Genetic Analysis of the CYP2D6 Gene in Patients With Parkinson's Disease

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To further investigate the association between Parkinson's disease (PD) and genetic polymorphism of the CYP2D6 gene, a mutant allele (CYP2D6J) frequently observed in the Japanese population and related to EM/PM polymorphism (phenotypically, individuals are either extensive metabolizers [EM] or poor metabolizers [PM] of debrisoquine) was investigated. The CYP2D6J gene with a nucleotide substitution from C to T at position 188 (the *Hph*I site in exon 1), which reduces CYP2D6 enzyme activity, was analyzed by polymerase chain reaction (PCR) and by digestion with *Hph*I. No significant relationship was observed between PD patients and controls for this mutation. This suggests that the EM/PM polymorphism of CYP2D6 contributes little to the pathogenesis of PD. To further study the molecular basis for the relationship between PD and CYP2D6, the heterogeneity of CYP2D6 was investigated by combined genotype analysis of the two mutant CYP2D6 genes (ie, CYP2D6J, the *Hph*I site mutation in exon 1, and CYP2D6L, the *Hha*I site mutation in exon 6). Although some characteristic patterns of the combined genotypes were observed in both PD patients and controls, a strong association between the heterogeneity of the CYP2D6 gene and PD was not shown by combined genotype analysis.
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THE ENZYME CYP2D6 is one of the enzymes responsible for metabolizing 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1,2,3,4-tetrahydroisoquinoline (TIQ), and possibly unknown environmental neurotoxins that cause Parkinson's disease (PD).[1-4] Genetic polymorphisms related to CYP2D6 activity have been characterized (ie, EM/PM polymorphism). Phenotypically, individuals are either extensive metabolizers (EM) or poor metabolizers (PM) of debrisoquine. It has been suggested that PMs fail to metabolize neurotoxins because of decreased CYP2D6 activity, and thus neurotoxins accumulate in dopaminergic neurons in the brain.[3,5] Whether genetic polymorphisms in the CYP2D6 gene are associated with PD is a topic of interest to both neurologists and pharmacologists. About 10% of caucasians are PMs,[6,7] and an association between EM/PM polymorphism and PD has been reported in caucasians.[5,8,9] We previously investigated the association of the CYP2D6A and CYP2D6B genes, which are the major contributors to the PM phenotype, in PD patients and healthy controls. No relationship was shown between PD in Japanese patients and EM/PM polymorphism in the CYP2D6 gene. However, a novel mutation was found at the *Hha*I site in exon 6 of the CYP2D6 gene (CYP2D6L). Individuals in the Japanese population homozygous for this mutation were at increased risk for PD.[10] Following this study, a novel mutant CYP2D6 gene (CYP2D6J) frequently observed in the Japanese population and related to EM/PM polymorphism was reported.[11-13] The CYP2D6J gene with a nucleotide substitution from C188 to T188 in exon 1 changes a conserved amino acid residue, Pro34, to Ser34 and reduces the enzyme activity of CYP2D6.[12]

In the present study, the CYP2D6J gene is analyzed in Japanese PD patients and controls to clarify whether EM/PM polymorphism is one of the contributors to PD. Moreover, the genetic heterogeneity of the CYP2D6 gene is investigated to understand the molecular basis of CYP2D6 in PD.

SUBJECTS AND METHODS

After obtaining informed consent, 63 PD patients (59 idiopathic and four juvenile) and 62 healthy volunteers were studied. The diagnosis of PD was based on international criteria[14]: the presence of two or more cardinal signs of the disease (muscle rigidity, tremor, bradykinesia, and postural instability) and responsiveness to levodopa therapy. Juvenile PD is determined by the time of onset, before age 40 years. There were four juvenile PD patients with no familial history of PD who were sensitive to drug therapy. Patients with multiple system atrophy and drug-induced or cerebrovascular parkinsonism were excluded from the study.

Genomic DNA from the peripheral leukocytes of PD patients and healthy controls was extracted by the modified method of Kan and Dozy.[15] The mutation at position 188 in exon 1, which is reported to reduce the enzyme activity of CYP2D6, was analyzed by polymerase chain reaction (PCR) and by digestion with *Hph*I. The PCR was performed in a total volume of 50 μL in the presence of 10 pmol of each primer (primer 1, 5′-CATATCCTGAACAAAGGAT-3′; primer 2, 5′-ATGCCCTTCTCCAGGACGT-3′), 1.25 mmol/L magnesium chloride, 10 mmol/L Tris-HCl (pH 8.3), 200 μmol/L of each dNTP, 400 ng genomic DNA, and 0.5 U Taq DNA polymerase (Promega, Madison, WI). The conditions for annealing, polymerization, and denaturation were 52°C for 1.5 minutes, 72°C for 1.5 minutes, and 94°C for 1 minute, respectively. The number of cycles amplified was 35. Amplified fragments were digested with *Hph*I (New England Biolabs, Beverly, MA), followed by electrophoresis in 2% agarose gel. The genotypes of the *Hha*I site mutation in exon 6 (CYP2D6L), mutation A (CYP2D6A) and mutation B (CYP2D6B) of the CYP2D6 gene have been previously described.[10] All subjects were of the EM genotype.

