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

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**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.

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**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.<sup>1</sup> Among these, mutation in the gene encoding parkin (*PARK2*) is the most common genetic risk factor for early-onset PD (EOPD).<sup>2,3</sup> 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,<sup>2</sup> whereas it has been reported to be 14–15% in patients with EOPD without a family history of PD.<sup>3,4</sup>

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.<sup>5</sup> 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.<sup>2,3,5-11</sup> 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

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PD patients.<sup>1-3,12,13</sup> Whether a single heterozygous mutation of *PARK2* is a risk factor for PD is controversial.<sup>14-20</sup> Pankratz et al.<sup>19</sup> 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.<sup>21</sup> 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.<sup>22</sup> EOPD was defined when the age at onset was  $\leq 55$  years. The patients were 6–67 years old ( $40.3 \pm 13.6$  years, mean  $\pm$  SD) at disease onset and 13–76 years old ( $44.6 \pm 10.9$  years) at blood sampling. Patients who had been included in our previous study were excluded from this study.<sup>7</sup> 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  $51.9 \pm 13.0$  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  $2^{-\Delta\Delta CT}$  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.<sup>7</sup>

### 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 ( $23.3 \pm 13.7$  years vs.  $28.5 \pm 8.3$  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  $\leq 30$  years (Table 2). In one EOPD and

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

Sex	Age at sample	Age at onset	Family history	Variants of <i>PARK2</i>	Zygosity
F	17	17	-	Ex1 del	Heterozygous
M	56	35	-	Ex1 del	Heterozygous
M	26	26	+	Ex1 del	Heterozygous
M	41	23	+	Ex1 del	Heterozygous
F	32	32	+	Ex1 del	Heterozygous
M	36	36	-	Ex2 del	Heterozygous
F	48	45	+	Ex2 dupl	Heterozygous
F	28	28	+	Ex3 del	Heterozygous
F	25	25	-	Ex4 del	Heterozygous
M	30	29	+	Ex4 del	Heterozygous
F	23	15	-	Ex5 dupl	Heterozygous
F	36	36	+	Ex6 del	Heterozygous
F	42	23	+	Ex7 del	Heterozygous
M	43	41	-	Ex10 del	Homozygous
F	25	6	-	Ex11 del	Homozygous
F	50	30	+	Ex4 del	Homozygous
M	34	28	-	Ex2 del/Ex4 del	Compound heterozygous
F	44	41.5	+	Ex7-10 del/Ex8-10 del	Compound heterozygous
M	24	16	-	Ex3 del/Ex1-4 del	Compound heterozygous
F	38	12	-	Ex7 dupl/c.101delAinsAG*	Compound heterozygous
F	48	12	+	G284R/c.674insT	Compound heterozygous
M	46	42	+	Ex2 del and Ex3 del	Phase unknown

\*rs55777503.

del: deletion, dupl: duplication, Ex: exon, F: female, M: male.

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

Age range (years old)	Early-onset Parkinson disease (n=137)	Familial Parkinson disease (n=52)
Younger than 31	7	6
31–40	2	3
41–50	1	3
Older than 50	0	0

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.<sup>23</sup> 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.<sup>5-9,21,24</sup> 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,<sup>25,26</sup> 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.<sup>6-11,21,24</sup> 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;<sup>27</sup> 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.<sup>3-7,9,15-17,21,28</sup> 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.<sup>14-20</sup> 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.<sup>21</sup>

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,<sup>15,16,18,19</sup> whereas those investigating idiopathic PD have generally found a negative association between heterozygous carriers of *PARK2* mutations and PD.<sup>14,20</sup> 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.<sup>20</sup> 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.<sup>19</sup> reported, a *PARK2* dosage mutation-but not a simple sequence variation-may be a risk factor for PD.<sup>19</sup> 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,<sup>19</sup> whereas heterozygous *PARK2* gene-dosage mutations are extremely rare in control subjects.<sup>14-16,18-20,29</sup> 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.<sup>15,16,19</sup> 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%).<sup>14,18,20,29</sup>

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.<sup>19,20</sup> 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.

