Mutation Analysis of the PINK1 Gene in 391 Patients With Parkinson Disease

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Objectives: To determine the frequency, distribution, and clinical features of Parkinson disease (PD) with PINK1 mutations.

Design: Retrospective clinical and genetic review.

Setting: University hospital.

Patients: We performed extensive mutation analyses of PINK1 in 414 PD patients negative for parkin mutations (mean [SD] age at onset, 42.8 [14.3] years), including 391 unrelated patients (190 patients with sporadic PD and 201 probands of patients with familial PD) from 13 countries.

Results: We found 10 patients with PD from 9 families with PINK1 mutations and identified 7 novel mutations (2 homozygous mutations [p.D297MfsX22 and p.W437R] and 5 single heterozygous mutations [p.A78V, p.P196QfsX25, p.M342V, p.W437R, and p.N542S]). No compound heterozygous mutations were found. The frequency of homozygous mutations was 4.26% (2 of 47) in families with autosomal recessive PD and 0.53% (1 of 190) in patients with sporadic PD. The frequency of heterozygous mutations was 1.89% (2 of 106) in families with potential autosomal dominant PD and 1.05% (2 of 190) in patients with sporadic PD. The mean (SD) age at onset in patients with single heterozygous mutations (33.6 [11.1] years; range, 39-69 years) was higher than that in patients with homozygous mutations (34.0 [20.3] years; range, 10-55 years). Myocardial iodine-123 metaiodobenzylguanidine uptake was low in patients with heterozygous mutations but not in those with homozygous mutations.

Conclusions: Our results suggest that homozygous PINK1 mutations tend to be diagnosed as the early-onset autosomal recessive form of PD. Single heterozygous mutations may contribute to the development of sporadic PD and also could be an additional genetic predisposition for developing familial PD. The reduced myocardial iodine-123 metaiodobenzylguanidine uptake observed in patients with single heterozygous PINK1 mutations is similar to that seen in patients with sporadic PD.

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Parkinson disease (PD) is predominantly characterized by degeneration of midbrain dopaminergic neurons, eventually leading to various motor dysfunctions, such as rigidity, tremor, bradykinesia, and postural instability. The etiology of PD is unknown but is presumably multifactorial, eg, perhaps having a genetic × environmental interaction.

Although most PD cases are sporadic, several causative genes have been identified in recent years in familial forms of PD. For example, alpha-synuclein (loci, PARK1 and PARK4), UCH-L1 (PARK5), and LRRK2/dardarin (PARK8) are reported to be the causative genes for autosomal dominant PD (ADPD); and parkin (PARK2), DJ-1 (PARK7), and PINK1 (OMIM 608309) (PARK6) are reported to be the causative genes for autosomal recessive PD (ARPD). Mutations in parkin are the major cause of ARPD, and the frequency of such mutations in families with ARPD is approximately 50%. In contrast, mutations in DJ-1 are rare (≤ 1%) in ARPD. Increasing numbers of patients with PINK1 mutations are being reported; however, there are no sufficiently large studies to define the frequency, age distribution, or clinical features of patients with PD associated with PINK1 mutations worldwide, especially not in Asia. Moreover, no association between PD and coding single nucleotide polymorphisms within PINK1 has been reported. The role of a single heterozygous PINK1 mutation in the clinical manifestation of Parkinsonism, such as age at onset, is not clear at present, mainly because previous reports have not identified substantial num-

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bers of PINK1 mutations. To clarify these aspects, we performed extensive mutation analysis in a large number of patients with PD in 13 countries.

## METHODS

### PATIENTS

We studied 414 parkin-negative PD patients (391 unrelated patients and 23 relatives) from 13 countries (249 Japanese, 55 Korean, 28 Israeli, 27 Taiwanese, 27 Chinese, 14 Tunisian, 5 Turkish, 3 Greek, 2 Moroccan, 1 Filipino, 1 Bulgarian, 1 Brazilian, and 1 Australian individual). Patients received clinical diagnoses of PD\(^\text{11}\) regardless of their familial history. The distribution of age at onset was as follows: younger than 50 years (early-onset) \((n=287 [69.3\%])\), 50 years or older (late-onset) \((n=121 [29.7\%])\). Hereditary information was obtained in 111 patients (from different hospitals) with an intravenous injection of 111 MBq of \(^{123}\)I-MIBG (Daichi Radioisotope Laboratories, Tokyo, Japan). Early images were obtained 15 minutes and delayed images were obtained 3 to 4 hours after injection. Whole myocardial \(^{123}\)I-MIBG uptake was measured on a planar image as the early and delayed heart to mediastinum activity ratio.

