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Original Article

Genetic association of cyclooxygenase-2 gene polymorphisms with Parkinson's disease susceptibility in Chinese Han population

Yi Dai[1], Yuquan Wu[2], Yansheng Li[3]

Departments of [1]Neurology, [2]Gerontology, 117 Hospital of PLA, Hangzhou, Zhejiang, China; [3]Department of Neurology, Renji Hospital Affiliated to Shanghai Jiao Tong University, Shanghai, China

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Abstract: Objective: The aim of this study was to explore the genetic association of cyclooxygenase-2 (*COX2*) gene promoter region polymorphisms with Parkinson's disease (PD) susceptibility in Chinese Han population. Methods: The genotyping of *COX2* gene polymorphisms was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 122 patients with PD and 120 healthy persons. The association strength of gene polymorphism with disease was measured by odds ratio (OR) and 95% confidence interval (95% CI) calculated using χ^2 test which also evaluated the Hardy-Weinberg equilibrium (HWE) of gene polymorphism in controls. The linkage disequilibrium and haplotype were also analyzed as evidence in the analysis of association. Results: On condition that the genotypes distributions of COX2 -1290A>G, -1195G>A, -765G>C in the control group all conformed to HWE, however, only the homozygous genotype AA of -1195G>A polymorphism showed an association with PD (OR=0.432, 95% CI=0.196-0.950). In addition, in haplotype analysis, G-A-C haplotype frequency in cases was significantly lower than the controls, compared with the common haplotype A-G-G (*P*=0.031, OR=0.375, 95% CI=0.149-0.940). Conclusions: *COX2* -1195G>A polymorphism might play a protective role in the onset of PD and G-A-C haplotype in this three promoter region polymorphisms also showed a negative association.

Keywords: Cyclooxygenase-2, polymorphism, promoter, parkinson's disease

Introduction

Parkinson's disease (PD), as known as paralysis agitans, is a kind of the second most common neuronal degeneration disease after Alzheimer disease (AD) [1, 2]. This disease mostly strikes the middle-aged adults, the prevalence is approximately 1% at age 65, furthermore, the percentage accounts for 5% at age 85 [3, 4]. The symptoms of PD usually are movement-related, containing shaking, bradykinesia, rigidity, gait impairment, postural instability [5]. It is not only a main cause of disability, but also impacts on the nervous system [6]. All the time, the onset of PD is considered to be impacted by multiple factors, the most glaring is genetic and environmental factors. Between them, the roles of genetic factors on PD have been disputed for many years. In 1997, genetic variant of α-synuclein gene was reported to lead to the genotypical PD for the first time [7], so far, the scholars have found many genes to be associated with the generation of PD [8]. But the etiology and pathology of PD still remain mysterious.

In addition, PD is also reported to be associated with cancer, the patients with PD have a low risk for the onset of cancer, compared with the normal persons [9, 10]. Cyclooxygenase-2 (*COX2*) is a key enzyme to synthesize prostaglandin in the inflammatory response which has been showed to involve in various cancers [11-13]. It is encoded by *PTGS2* gene located in chromosome 1q25.2-q25.3 [14]. *COX2* enzyme is expressed in the glomerular podocytes, renal arteries and veins endothelial, and smooth muscle cells to maintain the balance of hydropower medium in kidney. Meanwhile, it also protects the mucosa completeness of the gastrointestinal tract [15]. Unlikely the constitutive expression of COX1 enzyme, the *COX2* enzyme is induced-expressed by the inflammation environment [16]. Inflammation is considered as

Table 1. PCR primer sequences of *COX2* gene polymorphisms

Polymorphism		Primer sequence	Restriction enzyme
-1290A>G	Forward	5'-CAGGTTTTATGCTGTCATTTTCC-3'	*Rsa* I
	Reverse	5'-TAGTGCTCAGGGAGGAGCAT-3'	
-1195G>A	Forward	5'-CCCTGAGCACTACCCATGAT-3'	*Pvu* II
	Reverse	5'-GCCCTTCATAGGAGATACTGG-3'	
-765G>C	Forward	5'-TATTATGAGGAGAATTTACCTTTCGC-3'	*Hha* I
	Reverse	5'-GCTAAGTTGCTTTCAACAGAAGAAAT-3	

the joinpoint of PD and cancer, but the relative study of *COX2* polymorphisms and PD is very few.

In present study, we selected three important polymorphisms of *COX2* gene promoter region to explore the relevance with PD susceptibility. The objective aimed to provide the evidence for the association between the two and further explain the etiology of PD.

