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Detection and Analysis of Four Polymorphic Markers at the Human Monoamine Oxidase (MAO) Gene in Japanese Controls and Patients with Parkinson's Disease

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Monoamine oxidase (MAO), which exists in two forms (MAOA and MAOB), plays an important role in the oxidative metabolism of neurotransmitters such as dopamine, and has been implicated in the etiology of Parkinson's disease (PD). Individual variations in the activity of these enzymes appear to be genetically determined, and these genetic variations appear to be predominantly mediated by the MAO locus. Here, we detected and analyzed four polymorphic markers in the MAO gene using a polymerase chain reaction method in 228 Japanese controls (102 males and 126 females) and 68 patients with PD (30 males and 38 females). Although the analysis of the MAOA marker demonstrated no overall association between its alleles and PD, a significant difference in the frequency of one particular MAOA allele between controls and patients with PD was found. Moreover, in a comparison of the distribution of the full haplotypes at the MAOA locus, there was a significant difference in the frequency of one particular haplotype between male controls and patients with PD. In the MAOB polymorphism, there was no difference in the distribution of alleles between them. These findings support the hypothesis that the MAOA gene may affect the susceptibility of individuals to PD among MAOA polymorphic loci. © 1998 Academic Press

Parkinson's disease (PD) is a neurodegenerative disorder caused by death of dopaminergic neurons in the substantia nigra, and the symptoms are thought to occur with alterations of the dopamine levels in the striatum (1). Over the last decade, the role of genetic and environmental factors in the etiology of PD has been the subject of controversy (2-8). Recent studies have suggested that several genes encoding proteins of the dopaminergic pathway such as the dopamine transporter (9,10), the dopamine receptor D2 (11,12), the dopamine receptor D4 (13,14), the degradative enzyme catechol-O-methyltransferase (15,16) and the monoamine oxidase (MAO) (17-25) may be involved in the disease process. In particular, the primary enzyme MAO for the degradation of neurotransmitters such as dopamine, noradrenaline, and serotonin is thought to be associated with the susceptibility of individuals to PD. The enzyme exists in two forms, MAOA and MAOB, which can be distinguished on the basis of their differences in pharmacologic and biochemical characteristics. The genes for both forms of MAO are located in the same (p11.23-11.4) region of the human X chromosome (26). Individual variations in activity of these enzymes appear to be genetically determined, and these genetic variations appear to be predominantly mediated by the MAO locus (27).

To date, several expressed polymorphisms in the MAO gene have been identified and characterized, and four of them are as follows; i) a (GT)n dinucleotide repeat in intron 2 of the MAOA gene (MAOA-GT) (17), ii) the imperfectly duplicated 23-bp VNTR in intron 1 of the MAOA gene (MAOA-VNTR) (24), iii) an Fnu4HI-RFLP in exon 8 of the MAOA gene (MAOA-RFLP) (25), and iv) a (GT)n dinucleotide repeat in intron 2 of the MAOB gene (MAOB-GT) (18). Several association studies between these polymorphisms and PD have been performed, and a recent study found a significant difference in the frequency of MAOA haplotypes in male patients with PD compared with normal controls (22). However, whether these polymorphisms at MAO are directly involved in individual differences in susceptibility to PD have not yet been determined.

To clarify the potential implication in the pathogenesis of PD, we detected and analyzed the four identified polymorphisms in the MAO gene, and subsequently assessed the frequency of alleles for MAOA and MAOB in Japanese controls and patients with PD. Furthermore, we assessed the haplotype frequencies of MAOA

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Abbreviations: MAO, monoamine oxidase; PD, Parkinson's disease.