### GENETIC ANALYSIS

Genomic DNA was isolated from peripheral blood using standard protocols. For direct sequence analysis, DNA was amplified by polymerase chain reaction of each exon, using standard methods and published primers.\(^\text{14}\) Dideoxy sequencing was performed with Big Dye Terminator Chemistry (Applied Biosystems) and analyzed with DNA Sequence Analysis software (Applied Biosystems). Parkin mutations were examined by polymerase chain reaction, direct sequencing, and quantitative assays based on real-time polymerase chain reaction with TaqMan probes (Applied Biosystems) and analyzed with ABI PRISM 7700 sequence detection system (Applied Biosystems) in the 5 patients who had a heterozygous PINK1 mutation (patients E, F, G, H, and J) to rule out compound heterozygous mutations with other heterozygous exonic deletion or multiplication. We used the primer and the probe of Assay by Design (Applied Biosystems) according to a previously published report.\(^\text{14}\)

### MYOCARDIAL IODINE-123 METAOXIDOBENZYLGUANIDINE SCINTIGRAPHY

Myocardial iodine-123 metaiodobenzylguanidine (\(^{123}\)I-MIBG) scintigraphy was performed in 5 PINK1 mutation-positive patients (from different hospitals) with an intravenous injection of 111 MBq of \(^{123}\)I-MIBG (Daichi Radioisotope Laboratories, Tokyo, Japan). Early images were obtained 15 minutes and delayed images were obtained 3 to 4 hours after injection. Whole myocardial \(^{123}\)I-MIBG uptake was measured on a planar image as the early and delayed heart to mediastinum activity ratio.

### STATISTICAL ANALYSIS

Data are expressed as mean (SD). For continuous variables, such as age at onset, the \(t\) test was used to test for significant differences between the 2 groups. Categorical data, such as individual responses to each question on the diagnosis checklist and frequencies, were compared with the \(\chi^2\) test, with Yates correction when appropriate.

### RESULTS

We identified 10 patients with PD from 9 families with PINK1 mutations, including 7 novel mutations (Table 2). Three homozygous missense mutations were found in 4 patients: p.T313M, p.C388R, and a novel p.W437R. Previously, p.T313M and p.C388R had been reported.\(^\text{15,16}\) In addition, 3 novel single heterozygous missense mutations were found in 4 patients: p.A78V, p.M342V, and p.N542S. We also identified 1 novel homozygous deletion (p.D297MfsX22) and 1 novel single heterozygous deletion (p.P196QfsX25). We also found 1 patient with familial PD (without clear mode of inheritance) with a novel single heterozygous variant (p.V482M).

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### Table 1. Characteristics of 414 Analyzed Patients With Parkinson Disease

<table>
<thead>
<tr>
<th>Type of Disease</th>
<th>No. of Patients</th>
<th>Mean (SD) Age at Onset, Range, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic Parkinson disease</td>
<td>190 (105 males, 85 females)</td>
<td>37.2 (10.4), 7-81</td>
</tr>
<tr>
<td>Familial Parkinson disease</td>
<td>224 (201 probands, 23 relatives; 100 males, 124 females)</td>
<td>47.6 (15.5), 10-85</td>
</tr>
<tr>
<td>ARPD</td>
<td>55 (47 probands)</td>
<td>52.8 (13.8)</td>
</tr>
<tr>
<td>ADPD</td>
<td>121 (106 probands)</td>
<td>47.1 (15.8)</td>
</tr>
<tr>
<td>Unclear hereditary information</td>
<td>48</td>
<td>43.1 (16.0)</td>
</tr>
<tr>
<td>Total</td>
<td>414 (391 unrelated patients [190 patients with sporadic disease and 201 probands])</td>
<td>42.8 (14.3)</td>
</tr>
</tbody>
</table>

Abbreviations: ADPD, autosomal dominant Parkinson disease; ARPD, autosomal recessive Parkinson disease.
We did not find any of these mutations or variants in 300 chromosomes in a healthy Japanese population, and we did not detect exonic deletion or multiplication by gene dosage study. The aforementioned novel missense mutations and variants have not been reported as polymorphisms. In addition, we examined the homology regarding the PINK1 protein. The site of p.W437R mutation was highly conserved among various species. On the other hand, the p.V482M variant was not highly conserved (data not shown).