Materials and methods

Selection of subjects

The cases were 122 patients with PD diagnosed by pathology and selected from the department of neurology in The 117 Hospital of Chinese People's Liberation Army during April 2012 and May 2015, including 65 males and 57 females. Their age rang was 55-82 years old with an average age of 61.23±10.56. In addition, the patients with tumors or the history of tumors were excluded. 120 healthy persons enrolled in the healthy examination center of the same hospital in the same period with the cases were as the controls, containing 62 males and 58 females with the mean age of 59.87±9.21. The frequencies of age and gender in the two groups had no significant difference. In the meanwhile, the protocol was supported by the Research Ethics Committee of The 117 Hospital of Chinese People's Liberation Army and written consents were signed by all participants without a relationship by blood each other before collecting samples.

Sample operation

Every subject provided 3 ml fasting peripheral venous blood as the samples for this study and it was collected in the anticoagulative tube with EDTA-disodium salt. The blood DNA was extracted using the conventional method of the chloroform/isoamyl alcohol extraction and finally stored at -20°C.

The genotyping of COX2 gene promoter region polymorphisms

The genotyping of -1290A>G, -1195G>A, -765G>C polymorphisms was operated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR primer sequences were listed in the **Table 1** and synthesized by Sangon Biotech Co., Ltd. in Shanghai [17]. PCR reaction solution was a total of 25 μl and the program was predegeneration at 95°C for 2 min, followed by 35 cycles with 94°C degeneration for 30 s, 60°C (-1290A>G, -1195G>A) and 62°C (-765G>C) annealing for 30 s, 72°C extension for 45 s, and finally 72°C extension for 7 min.

The PCR products were digested by restriction enzymes *Rsa* I, *Pvu* II, *Hha* I, respectively and enzyme digestion for overnight in 37°C water bath. The products were separated by 2% agarose gel electrophoresis.

Statistical analysis

The genotype distributions of *COX2* promoter region -1290A>G, -1195G>A, -765G>C polymorphisms were checked whether were consistent with Hardy-Weinberg equilibrium (HWE) by χ^2 test in the control group. The data were represented with $\bar{x} \pm s$ or %. The strength of association between gene polymorphisms and disease was evaluated by odds ratio (OR) with 95% confidence interval (95% CI). Above steps were conducted by SPSS 18.0 software. Meanwhile the haploview software was used to measure the linkage disequilibrium (LD) and the role of haplotype.

Results

Characteristics of all subjects

A total of 242 participants were enrolled in this article, consisting of 122 patients with PD as the cases and 120 healthy persons as the controls. There was no significant difference between the case and control groups in sex and age. The distributions of the controls based on *COX2* -1290A>G, -1195G>A, -765G>C polymor-

Table 2. Genotype frequency comparison of *COX2* gene polymorphisms in case and control groups and the association strength with PD

Genotype/allele		Case, n (%)	Control, n (%)	OR (95% CI)	*P* value
-1290A>G	AA	92 (75.41)	83 (69.17)	1.000 (Ref.)	-
	AG	29 (23.77)	35 (29.17)	0.727 (0.410-1.288)	0.273
	GG	1 (0.82)	2 (1.66)	0.451 (0.040-5.066)	0.508
	A	213 (87.30)	201 (83.75)	1.000 (Ref.)	-
	G	31 (12.70)	39 (16.25)	0.750 (0.451-1.248)	0.268
-1195G>A	GG	38 (31.15)	34 (28.33)	1.000 (Ref.)	-
	GA	70 (57.38)	57 (47.50)	1.099 (0.615-1.962)	0.750
	AA	14 (11.47)	29 (24.17)	0.432 (0.196-0.950)	0.035
	G	146 (59.84)	125 (52.08)	1.000 (Ref.)	-
	A	98 (40.16)	115 (47.92)	0.730 (0.509-1.046)	0.086
-765G>C	GG	104 (85.25)	99 (82.50)	1.000 (Ref.)	-
	GC	18 (14.75)	21 (17.50)	0.816 (0.410-1.622)	0.561
	CC	0 (0)	0 (0)	-	-
	G	226 (92.62)	219 (91.25)	1.000 (Ref.)	-
	C	18 (7.38)	21 (8.75)	0.831 (0.431-1.601)	0.579

Table 3. Haplotypes analysis of *COX2* gene promoter region polymorphisms based on PD

Haplotype site1-site2-site3	Case, 2 n (%)	Control, 2 n (%)	OR (95% CI)	*P* value
A-G-G	146 (59.83)	125 (52.09)	1.000 (Ref.)	-
A-A-G	56 (22.95)	71 (29.58)	0.675 (0.442-1.032)	0.069
G-A-G	24 (9.84)	23 (9.58)	0.893 (0.481-1.660)	0.721
A-A-C	11 (4.51)	5 (2.08)	1.884 (0.637-5.567)	0.245
G-A-C	7 (2.87)	16 (6.67)	0.375 (0.149-0.940)	0.031

Note: site1: -1290A>G; site2: -1195G>A; site: -765G>C.

phisms all conformed to HWE (P>0.05) and the result showed that our study population possessed the representativeness and provided a reliable conclusion.

