146 (36.5)	241 (60.3)		628	172	
rs3025039	Genotype					Allele		
	N	TT (%)	CT (%)	CC (%)	*P*	C (%)	T (%)	*P*
PD	400	10 (2.5)	90 (22.5)	300 (75.0)	0.035	690	110	0.013
Control	400	7 (1.7)	63 (15.8)	330 (82.5)		723	77	
rs2010963	Genotype					Allele		
	N	GG (%)	CG (%)	CC (%)	*P*	C (%)	G (%)	*P*
PD	400	97 (24.2)	199 (49.8)	104 (26.0)	0.418	407	393	0.582
Control	400	111 (27.8)	182 (45.5)	107 (26.7)		396	404	

Table 3 Analysis of the three SNPs based on three genetic models

Locus	Genotype	Cases	CTLs	Additive		Dominant		Recessive	
				OR (95 % CI)	*P*	OR (95 % CI)	*P*	OR (95 % CI)	*P*
rs699947	AA	10	13	0.926 (0.676–1.269)	0.633	0.910 (0.670–1.237)	0.548	0.756 (0.307–1.861)	0.543
	CA	134	146						
	CC	256	241						
rs3025039	TT	10	7	1.489 (1.018–2.177)	0.040	1.538 (1.068–2.216)	0.021	1.911 (0.676–5.406)	0.222
	CT	90	63						
	CC	300	330						
rs2010963	CC	104	107	1.228 (0.854–1.768)	0.268	1.156 (0.822–1.625)	0.405	0.904 (0.644–1.268)	0.558
	CG	199	182						
	GG	97	111						

Cases: Parkinson's disease; CTLs: healthy controls; OR (95 %confidence intervals)

P value for logistic regression after adjusting for age and gender

Table 4 Distribution of rs3025039 polymorphisms observed in every PD patients and each healthy-matched controls subgroup

rs3025039	Genotype					Allele		
	N	TT (%)	CT (%)	CC (%)	*P*	C (%)	T (%)	*P*
Male PD	230	6 (2.6)	54 (23.5)	170 (73.9)	0.843	394 (85.7)	66 (14.3)	0.568
Famale PD	170	4 (2.4)	36 (21.2)	130 (76.5)		296 (87.1)	44 (12.9)	
Famale PD	170	4 (2.4)	36 (21.2)	130 (76.5)	0.712	296 (87.1)	44 (12.9)	0.416
Famale health	152	3 (2.0)	27 (17.8)	122 (80.3)		271 (89.1)	33 (10.9)	
Male PD	230	6 (2.6)	54 (23.5)	170 (73.9)	0.028	394 (85.7)	66 (14.3)	0.008
Male health	248	4 (1.6)	36 (14.5)	208 (83.9)		452 (91.1)	44 (8.9)	
EOPD	90	1 (1.1)	14 (15.6)	75 (83.3)	0.909	164 (91.1)	16 (8.9)	0.761
Health	400	7 (1.8)	63 (15.8)	330 (82.5)		723 (90.4)	77 (9.6)	
LOPD	310	9 (2.9)	76 (24.5)	225 (72.6)	0.006	526 (84.8)	94 (15.2)	0.002
Health	400	7 (1.8)	63 (15.8)	330 (82.5)		723 (90.4)	77 (9.6)	
EOPD	90	1 (1.1)	14 (15.6)	75 (83.3)	0.108	164 (91.1)	16 (8.9)	0.036
LOPD	310	9 (2.9)	76 (24.5)	225 (72.6)		526 (84.8)	94 (15.2)	

significant in genotype distribution between male PD and its healthy matched control subgroup ($P = 0.028$), In addition, the male PD patients showed a higher T allele frequency than its healthy-matched control (OR 1.721, 95 % CI 1.148–2.579, $P = 0.008$). It demonstrated a significant difference in genotype distribution between LOPD (diagnosed >50 years of age) and controls ($P = 0.006$) and the frequency of rs3025039 T allele was significantly increased in LOPD relative to healthy control subgroup (OR 1.678, 95 % CI 1.217–2.314, $P = 0.002$) (Table 4). Subgroup analyses were performed by gender and showed that significant effect between rs3025039 polymorphism and PD risk could be found in male, but not in the subgroup of female. Similarly, significant association could also be found in the subgroup of LOPD (Table 5). The EOPD (diagnosed ≤50 years of age) showed a lower T allele frequency than the LOPD (OR 1.832, 95 % CI 1.048–3.201, $P = 0.036$). In our study population, the VEGF polymorphisms (rs699947 and rs2010963, $D' = 0.531$, $r^2 = 0.073$; rs699947 and rs3025039, $D' = 0.121$, $r^2 = 0.008$; rs2010963 and rs3025039, $D' = 0.355$, $r^2 = 0.017$) did not suggest a strong linkage disequilibrium (LD). We found no significant differences in haplotype distributions between cases and controls (Table 6).

