Coding Polymorphisms in the Parkin Gene and Susceptibility to Parkinson Disease

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Background: Mutations in the parkin gene, an E3 protein-ubiquitin ligase, cause autosomal recessive early-onset Parkinson disease (PD). The role of polymorphisms in the parkin gene as risk factors for PD is still unclear, as the results in the literature are contradictory.

Patients: We compared the allele and genotype frequencies of the Ser167Asn, Arg366Trp, Val380Leu, and Asp394Asn polymorphisms in 194 patients with PD (92 familial and 102 sporadic) and 125 control subjects.

Results: Homozygous Val380 was significantly associated with sporadic PD (P=.008). There was also a trend toward an association of homozygous Asp394 with familial PD (P=.07).

Conclusions: Some parkin polymorphisms appear to be risk factors for sporadic or familial PD. The functional effects of these coding polymorphisms need to be established, and further studies on parkin polymorphisms in PD should be undertaken.

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THE CAUSE of the degeneration of the dopaminergic nigrostriatal pathway projections in Parkinson disease (PD) is largely unknown. In some rare families, PD is inherited as a Mendelian trait.1 To date, 9 disease loci, including 5 known genes (α-synuclein, parkin, UCHL1, DJ1, and Nurr1), have been described in monogenic forms, the most frequent of which is caused by parkin mutations.2 Mutations in the parkin gene, an E3 protein-ubiquitin ligase involved in the ubiquitin-proteasome pathway, result in a loss of function leading to an autosomal form of parkinsonism, with an early onset in most patients. Other forms of the disease are thought to be caused by a combination of environmental and genetic susceptibility factors. To identify susceptibility genes, various case-control or family-based association studies have been performed, including analysis of polymorphisms in some of the disease-causing genes just mentioned. Four polymorphisms that result in an amino acid change have been described in the parkin gene.3,4 For 3 of them, significant associations with PD have been reported at least once, but discordant results have also been reported, possibly because of ethnic differences or study limitations (eg, small sample size or inadequate matching between cases and controls).4,7 In a large case-control study, we tested the 4 known coding polymorphisms in the parkin gene that modify an amino acid for association with PD.

METHODS

We selected 194 European patients with PD, all white, according to the following criteria: symptoms of parkinsonism (at least 2 of the following: bradykinesia, rigidity, and rest tremor) with more than 30% improvement with levodopa and absence of atypical signs in the patients and the family members we examined. One hundred two patients had no affected relatives (sporadic PD [SPD] group). Of the 92 who had at least 1 affected relative (familial PD [FPD] group), 44 had 1 or more affected sibs, 40 had affected relatives in at least 2 successive generations, and 8 had more remotely related affected relatives. One hundred twenty-five matched white Europeans were used as control subjects (Table 1).

The polymorphisms studied were Ser167Asn (nt601G→A in exon 11), Arg366Trp (nt1197C→T in exon 10), Val380Leu (nt1239G→C, in exon 10), and Asp394Asn (nt1281G→A in exon 11). They were genotyped by polymerase chain reaction amplification of the respective exon (for primers see Kitada et alb), followed by digestion with AlwNI, NciI, BspI2861, and TaqI, respectively. Digestion products were analyzed by gel electrophoresis. Statistical analysis was done using the χ2 or Fisher
The age of the controls and the age at onset in patients were similar, as were the sex ratios, demonstrating adequate matching (\(P = .74\) and \(P = .69\), respectively) (Table 1). All 4 polymorphisms were in Hardy-Weinberg equilibrium in the control group.

The Arg366Trp polymorphism was omitted from further analysis because there was only a single heterozygous case in the FPD group. All other allele and genotype frequencies are presented in Table 2, Table 3, and Table 4. There was no linkage disequilibrium between the 3 remaining polymorphisms. The Val380 allele (nt1239G) was significantly more frequent in the overall PD sample (\(P = .03\); OR, 1.60; 95% CI, 1.05-2.42) and especially in the SPD group (\(P = .008\); OR, 2.02; 95% CI, 1.20-3.40). The predisposing genotype was G/G (\(P = .07\); OR, 1.56; 95% CI, 0.97-2.52 for the overall PD sample and \(P = .02\), OR, 2.07; 95% CI, 1.15-3.73 for the SPD group). This effect was independent of age at onset, sex, and presence of the 2 other polymorphisms (\(P = .07\), when corrected for these factors). Finally, a trend toward an association of the Asp394 allele (nt1281G) with FPD was detected (\(P = .07\); OR, 3.04; 95% CI, 0.85-10.94). Again, the most frequent genotype was G/G (\(P = .07\); OR, 3.15; 95% CI, 0.86-11.51), and the effect was independent of age at onset, sex, and presence of the 2 other polymorphisms (\(P = .12\), when corrected for these factors).

