Analysis of Dosage Mutation in PARK2 among Korean Patients with Early-Onset or Familial Parkinson’s Disease

Min Kyung Chu,¹ Won Chan Kim,² Jung Mi Choi,³ Jeong-Hoon Hong,³
Suk Yun Kang,⁴ Hyeo-Il Ma,⁴ Yun Joong Kim¹,⁴,⁵

¹Department of Neurology, Hallym University College of Medicine, Anyang, Korea
²Department of Neurology, CHA University College of Medicine, Pocheon, Korea
³Ilsong Institute of Life Science, Hallym University, Anyang, Korea
⁴Hallym Institute of Translational Genomics and Bioinformatics, Hallym University Medical Center, Anyang, Korea

Background and Purpose There is some controversy regarding heterozygous mutations of the gene encoding parkin (PARK2) as risk factors for Parkinson’s disease (PD), and all previous studies have been performed in non-Asian populations. Dosage mutation of PARK2, rather than a point mutation or small insertion/deletion mutation, was reported to be a risk factor for familial PD; dosage mutation of PARK2 is common in Asian populations.

Methods We performed a gene-dosage analysis of PARK2 using real-time polymerase chain reaction for 189 patients with early-onset PD or familial PD, and 191 control individuals. In the case of PD patients with heterozygous gene-dosage mutation, we performed a sequencing analysis to exclude compound heterozygous mutations. The association between heterozygous mutation of PARK2 and PD was tested.

Results We identified 22 PD patients with PARK2 mutations (11.6%). Five patients (2.6%) had compound heterozygous mutations, and 13 patients (6.9%) had a heterozygous mutation. The phase could not be determined in one patient. Three small sequence variations were found in 30 mutated alleles (10.0%). Gene-dosage mutation accounted for 90% of all of the mutations found. The frequency of a heterozygous PARK2 gene-dosage mutation was higher in PD patients than in the controls.

Conclusions Heterozygous gene-dosage mutation of PARK2 is a genetic risk factor for patients with early-onset or familial PD in Koreans.

Key Words Parkinson’s disease, PARK2, gene-dosage change, risk factor.

Introduction

Several causative genes for Parkinson’s disease (PD) have been identified in familial PD, with autosomal-dominant or autosomal-recessive inheritance patterns.⁴ Among these, mutation in the gene encoding parkin (PARK2) is the most common genetic risk factor for early-onset PD (EOPD).² The frequency of PARK2 mutations has been reported to be as high as 49% in patients with EOPD, with an autosomal-recessive mode of inheritance,⁷ whereas it has been reported to be 14–15% in patients with EOPD without a family history of PD.¹⁴

The types of mutations found in PARK2 are highly variable, such as point mutations, small deletions/insertions, and exonic rearrangement (either deletion or duplication), and have been reported in all exons of the gene.⁴ Notably, point mutations or small insertions/deletions, which are found in approximately 50% of Caucasian PD patients with PARK2 mutations, are infrequent in Asian populations.²,³,⁵,⁹,¹¹ Although PARK2 mutations were initially found in patients with familial PD with an autosomal-recessive mode of inheritance, heterozygous mutations were also not uncommonly found in
PD patients. Whether a single heterozygous mutation of PARK2 is a risk factor for PD is controversial. Pankratz et al. reported that PARK2 dosage mutation, rather than a point mutation or small insertion/deletion mutation, was a risk factor for familial PD, and may also be associated with a younger age at onset. Only a few previous studies have included control populations as well as PD patients in sequencing or gene-dosage analyses. Moreover, only one study screened for PARK2 gene-dosage mutation in 54 Asia populations that were included as controls. In the present study we assessed the heterozygosity of PARK2 mutations in relation to the risk of PD by performing gene-dosage analysis in 189 EOPD or familial PD patients and 191 control individuals.

Methods

Subjects
Fifty-two familial PD (44.2% male) and 137 early-onset PD (59.1% male) patients with sporadic onset were recruited from 5 movement-disorder clinics in Korea. The PD patients were diagnosed by movement disorders specialists according to the UK PD Brain Bank criteria. EOPD was defined when the age at onset was ≤55 years. The patients were 6–67 years old (40.3±13.6 years, mean±SD) at disease onset and 13–76 years old (44.6±10.9 years) at blood sampling. Patients who had been included in our previous study were excluded from this study. Furthermore, 191 healthy controls (34.6% male) who were asymptomatic when screened by a neurological examination were recruited from among the National Health Examinees at Hallym University Sacred Heart Hospital. The age of the control individuals at the time of blood sampling was 51.9±13.0 years. All of the subjects were of Korean ethnicity. The study was approved by the Institutional Review Boards at Hallym University Sacred Heart Hospital, and informed consent to participate was obtained from all of the subjects.