TABLE 1

Primer Sequences and PCR Thermal Conditions

Primer name [Ref. No.]	Sequence (5′–3′)	Denaturation	Annealing	Extension	Cycles
MAOA-GT 1 [17]	HEX-AGAGACTAGACAAGTTGCAC	94°C	60°C	72°C	30
MAOA-GT 2 [17]	CACTATCTTGTTAGCTCACT	30 sec.	45 sec.	60 sec.	
MAOA-VNTR 1 [24]	GGTAGACTCCTTTAAGAAAA	95°C	53°C	72°C	30
MAOA-VNTR 2 [24]	CAATAAATGTCCTACACCTT	30 sec.	40 sec.	40 sec.	
MAOA-RFLP 1 [25]	GACCTTGACTGCCAAGAT	95°C	57°C	72°C	30
MAOA-RFLP 2 [25]	CTTCTTCTTCCAGAAGGCC	30 sec.	45 sec.	60 sec.	
MAOB-GT 1 [18]	6-FAM-GAAGCATCGAAGTTAGGA	95°C	60°C	72°C	30
MAOB-GT 2 [18]	GTATTTGGCCTCATAGAGTTAG	30 sec.	30 sec.	60 sec.	

in male controls and patients with PD since males possess only one allele (on the X chromosome) which enables direct determination of the haplotype.

MATERIALS AND METHODS

Patients and controls. After obtaining informed consent, 68 unrelated Japanese patients with PD (38 males and 30 females, 48-90 years of age) were selected from Osaka University Medical Hospital. All patients met the diagnostic criteria for idiopathic PD. The control group consisted of 228 unrelated Japanese individuals (102 males and 126 females, 20-60 years of age). All controls were free of neurologic and psychiatric illness.

DNA extraction. Genomic DNA was extracted from the whole blood (28). After incubation with proteinase K (125 mg/ml) in lysis buffer (3 ml containing 50 mM Tris-HCl, 100 mM NaCl, 0.5% SDS, 1 mM EDTA) at 55°C for 3 hours, protein contaminants were removed by phenol/chloroform extraction, precipitated with ethanol, and then dried and stored at −20°C in 10 mM Tris-HCl (pH 7.6). Extracted DNA was quantified by measuring the optical density at 260 nm; a value of 1.0 was regarded to correspond to 50 mg/ml DNA.

DNA amplification by polymerase chain reaction. DNA amplification was performed using a Parkin-Elmer GeneAmp System 2400 in a total volume of 25 μl reaction mixtures containing 50 ng genomic DNA(template), 200 mM each of dATP, dTTP, and dCTP, 100 mM dGTP, 0.5 mM of each primer, 2.5 units of Taq DNA polymerase (Promega), and 1.5 mM $MgCl_2$. The oligonucleotide primers and PCR thermal conditions are shown in Table 1. Since MAOA-GT and MAOB-GT consist of short tandem repeat polymorphisms, a fluorescently labeled primer was used for PCR.

Laboratory genotyping. In MAOA-GT and MAOB-GT, PCR products were electrophoresed on a 6% denatured polyacrylamide gel in a Parkin-Elmer 373A DNA sequencer. Sizing and designation of alleles were performed using both the internal lane standard GeneScan Tamra 500 and ABI PRISM 672 analysis software.

The MAOA-VNTR products and the Fun4HI-digested products were electrophoresed for 3 hours at 150V in 2.0% GTG agarose gel (FMC Bio Products) and 3.0% Metaphor agarose gel (FMC Bio Products), respectively, and directly visualized with ethidium bromide under UV illumination. Each band size was calculated with the logarithmic regression curve determined by mobility of the standard size marker (100 base pair ladder; Parmacia-LKB).

The genomic organization of these genes and the relative position of the polymorphisms are shown in Figure 1.

Statistical analysis. Statistical analysis was performed by the Chi-square test and Fisher's exact test (29) to compare the allelic frequencies of MAOA and MAOB alleles in patients with PD and the control group.