Association analysis between COX2 gene polymorphisms and PD risk

As was shown in **Table 2**, *COX2* -1195G>A polymorphism AA genotype had a significantly lower frequency in cases than that of the controls (11.47% & 24.17%, P=0.035) and it obviously decreased the susceptibility to PD, compared with GG genotype (OR=0.432, 95% CI=0.196-0.950). But differently, both of -1290A>G, -765G>C polymorphisms showed that there were no significant associated with the generation of PD.

Linkage disequilibrium and haplotype analyses

The LD was checked among the three polymorphisms of *COX2* promoter region (-1290A>G, -1195G>A, -765G>C). A total of five haplotypes listed in the **Table 3** were found and analyzed, the result indicted that G_{-1290}-A_{-1195}-C_{-765} haplotype in *COX2* promoter region had a significantly lower frequency in cases, compared the haplotype A_{-1290}-G_{-1195}-G_{-765} (P=0.031) and remarkably decreasing the risk of PD development for the former was to compare it to the latter (OR =0.375, 95% CI=0.149-0.940).

Discussion

PD is a nervous system degenerative disease caused by the denaturation of the dopaminergic neuron in nigrostriatal pathway resulting in dopamine reduction and the hyperfunction of acetylcholine in middle-aged adults [18, 19]. The incidence of PD leads to disability even paralysis and brings about mental pressure and economic burden for patients themselves and their family. However, the etiology and pathology of PD are not clear completely, an increasing number of researchers think that both of genetic and environmental factors play a vital role in the generation and development of PD. Because only environmental factors don't account for the phenomenon that only some people suffer from PD, exposing to the same environment.

Recently, the role of genetic factors has been proved. In the study of Payami et al., the PD risk of first-degree relatives in patients with PD is 3.5 times higher than that of the persons without PD [20]. What's more, according to the

report of Gasser et al., the first- and second-degree relatives of 6%-30% PD propositions were found to suffer from PD and showed a positive association, that is, the larger the risk, the more patients with PD in family [21]. Wood et al. have demonstrated that the probability of identical twins suffering from PD simultaneously is larger than that of fraternal twins [22]. In addition, autosomal dominant or recessive inheritance in family with PD is reported at home and broad widely. Nowadays, a crowd of genes have ascertained to contribute to the onset of PD. The association of α-synuclein gene with PD was revealed firstly and then other genes are discovered one by one, such as *Parkin*, *LRRK2*, *DJ-1*, *NURR1*, *UCHL1*. Some polymorphisms of these genes are also associated with PD.

COX2 is also reported to involved in the onset of PD. Wang et al. showed the expression of *COX2* enzyme modulated by P38 signaling pathway had an influence on 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) -induced PD in the substantia nigra [23]. Teismann and his colleagues also draw an conclusion that *COX2* gene effects the neurodegenerative process and plays an important role in the pathogenesis of PD [24]. In 2014, Lopez de Maturana et al. found that leucine-rich repeat kinase 2 also influenced the inflammatory response in PD patients through regulating the expression of *COX2* enzyme [25]. But the role of genetic variant in *COX2* gene on the generation of PD is rarely studied.

In this article, three polymorphisms of *COX2* gene in promoter region (-1290A>G, -1195G>A, -765G>C) were measured the roles on PD development. AA genotype of -1195G>A polymorphism carriers might have lower possibility suffering from PD than GG genotypes carriers, but the other two polymorphisms didn't show any relevance to PD. Therefore, -1290A>G, -765G>C polymorphisms were not the independent risk factors. In the meanwhile, among polymorphisms of the same gene could exist the interaction and the conclusion was verified in our study. G_{-1290}-A_{-1195}-C_{-765} haplotype was associated with the decreased risk of PD. Further studies should be conducted to ensure the results with larger sample size and well-design, considering the interaction of gene-gene, gene polymorphisms, even gene-environment.

In addition, the relevance of PD and cancer has been reported. In previous studies, some known genes related to PD are found that their different polymorphisms may lead to different diseases: PD or cancer due to the differentiated background of neuronal and cancer cells [26-28]. As research continues, people think that the joinpoint of PD and cancer is inflammatory response and *COX2* is a key enzyme to synthesize prostaglandin and play role in degrading the neuron of PD patients. Furthermore, *COX2* may be the key enzyme to combine the inflammatory and cancer.

Disclosure of conflict of interest

None.

Address Correspondence to: Dr. Yi Dai, Department of Neurology, 117 Hospital of PLA, 14 Lingyin Road, Xihu District, Hangzhou 310013, Zhejiang, China. Tel: +86-571-87348698; Fax: +86-571-87348692; E-mail: daiyiswen@sina.com

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