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## REFERENCES

- Singleton AB, Farrer MJ, Bonifati V. The genetics of Parkinson's disease: progress and therapeutic implications. *Mov Disord* 2013;28:14-23.
- Lücking CB, Dürr A, Bonifati V, Vaughan J, De Michele G, Gasser T, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. *N Engl J Med* 2000;342:1560-1567.
- Periquet M, Latouche M, Lohmann E, Rawal N, De Michele G, Ricard S, et al. Parkin mutations are frequent in patients with isolated early-onset parkinsonism. *Brain* 2003;126(Pt 6):1271-1278.
- Hedrich K, Marder K, Harris J, Kann M, Lynch T, Mejia-Santana H, et al. Evaluation of 50 probands with early-onset Parkinson's disease for Parkin mutations. *Neurology* 2002;58:1239-1246.
- Hedrich K, Eskelson C, Wilmot B, Marder K, Harris J, Garrels J, et al. Distribution, type, and origin of Parkin mutations: review and case studies. *Mov Disord* 2004;19:1146-1157.
- Guo JF, Xiao B, Liao B, Zhang XW, Nie LL, Zhang YH, et al. Mutation analysis of Parkin, PINK1, DJ-1 and ATP13A2 genes in Chinese patients with autosomal recessive early-onset Parkinsonism. *Mov Disord* 2008;23:2074-2079.
- Choi JM, Woo MS, Ma HI, Kang SY, Sung YH, Yong SW, et al. Analysis of PARK genes in a Korean cohort of early-onset Parkinson disease. *Neurogenetics* 2008;9:263-269.
- Guo JF, Zhang XW, Nie LL, Zhang HN, Liao B, Li J, et al. Mutation analysis of Parkin, PINK1 and DJ-1 genes in Chinese patients with sporadic early onset parkinsonism. *J Neurol* 2010;257:1170-1175.
- Zhang BR, Hu ZX, Yin XZ, Cai M, Zhao GH, Liu ZR, et al. Mutation analysis of parkin and PINK1 genes in early-onset Parkinson's disease in China. *Neurosci Lett* 2010;477:19-22.
- Kim SY, Seong MW, Jeon BS, Kim SY, Ko HS, Kim JY, et al. Phase analysis identifies compound heterozygous deletions of the PARK2 gene in patients with early-onset Parkinson disease. *Clin Genet* 2012;82:77-82.
- Chung EJ, Ki CS, Lee WY, Kim IS, Kim JY. Clinical features and gene analysis in Korean patients with early-onset Parkinson disease. *Arch Neurol* 2006;63:1170-1174.
- Klein C, Pramstaller PP, Kis B, Page CC, Kann M, Leung J, et al. Parkin deletions in a family with adult-onset, tremor-dominant parkinsonism: expanding the phenotype. *Ann Neurol* 2000;48:65-71.
- Kann M, Hedrich K, Viergege P, Jacobs H, Müller B, Kock N, et al. The parkin gene is not involved in late-onset Parkinson's disease. *Neurology* 2002;58:835; author reply 835.
- Lincoln SJ, Maraganore DM, Lesnick TG, Bounds R, de Andrade M, Bower JH, et al. Parkin variants in North American Parkinson's disease: cases and controls. *Mov Disord* 2003;18:1306-1311.
- Clark LN, Afridi S, Karlins E, Wang Y, Mejia-Santana H, Harris J, et al. Case-control study of the parkin gene in early-onset Parkinson disease. *Arch Neurol* 2006;63:548-552.
- Lesage S, Lohmann E, Tison F, Durif F, Dürr A, Brice A, et al. Rare heterozygous parkin variants in French early-onset Parkinson disease patients and controls. *J Med Genet* 2008;45:43-46.
- Kay DM, Moran D, Moses L, Poorkaj P, Zabetian CP, Nutt J, et al. Heterozygous parkin point mutations are as common in control subjects as in Parkinson's patients. *Ann Neurol* 2007;61:47-54.
- Brooks J, Ding J, Simon-Sanchez J, Paisan-Ruiz C, Singleton AB, Scholz SW. Parkin and PINK1 mutations in early-onset Parkinson's disease: comprehensive screening in publicly available cases and control. *J Med Genet* 2009;46:375-381.
- Pankratz N, Kissell DK, Pauculo MW, Halter CA, Rudolph A, Pfeiffer RF, et al. Parkin dosage mutations have greater pathogenicity in familial PD than simple sequence mutations. *Neurology* 2009;73:279-286.
- Kay DM, Stevens CF, Hamza TH, Montimurro JS, Zabetian CP, Factor SA, et al. A comprehensive analysis of deletions, duplications, and copy number variations in PARK2. *Neurology* 2010;75:1189-1194.
- Wang C, Ma H, Feng X, Xie S, Chan P. Parkin dosage mutations in patients with early-onset sporadic and familial Parkinson's disease in Chinese: an independent pathogenic role. *Brain Res* 2010;1358:30-38.
- Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. *Neurology* 2001;57:1497-1499.
- Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum Mol Genet* 2009;18(R1):R48-R59.
- Wu RM, Bounds R, Lincoln S, Hulihan M, Lin CH, Hwu WL, et al. Parkin mutations and early-onset parkinsonism in a Taiwanese cohort. *Arch Neurol* 2005;62:82-87.
- Foroud T, Uniacke SK, Liu L, Pankratz N, Rudolph A, Halter C, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. *Neurology* 2003;60:796-801.
- Sun M, Latourelle JC, Wooten GF, Lew MF, Klein C, Shill HA, et al. Influence of heterozygosity for parkin mutation on onset age in familial Parkinson disease: the GenePD study. *Arch Neurol* 2006;63:826-832.
- Selikhova M, Williams DR, Kempster PA, Holton JL, Revesz T, Lees AJ. A clinico-pathological study of subtypes in Parkinson's disease. *Brain* 2009;132(Pt 11):2947-2957.
- Hedrich K, Kann M, Lanthaler AJ, Dalski A, Eskelson C, Landt O, et al. The importance of gene dosage studies: mutational analysis of the parkin gene in early-onset parkinsonism. *Hum Mol Genet* 2001;10:1649-1656.
- Brüggemann N, Mitterer M, Lanthaler AJ, Djarmati A, Hagenah J, Wiegers K, et al. Frequency of heterozygous Parkin mutations in healthy subjects: need for careful prospective follow-up examination of mutation carriers. *Parkinsonism Relat Disord* 2009;15:425-429.