Molecular analysis
Genomic DNA was extracted from the peripheral lymphoblasts of each subject according to a standard protocol. Quantitative real-time polymerase chain reaction (PCR) was performed using the StepOnePlus Real-time PCR System (Applied Biosystems, Foster, CA, USA). Either commercial kits (Taqman Copy Number Assays, Applied Biosystems, Foster, CA, USA) or custom-made primers and probes were used (Supplementary Table 1 and 2). RNase P was used as the endogenous control. All PCR reactions were carried out with the following program: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C. The relative change in PARK2 expression was calculated using the 2-ΔΔCT method. In the case of PD patients with a confirmed PARK2 dosage mutation, variants were screened in all exons of the gene to detect point mutations or small insertions/deletions, by PCR and direct sequencing by using previously described conditions.

Statistical analysis
The frequency of a single heterozygous mutation was compared between PD and control individuals using Fisher’s exact test in order to determine whether gene-dosage mutation in one allele increases the risk for PD. The Mann-Whitney U-test or ANOVA were used to compare age at onset between or among the groups. The cutoff for statistical significance was set at p<0.05.

Results
We identified PARK2 mutations in at least 1 allele in 22 of the 189 patients (11.6%) (Table 1). Eight of these patients (36.4%) had mutations in both alleles (5 compound heterozygous and 3 homozygous mutations), and 13 patients (59.1%) had heterozygous mutations. The phase could not be determined in a patient with deletions of exons 2 and 3. Of the patients with PARK2 mutations, 12 had a family history of PD (54.5%) and 10 had EOPD (45.5%). Those with familial PD comprised two compound heterozygous mutations, eight heterozygous mutations, one homozygous mutation, and one phase-unknown mutation, while the EOPD cases comprised three compound heterozygous mutations, two homozygous mutations, and five heterozygous mutations. Furthermore, a family history of PD in first- and second-degree relatives was present in 11 and 1 of the 12 familial PD patients with PARK2 mutations, respectively.

Small sequence variations were found in 3 (10.0%) of the 30 mutated alleles, with the majority (90.0%) of the mutations being exonic rearrangements. In a patient with a point mutation and an insertion (G284R/c.674insT), sequence variations in both alleles were found incidentally because a mutation was located in the binding region of the probe used in the analysis. In 27 alleles with gene-dosage mutations, 24 were deletions and 3 were duplications. All of the observed exonic rearrangements occurred in exons 1–11; no mutations were found in exon 12, and exons 1 and 4 were the most common sites of gene-dosage mutation (n=6, respectively). Although the mean age at PD onset appeared to be younger in patients with PARK2 mutation in two alleles than in those with PARK2 mutations in a single allele (excluding a case with unknown phase), the difference was not statistically significant (23.3±13.7 years vs. 28.5±8.3 years; Mann-Whitney U-test, p=0.05). In 13 out of 22 patients (59.1%) with a PARK2 mutation, the age at PD onset was ≤30 years (Table 2).
Dosage Mutation of PARK2

Table 1. Demographic characteristics of patients with PARK2 mutations, and the type and location of the mutations