RESULTS

Allele frequencies for each of the three MAOA markers in controls and patients with PD are shown in Table 2. Analysis of the MAOA marker demonstrated no overall association between alleles and PD (MAOA-GT:Total; χ^2 =13.02, df=8, p=0.11. Male; χ^2 =12.58, df=7, p=0.08. Female; χ^2=12.67, df=8, p=0.12., MAOA-VNTR:Total; χ^2 =1.17, df=2, p=0.59. Male; χ^2=2.57, df=2, p=0.28. Female; χ^2 =2.68, df=2, p>0.99., MAOA-RFLP:Total, Male and Female; p>0.99.). However, further analysis of each allele separately using Fisher's exact test demonstrated a significant deviation in the MAOA-GT allele "119" (Total; χ^2=10.02, df=1, p=0.004. Male; χ^2=7.33, df=1, p=0.019. Female; χ^2=6.50, df=1, p=0.025. See Table 2.) between controls and patients with PD. This difference reached the significant level after Bonferroni's correction in total (nine alleles, critical *P* value = 0.0056). The incidence of allele 119 was almost three, ten and 2.5 times more frequent in patients with PD than controls in total, males and females, respectively.

Table 3 shows the distribution of haplotypic combinations at the MAOA locus in male controls and patients with PD. In a comparison of the distribution of the full haplotypes [(GT)n+VNTR+RFLP] at the MAOA locus, there was a significant difference in the frequency of haplotype "119-C-R2" between controls and patients with PD (χ^2 = 11.05, df=1, p=0.005). However, the overall distribution of these haplotypes was not significantly different between them (χ^2=21.55, df=24, p=0.61).

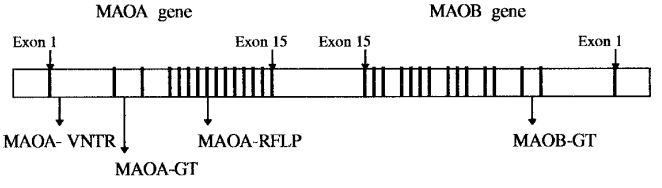

FIG. 1. Genomic organization (not in scale) of the human MAO genes. The relative position of the MAO polymorphisms are indicated (30).

TABLE 2

Distribution of Alleles at the MAOA Locus in Controls and Patients with Parkinson's Disease

	Total		Male		Female	
Allele	Control (N = 228) n	Patients (N = 68) n	Control (N = 102) n	Patients (N = 38) n	Control (N = 126) n	Patients (N = 30) n
			MAOA-GT			
113	9 (2.5)	3 (3.1)	3 (2.9)	0 (0)	6 (2.4)	3 (5.0)
115	85 (24.0)	20 (20.4)	21 (20.6)	8 (21.1)	64 (25.4)	12 (20.0)
117	63 (17.8)	17 (17.3)	13 (12.7)	8 (21.1)	50 (19.8)	9 (15.0)
119	20 (5.6)	15 (15.3)*	1 (1.0)	4 (10.5)**	19 (7.5)	11 (18.3)***
121	28 (7.9)	8 (8.2)	13 (12.7)	2 (5.3)	15 (6.0)	6 (10.0)
123	96 (27.1)	25 (25.5)	37 (36.3)	10 (26.3)	59 (23.4)	15 (25.0)
125	42 (11.9)	8 (8.2)	9 (8.8)	5 (13.2)	33 (13.1)	3 (5.0)
127	9 (2.5)	2 (2.0)	5 (4.9)	1 (2.6)	4 (1.6)	1 (1.7)
129	2 (0.6)	0 (0)	0 (0)	0 (0)	2 (0.8)	0 (0)
			MAOA-VNTR			
A	12 (3.4)	2 (2.0)	8 (7.8)	1 (2.6)	4 (1.6)	1 (1.7)
B	212 (59.9)	55 (56.1)	56 (54.9)	18 (47.4)	156 (62.0)	37 (61.7)
C	130 (36.7)	41 (41.8)	38 (37.3)	19 (50.0)	92 (36.5)	22 (36.7)
			MAOA-RFLP			
R1	209 (59.0)	58 (59.2)	58 (56.9)	20 (52.6)	151 (59.9)	38 (63.3)
R2	145 (41.0)	40 (40.8)	44 (43.1)	18 (47.4)	101 (40.1)	22 (36.7)

Note. Number in parentheses indicates percentage. N: number of individuals; n: number of X chromosomes analyzed.
*: $\chi^2 = 10.02$, df = 1, p = 0.004. **: $\chi^2 = 7.33$, df = 1, p = 0.019. ***: $\chi^2 = 6.50$, df = 1, p = 0.025. (Fisher's exact test.)

