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Catechol-*O*-methyltransferase genotype and susceptibility to Parkinson's disease in Japan

Short Communication

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Summary. We report −108Met/Val polymorphism of the COMT gene in Japanese patients with Parkinson's disease (PD). The allele frequency for −108Val was higher in PD patients compared with controls, although the differences did not reach the statistical significance. However, the frequency of −108Val homozygotes was significantly higher in PD patients (56.8%) than in control subjects (44.2%), and heterozygotes of −108Met/Val were less in PD. COMT gene polymorphism may constitute a genetic risk factor for PD among Japanese.

Keywords: Catechol-*O*-methyltransferase, polymorphism, Parkinson's disease.

Introduction

Catechol-*O*-methyltransferase (COMT) metabolizes catechol compounds, including dopamine and levodopa. A decrease in COMT activity may result in an increase in the metabolism of dopamine to neuromelanin that can promote the formation of cytotoxic radicals contributing to neuronal degeneration (Jellinger et al., 1993). Therefore, functional polymorphism of the COMT gene may constitute a genetic risk factor for Parkinson's disease (PD).

Two isoforms of COMT are known, i.e., the thermolabile low activity form ($COMT^{L}$) and the thermostable high activity form ($COMT^{H}$) (Grossman et al., 1992a,b). Recently, the rat and human COMT cDNAs were cloned (Bertocci et al., 1991; Lundstrom et al., 1992), and the two isoforms of human COMT were shown to differ in one amino acid at exon 4 (−108Val is replaced by Met). The catalytic activity of the −108Val and −108Met COMT enzymes was essentially similar, but the −108Met enzyme was more thermolabile at physiological temperatures (Lotta et al., 1995).

Regarding the association between diseases and the COMT gene, it was reported that COMT was deleted in almost all patients with velo-cardio-facial syndrome (Dunham et al., 1992) and homozygotes of low activity form of

enzyme was a risk factor for obsessive-compulsive disorder (Karayiorgou et al., 1997). Recently, Hoda et al. (1996) found no difference in allele frequency or genotype distribution of the COMT gene between PD patients and the control subjects. In the present study, we analyzed this biallelic polymorphism in Japanese PD patients.

Subjects and methods

Blood samples were collected from 176 PD patients and 156 control subjects who gave informed consent at the Neurology Service of Juntendo University Medical School. Both PD patients and the control subjects studied are not blood related each other. The diagnosis of PD was based on the consensus statement (CAPIT Committee, 1992); the average age of the patients was 62.7 ± 8.8 years and the average duration of the disease 7.1 ± 5.6 years. The control group included normal volunteers and patients with various diseases but neurodegenerative disorders; the average age was 57.9 ± 16.2 years. All the subjects were ethnically Japanese.

Human genomic DNA was prepared as described previously (Miller et al., 1988). DNA typing for biallelic polymorphism (GTG for valine and ATG for methionine at exon 4) was performed by the miss-match primer-PCR method using 4-miss U; ATCACCCAGCGGATGGTGGATTTCGCTGTC and 4-miss R; TGGTGATAGTGGGTTTTCAGTGAACGTGGT as the primer set to create a new BspHI restriction site in the mutant allele. The PCR condition involved 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min, followed by extension at 72°C for 10 min. Then the PCR products were digested with B*sp*HI (New England Biolabs, Inc., MA, USA), and subjected to electrophoresis on 3% agarose gel.

Results

The results are summarized in Table 1. The allele frequency of −108Met was 25.6% in PD patients and 31.1% in the control subjects; the difference was not statistically significant ($\chi^2 = 2.49$, $p = 0.115$). As shown in Table 1, a significant difference in the genotype distribution was noted between the two groups ($\chi^2 = 6.79$, $p = 0.033$). The frequency of −108Val homozygotes was significantly higher in PD than in the controls ($p = 0.022$). The risk of developing PD in the presence of −108Val homozygosity was calculated as 1.66 (95% CI; 1.079–2.555). The frequency of −108Met homozygotes ($COMT^L$) did not differ significantly between the two groups. While −108Val/Met heterozygotes were significantly less common among the PD patients compared with the controls ($p = 0.009$). In another words, 62 of the 139 subjects with this heterozygosity (44.6%) had PD; while among the 193 subjects with homozygosity for either −108Met or −108Val, 114 (69.9%) had PD ($\chi^2 = 6.79$, $p = 0.009$). The genotype distribution was within the expected range when calculated from the allele frequency in the respective groups.