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age at sample</th>
<th>Age at onset</th>
<th>Family history</th>
<th>Variants of PARK2</th>
<th>Zygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>17</td>
<td>17</td>
<td>-</td>
<td>Ex1 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>M</td>
<td>56</td>
<td>35</td>
<td>-</td>
<td>Ex1 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>M</td>
<td>26</td>
<td>26</td>
<td>+</td>
<td>Ex1 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>M</td>
<td>41</td>
<td>23</td>
<td>+</td>
<td>Ex1 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>F</td>
<td>32</td>
<td>32</td>
<td>+</td>
<td>Ex1 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>36</td>
<td>-</td>
<td>Ex2 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>F</td>
<td>48</td>
<td>45</td>
<td>+</td>
<td>Ex2 dupl</td>
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<tr>
<td>F</td>
<td>28</td>
<td>28</td>
<td>+</td>
<td>Ex3 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>F</td>
<td>25</td>
<td>25</td>
<td>-</td>
<td>Ex4 del</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>M</td>
<td>30</td>
<td>29</td>
<td>+</td>
<td>Ex4 del</td>
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</tr>
<tr>
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<td>+</td>
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<td>23</td>
<td>+</td>
<td>Ex7 del</td>
<td>Heterozygous</td>
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<tr>
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<td>43</td>
<td>41</td>
<td>-</td>
<td>Ex10 del</td>
<td>Homozygous</td>
</tr>
<tr>
<td>F</td>
<td>25</td>
<td>6</td>
<td>-</td>
<td>Ex11 del</td>
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<tr>
<td>F</td>
<td>50</td>
<td>30</td>
<td>+</td>
<td>Ex4 del</td>
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</tr>
<tr>
<td>M</td>
<td>34</td>
<td>28</td>
<td>-</td>
<td>Ex2 del/Ex4 del</td>
<td>Compound heterozygous</td>
</tr>
<tr>
<td>F</td>
<td>44</td>
<td>41.5</td>
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<tr>
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<td>16</td>
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<td>Ex3 del/Ex1-4 del</td>
<td>Compound heterozygous</td>
</tr>
<tr>
<td>F</td>
<td>38</td>
<td>12</td>
<td>-</td>
<td>Ex7 dupl/c.101delAinsAG*</td>
<td>Compound heterozygous</td>
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<tr>
<td>F</td>
<td>48</td>
<td>12</td>
<td>+</td>
<td>G284R/c.674insT</td>
<td>Compound heterozygous</td>
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<tr>
<td>M</td>
<td>46</td>
<td>42</td>
<td>+</td>
<td>Ex2 del and Ex3 del</td>
<td>Phase unknown</td>
</tr>
</tbody>
</table>

*rs55777503.


Table 2. Distribution of age-at-onset of 22 Parkinson disease patients with PARK2 mutation

<table>
<thead>
<tr>
<th>Age range (years old)</th>
<th>Early-onset Parkinson disease (n=137)</th>
<th>Familial Parkinson disease (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger than 31</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>31–40</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>41–50</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Older than 50</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

three familial PD patients, the age at PD onset was >40 years. There were no patients with a PARK2 mutation whose age at onset was >45 years.

The risk for PD posed by gene-dosage mutation in only one allele of PARK2 was assessed, as was the existence of PARK2 gene-dosage mutation in the 191 control individuals. None of the non-PD control individuals had a PARK2 gene-dosage mutation. For an association study, we excluded PD patients with PARK2 mutations in both alleles or of unknown phase. Since the frequency of PARK2 gene-dosage mutation in each exon is so low that it cannot provide sufficient power to test separately for an association, the frequency of gene-dosage mutations in a single allele of PARK2, in any exon, was compared between the PD patients and control individuals. The frequency of a single PARK2 mutation due to exonic rearrangement was higher among the PD patients than among the control individuals (7.2% vs. 0.0%, p<0.0001, Fisher’s exact test).

Discussion

Mutation of PARK2 is reportedly the most common genetic cause for familial PD or EOPD.23 Although the frequency of PARK2 mutations in our study was rather low, the types of mutation and their location were consistent with the results reported previously for both Asian and other populations.5-9,21,24 However, although in our study the age at onset tended to be younger in patients with mutations in two alleles compared to those with mutations in a single allele, as found in previous studies,25,26 the difference was not statistically significant. This difference in findings may be attributable to the smaller number of PARK2 mutation cases in our series. However, the potential contribution of other factors such as ethnic background or the type of mutation cannot be excluded.

In Asian populations, the frequency of PARK2 mutations, regardless of zygosity, varies between 5.6% and 48.3% depending on the characteristics of the study population such as the family history or age at onset.6,11,21,24 The frequency of
PARK2 mutations in our study was 11.6%, which is lower than that found in other studies. We believe that the low mutation frequency observed in our cohort can be attributed to our selection criteria; the age at onset for EOPD in this study was older than in other studies. Although we failed to demonstrate that PD patients carrying a PARK2 mutation have a younger age at PD onset than noncarriers, 59.1% of patients with a PARK2 mutation developed PD before the age of 31 years, and none developed PD after the age of 45 years. These findings suggest that PARK2 mutations are related to a younger age at PD onset.