There was no difference in the distribution of MAOB alleles between controls and patients with PD as shown in Table 4.

DISCUSSION

The purpose of this study was to demonstrate whether MAO polymorphisms are directly involved in individual differences in susceptibility to PD. MAO plays an important role in metabolizing several neurotransmitters such as dopamine, and exhibits more than 50-fold variations in activity levels among normal humans (30,31). In view of this trait, we hypothesized that MAO polymorphisms may affect some gene expression in forms of various haplotypic combinations among MAO polymorphic loci, thus resulting in wide variations in activity in humans.

The result of the experiment was that we found significant differences between controls and patients with PD in the MAOA-GT allele "119" and MAOA haplotype "119-C-R2". However, regarding the MAOB gene, we found no such difference.

Previously, Nanko et al. (20) reported that significant associations were found between PD and allele A4 of MAOA-GT, and between PD and allele B4 of MAOB-GT in the frequency of each allele, although both differences did not reach the significant level after Bonferroni's correction. They detected a total of nine alleles (from A1 to A9) in the Japanese population in MAOA-GT but the sizes of the alleles were not described. Therefore, we could not directly compare our Japanese data with theirs. However, as the A4 allele of MAOA that they reported is the same as the "119" allele that we reported here, susceptibility of individuals to PD mediated by the levels of MAOA activity may be associated with the MAOA-GT "119" allele in Japanese. On the other hand, we did not find an association between PD and alleles of MAOB-GT because our investigation was performed in a larger sample size than their investigation. They detected a total of eight alleles in 211 chromosomes in MAOB-GT, compared to twelve alleles in 393 chromosomes in the present study.

Meanwhile, Kurth et al. (6) reported that no allelic association between patients and controls for MAOA-RFLP was found in the mixed European white population. This finding are in agreement with our Japanese data. At the same time, they found a significant association between a MAOB polymorphism defined by a single-strand conformation polymorphism and PD although Ho et al. (7) failed to find any association between them. After a while, Morimoto et al. (21) investigated this MAOB polymorphism in the Japanese

TABLE 3

Distribution of Haplotypic Combinations at the MAOA Locus in Male Controls and Patients with Parkinson's Disease

Haplotypes	Controls (n = 102) n (%)	Patients (n = 38) n (%)
113-C-R1	3 (2.9)	0 (0)
115-B-R1	2 (2.0)	1 (2.6)
115-C-R1	15 (14.7)	5 (13.2)
115-C-R2	4 (3.9)	2 (5.3)
117-B-R2	1 (1.0)	1 (2.6)
117-C-R1	9 (8.8)	5 (13.2)
117-C-R2	3 (2.9)	2 (5.3)
119-B-R2	1 (1.0)	0 (0)
119-C-R2	0 (0)	4 (10.5)*
121-A-R2	1 (1.0)	0 (0)
121-B-R1	5 (4.9)	1 (2.6)
121-B-R2	6 (5.9)	1 (2.6)
121-C-R2	1 (1.0)	0 (0)
123-A-R1	1 (1.0)	0 (0)
123-A-R2	4 (3.9)	1 (2.6)
123-B-R1	15 (14.7)	4 (10.5)
123-B-R2	14 (13.7)	4 (10.5)
123-C-R1	1 (1.0)	1 (2.6)
123-C-R2	2 (2.0)	0 (0)
125-A-R1	1 (1.0)	0 (0)
125-A-R2	1 (1.0)	0 (0)
125-B-R1	3 (2.9)	3 (7.9)
125-B-R2	4 (3.9)	2 (5.3)
127-B-R1	3 (2.9)	0 (0)
127-B-R2	2 (2.0)	1 (2.6)