Discussion

In the present study, the allele frequency of the COMT gene did not differ significantly between PD patients and the controls. On the other hand, the genotype distribution differed significantly; the frequency of −108Val homozygotes was significantly higher in PD patients (56.8%) compared with

Table 1. COMT allele and genotype frequencies in Parkinson's disease (PD) patients and controls

	Control		Parkinson		Total	
No of subjects	156		176		332	
No of chromosome	312		352		664	
	No	%	No	%	No	%
Allele frequency						
−108Met	97	31.1	90	25.6	187	28.2
−108Val	215	68.9	262	74.4	477	71.8
		$\chi^2 = 2.49$, p = 0.115				
Genotype frequency						
Met/Met	10	6.4	14[1]	8.0	24	7.2
Met/Val	77	49.4	62[2]	35.2	139	41.9
Val/Val	69	44.2	100[3]	56.8	169	50.9
		$\chi^2 = 6.79$, p = 0.033				

[1]The frequency of Met homozygotes did not differ significantly between the two groups compared with other two genotypes combined ($\chi^2 = 0.29$, $p = 0.587$). [2]The frequency of −108Val/Met heterozygotes was significantly lower in PD compared with other homozygotes combined ($\chi^2 = 6.79$, $p = 0.009$). [3]The frequency of Val homozygotes was significantly higher in PD compared with other genotypes combined ($\chi^2 = 5.24$, p = 0.022). The genotype frequency in each group was within the Hardy-Weinberg equilibrium

the controls (44.2%); while the frequency of Val/Met heterozygotes was significantly lower in PD patients. Therefore, this COMT gene polymorphism appears to constitute a genetic risk factor for PD in Japanese in contrast to the results on Caucasian population by Hoda et al. (1996). The frequency of −108Val and the incidence of −108Val homozygosity are much lower among Caucasians compared with the Japanese. According to Hoda et al. (1996), the −108Val allele frequency was 47.1% among Caucasians and the incidence of −108Val homozygosity was 23.1%. These differences probably arise from the ethnic backgrounds of the subjects in the two studies. Our −108Met frequency was consistent with the distribution of $COMT^H$ and $COMT^L$ in erythrocytes among Orientals (Rivera-Calimlim and Reilly, 1984; McLeod et al., 1994).

According to the neuromelanin toxicity theory (Jellinger et al., 1992) $COMT^L$ may constitute a genetic risk factor and the higher prevalence of PD among Caucasians who have a higher $COMT^L$ frequency is consistent with this hypothesis. However, $COMT^L$ was not a risk factor in the present study as well as in the study of Hoda et al. (1996). If −108Val is a genetic risk factor for PD, this homozygosity results in $COMT^H$ at the protein level, so the proportion of dopamine metabolized to 3-methoxytyramine is expected to increase. According to our in vitro studies (Morikawa et al., 1996), 3-methoxytyramine is a weak inhibitor of mitochondrial complex I and NADH-linked state 3

respiration. This could be a reason for increased susceptibility to PD among patients who have −108Val homozygosity. Another possibility is that −108Val has nothing to do with susceptibility of PD, but that gene closely linked to the COMT gene is the risk factor.

Another interpretation of our results is that −108Met/Val heterozygosity may be a neuroprotective factor for PD, because −108Met/Val heterozygotes had a significantly lower prevalence of PD. Of the 139 subjects with this heterozygosity, 62 (44.6%) had PD. On the other hand, among 193 with homozygosity for either −108Met or −108Val, 114 (69.9%) had PD (χ^2 = 6.786, p = 0.0092). Thus, intermediate activity of COMT may in some way be a neuroprotective factor for PD and some of the conflicting data on COMT polymorphism may be explained by this concept.

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