RESULTS

The distribution of each genotype of the *Hph*I site mutation (CYP2D6J gene) and the statistical analysis are shown in Table 1. The frequency of the CYP2D6J allele is 36.5% in PD patients and 41.9% in controls. A χ^2 contingency-table analysis for all three genotype frequencies in PD patients and controls showed that the frequency of the CYP2D6J gene did not differ between the two groups ($\chi^2 = 5.5$, $df = 2$, $P > .05$). The relative risk

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Table 1. Genotype Distribution and Statistical Analysis of the *Hph*I Site Mutation in Exon 1 of the CYP2D6 Gene

	w/w	w/m	m/m	χ^2	Relative Risk	95% CI
PD patients (n)	32	16	15	5.5 ($P > .05$)	1.3	0.6-3.1
Healthy controls (n)	22	28	12			

NOTE. χ^2 indicates the contingency-table test statistic for 3 × 2, comparing all 3 genotype frequencies in PD patients and controls. The relative risk represents the risk factor of the mutant homozygote for PD.

Abbreviations: w/w, w/m, and m/m, wild-type homozygote, heterozygote, and mutant-type homozygote, respectively.

associated with the homozygous CYP2D6J in PD patients was 1.3 (95% confidence interval, 0.6 to 3.1). No significant differences between PD patients and controls were observed for the genotype distribution of the CYP2D6J gene related to EM/PM polymorphism.

The combined genotype analysis of the two mutant CYP2D6 genes (CYP2D6J and CYP2D6L) was performed to investigate CYP2D6 gene heterogeneity between PD patients and controls. Table 2 shows a combined analysis of genotype distribution of the *Hph*I site mutation in exon 1 (CYP2D6J gene) and the *Hha*I site mutation in exon 6 (CYP2D6L gene). Genotype distribution was statistically compared. The double-mutant homozygote (m/m/m/m), the mutant homozygote of the *Hph*I site mutation with the mutant heterozygote of the *Hha*I site mutation (m/m/w/m), and the mutant heterozygote of the *Hph*I site mutation with the mutant homozygote of the *Hha*I site mutation (w/m/m/m) were not observed. The frequency of both the heterozygote for the *Hph*I site mutation and the wild-type homozygote for the *Hha*I site mutation (w/m/w/w) was 14.3% (9 of 63) in PD patients and 37.1% (23 of 62) in controls, and was statistically significant ($\chi^2 = 8.5$, $df = 1$, $P < .005$).

DISCUSSION

Most studies on the relationship between CYP2D6 and PD focus on the enzyme activity of CYP2D6.[5,8,9] Most Japanese are phenotypically EMs, but the logarithmic metabolic ratio (MR) of Japanese EMs varies from −1.0 to 1.0.[6] Because the CYP2D6J gene with the *Hph*I site mutation in exon 1 reduces the enzyme activity,[12] the variability in the MR is partly derived from this mutation in Japanese EMs. If this polymorphism is involved in the pathogenesis of PD, genotype distribution of the *Hph*I site mutation should be different between PD patients and controls. No significant difference between PD patients and controls was observed for the *Hph*I site mutation in exon 1 related to EM/PM polymorphism (Table 1). This result suggests that EM/PM polymorphism of CYP2D6 is associated less with the pathogenesis of PD in the Japanese population than in caucasians.[5,8,9]

Table 2. Combined Analysis of Genotype Distribution of the *Hph*I Site Mutation in Exon 1 and the *Hha*I Site Mutation in Exon 6 of the CYP2D6 Gene

Genotype of *Hph*I Site Mutation	Genotype of the *Hha*I Site Mutation						
	w/w		w/m		m/m		
	No.	%	No.	%	No.	%	Total
w/w							
PD	19	30.2	6	9.5	7	11.1	32
Control	14	22.6	6	9.7	2	3.2	22
w/m							
PD	9	14.3	7	11.1	0		16
Control	23	37.1	5	8.1	0		28
m/m							
PD	15	23.8	0		0		15
Control	12	19.3	0		0		12
Total							
PD	43		13		7		63
Control	49		11		2		62

Abbreviations: w/w, w/m, and m/m, wild-type homozygote, heterozygote, and mutant-type homozygote, respectively.

Our present study does not exclude involvement of CYP2D6 in the onset of PD. First, because we have previously reported a novel mutation in the CYP2D6 gene (CYP2D6L) that is associated with an increased risk for PD.[10] However, this mutation does not reduce CYP2D6 enzyme activity.[16] Second, interethnic differences in CYP2D6 gene polymorphism might affect the association study of CYP2D6 and PD. Therefore, factors other than the enzyme activity of CYP2D6 should be taken into account. One such possible factor, heterogeneity of the CYP2D6 gene, was investigated by combined genotype analysis. One axis is the *Hph*I site mutation, which is associated with the enzyme activity of CYP2D6.[11-13] The other axis is the *Hha*I site mutation, which has been shown to be a high risk factor for PD in the Japanese population[10] and did not reduce the enzyme activity of CYP2D6.[16] CYP2D6 gene heterogeneity was observed in both PD patients and controls (Table 2). Neither the double-mutant homozygote (m/m/m/m), nor the mutant homozygote of the *Hph*I site mutation with the mutant heterozygote of the *Hha*I site mutation (m/m/w/m), nor the mutant heterozygote of the *Hph*I site mutation with the mutant homozygote of the *Hha*I site mutation (w/m/m/m) were observed. A significant difference in the mutant homozygote of the *Hha*I site mutation in PD patients was discussed previously.[10] In this previous study, individuals were all wild-type homozygotes for the *Hph*I site mutation, suggesting that CYP2D6 enzyme activity was not decreased. Another significant difference was observed when the mutant heterozygote of the *Hph*I site mutation was found with the wild-type homozygote of the *Hha*I site mutation (w/m/w/w) in PD patients. This difference cannot be attributed to CYP2D6 enzyme activity, since there is no significant difference in the frequency of the mutant homozygote of the *Hph*I site mutation (m/m/w/w), which has less CYP2D6 enzyme activity in PD patients versus controls. It is difficult to give a reasonable explanation for this difference at present. Further investigation is required to clarify the association between CYP2D6 gene heterogeneity and PD.

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