Note. n: number of X chromosomes analyzed.
* Significant (p = 0.005. Fisher's exact test.)

population using the same method described by Kurth et al.. They found that no allelic association between patients and controls, and there was an ethnic difference in the MAOB allelic frequency between the Japanese and Caucasian populations, suggesting that environmental factors may play some roles in the prevention of PD in Japanese with genetic susceptibility for PD. We also support their idea that environmental factors may be involved in the development of PD since many discordant results have been reported with regard to MAO polymorphisms.

In other previous studies, Planté-Bordeneuve et al. (8) reported that no allelic association was found between MAOA-GT, MAOB-GT and sporadic PD in the mixed European white population. On the contrary, Hotamisligil et al. (22) reported that the haplotype "C122" was markedly different in the frequency of MAOA haplotypes between controls and patients with PD, and Hsu et al. (23) suggested that since this "C122" allele which is associated with high activities of MAOA occurred less frequently among patients with PD than among controls, low levels of MAOA activity may be implicated in the susceptibility of individuals to PD in some patients. In Japanese, the frequency of "122" allele of MAOA was not different between patients and controls. However, taking the ethnic difference into consideration, the "119" allele (especially MAOA haplotype "119-C-R2") that we reported here may be involved in the susceptibility of individuals to PD in Japanese, although the association between this haplotype and the level of MAOA activity was not demonstrated in our study.

These findings support the hypothesis that the MAOA gene may affect the susceptibility of individuals to PD among MAOA polymorphic loci. In this sense, we expect that more haplotypes of the MAOA locus will be discovered in patients with PD, and further investigation should be undertaken in a larger sample size.

TABLE 4

Distribution of Alleles at the MAOB Locus in Controls and Patients with Parkinson's Disease

	Total		Male		Female	
Allele	Control (N = 196) n	Patients (N = 68) n	Control (N = 97) n	Patients (N = 38) n	Control (N = 99) n	Patients (N = 30) n
MAOB-GT						
166	1 (0.3)	1 (1.0)	0 (0)	0 (0)	1 (0.5)	1 (1.7)
168	2 (0.7)	3 (3.1)	1 (1.0)	2 (5.3)	1 (0.5)	1 (1.7)
170	28 (9.5)	13 (13.3)	14 (14.4)	8 (21.1)	14 (7.1)	5 (8.3)
172	16 (5.4)	4 (4.1)	7 (7.2)	2 (5.3)	9 (4.5)	2 (3.3)
174	5 (1.7)	3 (3.1)	1 (1.0)	1 (2.6)	4 (2.0)	2 (3.3)
176	8 (2.7)	3 (3.1)	2 (2.1)	1 (2.6)	6 (3.0)	2 (3.3)
178	19 (6.4)	8 (8.2)	7 (7.2)	3 (7.9)	12 (6.1)	5 (8.3)
180	22 (7.5)	6 (6.1)	9 (9.3)	3 (7.9)	13 (6.6)	3 (5.0)
182	54 (18.3)	16 (16.3)	12 (12.4)	3 (7.9)	42 (21.2)	13 (21.7)
184	101 (34.2)	30 (30.6)	39 (40.2)	13 (34.2)	62 (31.3)	17 (28.3)
186	30 (10.2)	8 (8.2)	4 (4.1)	1 (2.6)	26 (13.1)	7 (11.7)
188	9 (3.1)	3 (3.1)	1 (1.0)	1 (2.6)	8 (4.0)	2 (3.3)

Note. Number in parentheses indicates percentage. N: number of individuals; n: number of X chromosomes analyzed.

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