There are currently no guidelines or indications for PARK2 genetic screening for mutations, but the present findings suggest that an age at PD onset younger than 30 years is a strong indicator for such screening. Alternatively, PD with an age at onset older than 45 years might not be justifiable for PARK2 mutation screening. We did not sequence PARK2 in all PD patients, and so the frequency of PARK2 mutations might have been underestimated. However, we do not expect that complete sequencing in the case of all PD patients is likely to markedly alter the frequency of the observed PARK2 mutations. Unlike Caucasian populations, point mutations of PARK2 are not common in Asian populations; most of those that are observed in Asians are exonic rearrangements. Point mutations or small insertions/deletions, which have been reported to represent 8.0–16.7% of all PARK2 mutations in Asian PD patients, were found in two patients (9.1%) in our case series. The frequency of heterozygous mutations among PARK2 mutation carriers in our PD cohort (59.1%) is within the frequency range described by previous studies in Asian PD patients (16.7–75%).

To exclude compound heterozygous mutations due to sequence variation in patients with heterozygous gene-dosage mutation, we sequenced all exons of PARK2; small sequence variations were found in only two patients. We did not perform Sanger sequencing in all PD patients with no PARK2 gene-dosage changes, although the frequency of PARK2 point mutations is low in Asian populations, and so the data might have underestimated the importance of such point mutations in Asian PD. Heterozygous PARK2 gene-dosage mutations in PD have been reported worldwide. However, whether heterozygous mutations of PARK2 are genetic risk factors for PD remains a matter of controversy, even though some studies performed PARK2 genotyping in control populations. Since most of the subjects who participated in these studies were not Asian, we cannot directly compare our results with those of these other studies. Gene-dosage mutation of PARK2 was analyzed in a small number of healthy Asian controls (n=54) in only one study.

A possible explanation for the lack of consensus lies with the selected study populations of PD patients, because age at PD onset appears to be an important factor. Studies of EOPD or familial PD have found a positive association or a trend toward an association, whereas those investigating idiopathic PD have generally found a negative association between heterozygous carriers of PARK2 mutations and PD. A recent comprehensive analysis of PARK2 mutations in 1686 controls and 2091 PD patients found that the frequency of PARK2 mutations among PD patients varied with the age at PD onset, whereas that among controls remained constant across all age groups. The frequency of PARK2 mutations was found to be extremely high in EOPD patients, declining sharply with increasing age at onset. By an age 45 years (and thereafter), the mutation frequency in PD and control subjects are completely superimposed.

The type of mutation is another potentially confounding factor, because as Pankratz et al., reported, a PARK2 dosage mutation—but not a simple sequence variation—may be a risk factor for PD. The reported frequencies of heterozygous mutations in controls vary considerably depending on the type of mutation. Heterozygous point mutations of PARK2 are found in as many as 3.4% of controls, whereas heterozygous PARK2 gene-dosage mutations are extremely rare in control subjects. Three of seven studies that performed PARK2 gene-dosage analysis in controls found no PARK2 gene-dosage mutation, as was the case in the current investigation. In the other four studies, although rare, heterozygous gene-dosage mutations were found in controls, with the average frequency of heterozygous carriers being 0.85% (range, 0.52–1.09%).

Several caveats in addition to the sequencing issue mentioned above must be considered when drawing conclusions from the present findings. First, the mean age of the controls (51.9 years) is younger than the previously reported mean age at onset of idiopathic PD (58–62 years). It is possible that some of the controls will develop PD in the future. Second, we studied only EOPD or familial PD patients, and therefore our results cannot be generalized to the entire PD population. Third, the samples in the present study were smaller than those in two recent comprehensive analyses. However, given that gene-dosage mutation is a common form of PARK2 mutation in Asian populations, we believe that heterozygous gene-dosage mutation as a risk factor for EOPD or familial PD has important genetic and clinical implications.

In conclusion, heterozygous gene-dosage mutation of PARK2 is a genetic risk factor for PD in Korean patients with EOPD or familial PD.

Conflicts of Interest

The authors have no financial conflicts of interest.
REFERENCES