To our knowledge, this is the first study that comprehensively analyzes all known coding polymorphisms in the parkin gene (Ser167Asn, Arg366Trp, Val380Leu, and Asp394Asn) in which their effects on FPD and SPD, age at onset, and interaction between the polymorphisms were tested in a white European population.
The Arg366Trp polymorphism was too rare to be tested in our population, precluding replication of the findings of this polymorphism by Wang et al in a Japanese population. The 3 remaining polymorphisms were not in linkage disequilibrium, despite their location within the same gene, suggesting the existence of frequent recombinations within the large introns of the parkin gene.

As in previous studies in Asian and white populations, we did not find an association between PD and the allele or the genotype frequency of the Ser167Asn polymorphism. Interestingly, the frequency of this polymorphism appears to be highly dependent on ethnic origin (40% in Asian populations vs 2% in the present study). This underscores the need for ethnically matched controls and raises the hypothesis that presence of the allele associated with PD may be related to genetic susceptibility.

In this study, we detected a positive association between SPD and homozygosity for the Val380 allele. An association between this polymorphism and early-onset SPD (age at onset, <50 years) has been reported, but the allele associated with SPD was not clearly specified. We did not, however, find an effect of the Val380 allele on age at onset. No associations with this allele were found in 2 Asian PD populations, in which the frequency of the variant allele was low (4%-5%), or in studies in a Finnish population in which allele frequencies were similar to ours. As for homozygosity of the Asp394 allele, we detected a trend toward association with FPD. This polymorphism was analyzed only once before, with no differences in frequency found between patients with PD and controls. These discrepancies demonstrate the need for replication of association studies, as recently underscored.

For the Val380Leu and the Asp394Asn polymorphisms, presence of the most frequent alleles (found in 79% of controls for Val380 and in 93% for Asp394) increased the risk of PD, suggesting that the rare alleles might be protective. An association with the most common haplotype was also found between polymorphisms in the tau gene and progressive supranuclear palsy. We could not find an interaction between the Val380Leu and Asp394Asn polymorphisms. Because they are both located in the RING-IBR-RING domain of parkin, one might speculate that they could affect the binding of substrates or E2 protein–ubiquitin–conjugating enzymes (UbCH7 and 8).

Table 4. Allele and Genotype Frequencies of the Asp394Asn Polymorphism

<table>
<thead>
<tr>
<th>Asp394Asn</th>
<th>Allele G</th>
<th>Allele A</th>
<th>G/G</th>
<th>G/A</th>
<th>Odds Ratios (95% Confidence Interval)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases (N = 194)</td>
<td>378 (97.4)</td>
<td>10 (2.6)</td>
<td>184 (94.8)</td>
<td>10 (5.2)</td>
<td>G vs A: 1.91 (0.81-4.48)</td>
<td>.13</td>
</tr>
<tr>
<td>Familial Parkinson disease (n = 92)</td>
<td>181 (98.4)</td>
<td>3 (1.6)</td>
<td>89 (96.7)</td>
<td>3 (3.3)</td>
<td>G vs A: 3.04 (0.85-10.94)</td>
<td>.07</td>
</tr>
<tr>
<td>Sporadic Parkinson disease (n = 102)</td>
<td>197 (96.6)</td>
<td>7 (3.4)</td>
<td>95 (93.1)</td>
<td>7 (6.9)</td>
<td>G vs A: 1.41 (0.55-3.67)</td>
<td>.47</td>
</tr>
<tr>
<td>Controls (N = 125)</td>
<td>238 (95.2)</td>
<td>12 (4.8)</td>
<td>113 (90.4)</td>
<td>12 (9.6)</td>
<td>G vs A: 1.44 (0.55-3.81)</td>
<td>.46</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) unless otherwise indicated. All comparisons are made between the group and the controls. Trends are in italics.
†χ² Test.

Correction

In the Original Contribution by Lee et al titled “Frequency Analysis and Clinical Characterization of Spinocerebellar Ataxia Types 1, 2, 3, 6, and 7 in Korean Patients,” published in the June issue of the ARCHIVES (2003;60:858-863), an error occurred on page 862. The corresponding author was correctly listed as Won Yong Lee, MD, PhD, Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-ku, Seoul 135-710, Korea (e-mail: wylee@smc.samsung.co.kr). However, the contact person for reprints should have been listed as Dong Kyu Jin, MD, PhD, Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-ku, Seoul, 135-710, Korea (e-mail: dkjin@smc.samsung.co.kr). The journal regrets the error.