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Association of the Dopamine Transporter Gene with Parkinson's Disease in Korean Patients

Dopamine transporters (DAT) uptake neurotoxic substances such as 1-methyl-4-phenylpyridinium (MPP^+) in the dopaminergic nerve terminals and may confer susceptibility to cytotoxic effects of neurotoxic substance. The association of a variable number tandem repeat polymorphism in the DAT gene with Parkinson's disease (PD) in a Korean population was studied. The 10-copy allele was the most common, accounting for 77.2% and 81.6% of alleles in PD patients and control subjects, respectively. The rare 11-copy allele was more common in the patients (odds ratio = 2.5, 95% confidence interval = 1.1-5.7, $p<0.02$). It is suggested that the 11-copy allele of the DAT gene may confer susceptibility to PD for some patients in Korea.

Key Words: *Dopamine; Minisatellite Repeats; Variable Number Tandem Repeat; Polymorphism (Genetics); Parkinson Disease*

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INTRODUCTION

Parkinson's disease (PD), a neurodegenerative disease, is characterized by rigidity, bradykinesia and rest tremor. However, the etiology of PD remains unknown. A case-control study demonstrated that environmental neurotoxins played an important role in the pathogenesis of PD (1). Another study strongly supports this view by showing that the intravenous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) caused parkinsonism (2). Nevertheless, there have been no reports of any toxin that could explain most cases of PD. Recently, the contribution of genetics to the pathogenesis of PD has also been recognized. The alpha-synuclein and Parkin gene were identified to cause hereditary PD (3, 4). However, the mutation of these genes has been found to be responsible for a minority of familial PD, and no single gene responsible for the majority of cases of PD has been identified. Both environmental and genetic causes have been sought and the etiology is likely to be an interaction between these factors.

Case-control analysis using polymorphism in candidate genes is a powerful tool for investigating genetic factors in complex disease. The dopamine transporters (DAT) are found mainly on the terminals of neurons that originate in the substantia nigra and release dopamine into the striatum. Furthermore, the DAT uptake 1-methyl-4-phenylpyridinium (MPP^+), the neurotoxic metabolite of MPTP, into these neurons, thus explaining the selective toxicity of MPTP for dopaminergic cells (5). Thus, the DAT are a plausible candidate gene for PD because it may alter susceptibility to neurotoxins. The DAT gene contains a variable number tandem repeat (VNTR) in the 3'-untranslated region (6). The association of this polymorphism with several dopaminergic disorders has been investigated (7, 8). Different studies have been done on the association of polymorphism in the DAT gene with PD depending on the races (8, 9).

The aims of this study are to compare VNTR polymorphism of Korean parkinsonian patients with that of other countries and to investigate the association of VNTR polymorphism with PD in Korean patients.

MATERIALS AND METHODS

Korean patients (n=116) with PD were enrolled from the Department of Neurology at Dong-A University Hospital. PD was diagnosed according to the criteria of Calne et al. (10). Control subjects (n=128) were matched with the patients according to age and gender. Venous blood samples were collected into tubes containing

EDTA from all subjects and stored at -70℃. Genomic DNA was extracted from leukocytes and a segment of the DAT gene containing the polymorphic VNTR region and an additional 80 base pairs (bp) of flanking sequence were amplified by polymerase chain reaction (11). The 6-, 7-, 8-, 9-, 10-, and 11-copy alleles had molecular weights of 320, 360, 400, 440, 480, and 520 bp, respectively. DNA was amplified in 50 μL of buffer containing $MgCl_2$ (1.35 mM), deoxynucleotide triphosphate (0.16 mM), 10× Tris-buffered saline (5 μL), acetamide (10%), 1 unit *Taq* polymerase and oligonucleotide primers (0.5 mM, 5'-TGTGGTGTAGGGAACGGCCTGAG-3' and 5'-CTTCCTGGAGGTCAGGGCTCAAGG-3'). The reactants were subjected to 35 thermal cycles: 94℃ for 45 sec, 67℃ for 90 sec, and 73℃ for 35 sec. The size of the reaction products was determined by electrophoresis on a 2% agarose gel and comparison with molecular weight standards.

Genotypic and allelic distributions were analyzed using the χ^2 test. $p<0.05$ was considered statistically significant.