Discussion

In this study, the association between the three common VEGF polymorphisms (rs3025039, rs699947 and rs2010963) and sporadic PD in Han Chinese population has been investigated, and we found only rs3025039 polymorphisms may affect the development of sporadic PD in Han Chinese population. These results suggest that rs3025039 polymorphism may increase the risk of

Table 5 Analysis of the rs3025039 polymorphism subgroup based on three genetic models

rs3025039	Genotype			Additive		Dominant		Recessive	
	TT	CT	CC	OR (95 % CI)	*P*	OR (95 % CI)	*P*	OR (95 % CI)	*P*
Female PD	4	36	130	1.157 (0.638–2.097)	0.632	1.234 (0.697–2.185)	0.470	2.134 (0.452–10.717)	0.357
Female health	3	27	122						
Male PD	6	54	170	1.786 (1.078–2.899)	0.024	1.789 (1.113–2.874)	0.016	0.562 (0.145–2.184)	0.405
Male health	4	36	208						
EOPD	1	14	75	0.687 (0.204–2.318)	0.545	0.685 (0.218–2.150)	0.517	0.714 (0.043–11.805)	0.814
Health	7	63	330						
LOPD	9	76	225	1.624 (1.108–2.380)	0.013	1.663 (1.153–2.937)	0.006	1.853 (0.666–5.157)	0.237
Health	7	63	330						

P value for logistic regression after adjusting for age and gender; OR (95 % confidence intervals)

Table 6 Frequency distribution of the VEGF haplotype alleles between cases and controls; frequency ≤0.03 was ignored in analysis

Genotypes	Cases (frequency)	Controls (frequency)	Chi square	*P* value
CCC	339.8 (0.425)	330.2 (0.413)	0.237	0.626
CGC	231.0 (0.289)	242.6 (0.303)	0.409	0.522
AGC	89.6 (0.112)	107.8 (0.135)	1.915	0.166
CGT	39.9 (0.050)	33.7 (0.042)	0.542	0.462
ACC	29.6 (0.037)	42.4 (0.053)	2.355	0.125
CCT	35.4 (0.044)	21.5 (0.027)	3.523	0.061
AGT	32.5 (0.041)	19.8 (0.025)	3.199	0.074

development of PD, and rs3025039 T allele may be a risk factor of sporadic PD in Han Chinese population. However, none of the three haplotypes identified in our population showed significant association with PD.

As to the subgroup, rs3025039 showed significant association between male and healthy-matched control subgroup. We can infer that the pathogenesis of male and female PD might be different and rs3025039 T allele may be a risk factor of male PD. The male with rs3025039 missense mutation may be susceptible to PD. In addition, our study demonstrated a significant association between LOPD and controls. So, we indicate that genetic factors may affect the development of PD, that age at onset >50 years. While for rs699947 and rs2010963, there was no statistically significant difference between any subgroups.

The pathophysiology of PD is still poorly understood. Gene polymorphisms, oxidative damage, perfusion deficits and neuronal apoptosis have been hypothesized to play roles in the pathophysiological mechanism [2]. Based on the pivotal role of the VEGF gene in the progressive damage of nigral dopaminergic neurons, VEGF polymorphisms might be a good candidate in determining or modifying the risk of developing PD. To our knowledge, this article is the first description of a significant association between rs3025039 polymorphism and the risk for development of PD. The mechanism about the rs3025039 polymorphism and how it affects VEGF levels and expression is still not clear. The rs3025039 C allele carries a potential binding site for activator protein 4 (AP-4), which is abolished in the rs3025039 T allele. AP-4, as a transcription factor, can enhance the expression of genes by binding to the specific site. Deficiency of this potential binding site could be in charge of decreased VEGF expression by the T-allele [15, 17]. It is possible that the dysregulated VEGF expression could be implicated in the pathogenesis of PD. Thus, we conjecture that the rs3025039 polymorphism can contribute to the differences between individuals in terms of susceptibility to PD. Further studies are needed to clarify this matter.

Two other studies have investigated the association of VEGF polymorphisms with PD, Mihci et al. investigated polymorphic variations in the VEGF gene in Turkish population with PD ($n = 126$) and controls ($n = 88$), neither the promoter polymorphisms (rs3025039, rs699947 and rs2010963) in VEGF, nor its decreased serum levels are associated with increased risk of developing idiopathic PD [20]. There was no significant differences in the tested SNPs between PD ($n = 188$) and lung cancer ($n = 321$) in Korean patients; but one haplotype was significantly different in comparisons between the two diseases. In terms of these results, they suggested that VEGF genetic polymorphisms might help understand the low incidence of lung

cancer in the patients with PD [21]. According to these data, although the evidence is unclear for inferring VEGFs as a factor, it is possible that ethnicity, age, gender and country can impact disease susceptibility.

In recent years, an increasing number of studies between the VEGF gene polymorphisms and neurodegenerative diseases have been reported. The VEGF rs699947 (−2578A/A) genotype was associated with an increased risk for sporadic AD in Italian population, independently of apolipoprotein E genotype [18, 22]. Similarly, another study concluded that in the absence of ApoEε4, the −1154G allele and the −2549D/−1154G haplotype of the VEGF promoter may increase the risk for SAD in Northern Chinese Han population [23]. In contrast, in a large French case–control population, no association of the VEGF promoter polymorphism with the risk of developing AD was observed and negative findings in the Spanish population argue against the hypothesis that the VEGF (rs699947) polymorphism is causally related to AD [24, 25]. Coincidentally, there were conflicting data about association between VEGF promoter polymorphism and risk of developing ALS. A case–control study found a threefold increased risk among individuals homozygous for the AAG or AGG haplotypes [19]. While, Van Vught et al. failed to find an association between the VEGF genotype and ALS [26]. Taken together, all these studies may confer that different ethnicities are related to the susceptibility to neurodegenerative disease. VEGFs could be the part of the crucial mechanism in these diseases.

In conclusion, our study suggests that rs3025039 polymorphism is associated with increased risk of developing sporadic PD, male PD and LOPD and the T allele of rs3025039 may be a risk factor for PD. However, relatively small sample size for both cases and controls is a limitation of this study. More studies are needed to confirm the association of VEGF polymorphisms with PD in larger populations and in other ethnic groups.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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