The affected relatives of patients G, H, and J could not be tested for cosegregation of the same heterozygous mutation that was found in the probands. Thus, we could not exclude that the mutation does not cosegregate in 1 or more of these families. No cosegregation of the p.V482M variant was observed among patients in the same family. Therefore, the role of this variant in this family was not clear.

The frequency of homozygous PINK1-positive patients was 1.02% (4 of 391) [1 patient with sporadic PD + 3 familial PD probands]/[1190 patients with sporadic PD + 201 familial PD probands]) among the entire group of PD patients. Furthermore, the frequency of homozygous PINK1-positive patients was 4.26% (2 of 47) in ARPD families and 0.53% (1 of 190) in patients with sporadic PD. Homozygous mutations were not detected in patients with ADPD. However, the frequency of single heterozygous PINK1-positive patients was 1.28% (5 of 391) among the entire group of PD patients, 1.89% (2 of 106) in ADPD families, and 1.05% (2 of 190) among patients with sporadic PD. No single heterozygous mutations were detected in patients with ARPD.

**CLINICAL ANALYSIS**

Table 2 lists the clinical features of 10 PINK1-positive patients and the Figure shows the pedigree of families with the PINK1 mutation. In this study, the family with no cosegregation of p.V482M was excluded from Table 2 and the Figure, because the role of the V482M variant in this
family was not clear. Among the PINK1-positive families, consanguineous marriages were noted in 5 patients (patients B, C, D [pedigree not available], I, and J).

The mean age at onset of patients with homozygous PINK1 mutations was 34.0 (20.3) years (range, 10-55 years), and that of patients with a single heterozygous PINK1 mutation was 53.6 (11.1) years (range, 39-69 years). The age at onset was significantly lower in the homozygous PINK1-positive patients compared with the single heterozygous PINK1-positive and PINK1-negative patients.

As presented in Table 2, motor dysfunction was comparatively mild in many PINK1-positive patients. The mean Hoehn-Yahr stage of homozygous PINK1-positive patients was 1.7 (0.4) in the on state and 3.3 (0.6) in the off state. In contrast, the average Hoehn-Yahr stage of patients with a single heterozygous PINK1 mutation was 2.9 (0.5) in the on state and 3.0 (0.0) in the off state. Even in patient E, who had had PD for 21 years, the Hoehn-Yahr stage was 2.5. None of the patients had a Hoehn-Yahr stage of 5.0.

Patient I had a homozygous 1-base deletion mutation and patient J had a single heterozygous 1-base deletion mutation. These 2 patients had similar deletion mutations that caused stop codons within the serine/threonine kinase domain of PINK1, but age at onset was clearly different: 58 years for patient J (the latest) and 10 years for patient I (the earliest among PINK1-positive patients). Although both patients had hyperflexia, patient J did not have dystonia at onset, while patient I had dystonia at onset. To date, none of the PINK1-positive patients in this study were investigated pathologically.

**MYOCARDIAL 123I-MIBG SCINTIGRAPHY**

Myocardial 123I-MIBG scintigraphy was performed in 5 PINK1-positive patients (patients B, C, E, H, and J). The early and delayed heart to mediastinum ratios of these patients are listed together with the age-matched standard values in Table 2. Myocardial 123I-MIBG uptake was normal in patients with homozygous PINK1 mutations (patients B and C), whereas it was decreased in patients with single heterozygous PINK1 mutations (patients E, H, and J).

**COMMENT**

Combining the results of our previous studies14,15,17 and this study, the frequency of PINK1-positive families with 2 allele mutations (homozygous mutations and compound heterozygous mutations) among parkin-negative ARPD was 11.5% (10 of 87). Among heterozygous mutations, many were single heterozygous rather than compound heterozygous. Our results showed that not only a Japanese individual but 1 Greek and 1 Turkish individual had PINK1 mutations (Table 2), which suggests that the mutation is possibly distributed worldwide, similar to parkin mutations.10,11 Considering previous reports on the frequencies of parkin10,11 and DJ-118,19 mutations, we propose that we should first screen patients with PD for parkin mutations, including gene dosage study, then screen for PINK1 mutations, and finally screen for DJ-1 in ARPD.