RESULTS

The patients and controls were matched according to age (58.6±11.2 and 60.2±10.5 years) and gender (men-to-women ratio of 1:1.6 and 1:1.7). The frequencies of the alleles and genotypes are shown in Table 1. The 10-copy alleles were the most common in both patients and controls. The 11-copy allele was more common in patients than in control subjects (odds ratio = 2.5, 95% confidence interval = 1.1-5.7, $p<0.02$).

Table 1. Distribution frequency of the dopamine transporter gene variable number tandem repeat genotype and alleles in controls and patients with Parkinson's disease

VNTR copy number	Patients n=116 (%)	Controls n=128 (%)
Allelic frequency		
9	32 (13.8)	37 (14.5)
10	179 (77.2)	209 (81.6)
11*	21 (9.1)	10 (3.9)
Genotypic frequency		
9/9	12 (10.3)	15 (11.7)
10/9	7 (6.0)	6 (4.7)
10/10	84 (72.4)	101 (78.9)
11/9	1 (0.8)	1 (0.8)
11/10	4 (3.4)	1 (0.8)
11/11	8 (6.9)	4 (3.1)

*$p<0.02$, odds ratio=2.45 (95% confidence interval, 1.1-5.7)
VNTR, variable number tandem repeat

DISCUSSION

A number of putative candidate genes involved in dopamine synthesis, metabolism and mitochondrial function were analysed. These candidate genes include CYP2D6 (12), monoamine oxidase (13), dopamine receptors (14), superoxide dismutase 1 (15) and glutathione peroxidase (14). However, there is no definitive evidence pointing to any one of these genes of being the putative cause in the etiology of PD.

The DAT gene, which is located at 5p15.3, contains a VNTR in the 3'-untranslated region (6). Between 3 and 11 copies of the 40-bp repeat element in DAT gene have been reported in normal populations (11). In our study, the 10-copy allele was the most common in a Korean population, accounting for 77.2% and 81.6% of the alleles in patients with PD and control subjects, respectively. The 10-copy allele was described as more common in other Asian populations and less common in Caucasian. Li et al. (16) reported that 92% of DAT alleles were 10-copy in a Chinese population, and Sano et al. (17) reported a value of 93% in a Japanese population. In Caucasian and black populations, the 10-copy allele was less common, accounting for only about 70% of the alleles (6). Such racial differences in noncoding regions of the genome have been well recognized (9).

We found that the 11-copy allele was more common in Korean parkinsonian patients and was associated with a 2.5-fold increase in the PD risk. It accounted for 9.1% of alleles in the patients and 3.9% in the controls. It could explain approximately 11% of cases. The frequencies of the 11-copy allele both in patients and controls were higher in a Korean population than in populations of other Asian countries and Caucasian (9). The difference in the results among races could have occurred as a result of methodological problems such as selection bias. However, it also suggests that the etiology and pathogenesis of PD may differ among races. Such a notion is strengthened by observations showing that several mechanisms such as different environmental neurotoxins, different exposure to protective factors and different genetic risk factors or protective genes, can influence the pathogenesis of PD.

Several possible mechanisms for an association of the 11-copy allele with PD have been described. First, polymorphism could be genetically linked to other mutations. Mutations in the mouse DAT gene increased both the uptake velocity and affinity of MPP^+ with little effect on the transport of dopamine (18). Similar mutations, however, in the human DAT gene have not been reported. Polymorphic VNTRs can influence gene expression directly (19). A VNTR in the 5'-untranslated region of the insulin gene is associated with susceptibility to dia-

betes mellitus (20). The longest repeats have the greatest effect on gene expression, possibly because of extra binding sites for transcription factors. The 11-copy polymorphism of the DAT gene may result in increased expression, thus increasing transport of MPP^+-like compounds into nerve terminals (8). On the other hand, the 11-copy allele may allow decreased activity of DAT, leading to increased extracellular dopamine and oxidative stress to neurons (21).

Case-control studies are open to a number of biases, particularly related to selection bias, and this confusion is reflected in the literature. This study was performed based on a population enrolled from our hospital and might therefore have some limitations. Accordingly, these results must be considered preliminary, and further studies will be required to confirm the magnitude of this risk.

Our studies suggest that the susceptibility of some people to PD in Korea may be conferred by polymorphism in the DAT gene that could lead to increased cellular accumulation of neurotoxic compounds in dopaminergic neurons. However, the precise nature of DAT gene mechanism in the pathogenesis of PD and its relative contribution in sporadic PD have yet to be determined.

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