In the present study, we did not screen fully for heterozygous PINK1 deletion mutations and multiplications by the gene dosage study using TaqMan assay to save time in screening all patients. Homozygous PINK1 deletion mutation of more than 1 exon structure had been reported in only 1 case so far.13 PINK1 and DJ-1 deletion mutations seem to be less frequent than parkin deletion mutations even if these heterozygous deletion mutations are to be included. In this regard, we think that gene dosage study of PINK1 may not be as important as that of parkin.

Although the prevalence was rare, our study and others20,22 showed that homozgyous mutations as well as single heterozygous PINK1 mutations are found not only in ARPD but also in ADPD families and patients with sporadic PD. These results suggest that screening for PINK1 mutations may also be necessary in patients with potential ADPD and sporadic PD.

Although heterozygous carriers are clinically unaffected in most autosomal recessive disorders, higher preponderance of heterozygous PINK1 mutations in patients with sporadic PD, compared with matched controls, has been reported.21-23 Accordingly, although it is difficult to make a firm conclusion about the frequencies of heterozygous PINK1 mutations in patients vs controls, all the single heterozygous PINK1 mutations were found only in Japanese patients with PD but not in Japanese controls. Moreover, in the positron-emission tomographic study, carriers of heterozygous PINK1 mutations showed significant reductions in caudal and putaminal fluorodeoxyglucose F18 uptake (mean of 20%-30% lower than the controls), indicating increased susceptibility for the de-
In addition, our data showed that the age at onset of patients with heterozygous PINK1 mutations was higher than that of patients with homozygous PINK1 mutations and was similar to that of classic sporadic PD. Thus, the previous findings and our data emphasize the importance of heterozygous PINK1 mutations as a possible risk factor for developing the common classic form of sporadic PD. However, we could not exclude other possibilities, eg, that these mutations could be coincidental findings or even be a cause of ADPD, because we did not perform the genetic tests in the relatives of the patient with a single heterozygous mutation or in controls outside of the Japanese population. In addition, we could not exclude the possibility of digenic inheritance or technical limitations in detecting all possible mutations (eg, in the introns and promoter).

Table 3 lists the clinical symptoms of the patients in this study and patients reported previously by our group.13,15,17 Thus, we could compare 23 PINK1-positive patients with 404 PINK1-negative patients and compare 18 patients with 2 allele PINK1 mutations (16 patients with homozygous PINK1 mutations and 2 patients with compound heterozygous mutations) with 5 patients with 1 allele PINK1 mutation. The data in Table 3 show that most PINK1-positive patients develop early-onset parkinsonism. Moreover, the mean age at onset of patients with 1 allele PINK1 mutation was higher than that of patients with 2 allele mutations.

Age at onset, hyperreflexia, and gait disturbances were significantly more frequent in homozygous PINK1-positive patients than in PINK1-negative patients. Indeed, these symptoms were also significantly different in patients with or without PINK1 mutations. However, there were no statistical differences in pathognomonic symptoms between patients with 1 or 2 allele PINK1 mutations, except for age at onset. These data indicate that the phenotypes of patients with a single heterozygous PINK1 mutation are more likely to be similar to those of homozygous PINK1-positive patients, except for age at onset.

Myocardial 123I-MIBG scintigraphy is one of the most supportive diagnostic tools used in differentiating PD from Table 3. Clinical Features of 23 Patients With PINK1 Mutations in Current and Past Studies

<table>
<thead>
<tr>
<th>Measure</th>
<th>No of Patients</th>
<th>PINK1-Mutation Positive</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No PINK1 Mutation</td>
<td>Homozygous (n=16)</td>
</tr>
<tr>
<td>Sporadic PD</td>
<td>187</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ARPDa</td>
<td>52</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>ADPD</td>
<td>119</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patients with familial PD, unclear hereditary information</td>
<td>46</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Age at onset, mean (SD), y</td>
<td>42.8 (14.3)</td>
<td>32.6 (8.5)</td>
<td>18.5 (0.7)</td>
</tr>
<tr>
<td>Resting tremor</td>
<td>293</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Rigidity</td>
<td>366</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>368</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Postural instability</td>
<td>244</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Gait disturbance</td>
<td>268</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Frozen gait</td>
<td>NA</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Wearing off</td>
<td>227</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>On/off states</td>
<td>NA</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Asymmetry at onset</td>
<td>293</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Orthostatic hypotension</td>
<td>43</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Incontinence</td>
<td>30</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Urinary urgency</td>
<td>63</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Levodopa-induced dyskinesia</td>
<td>170</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Sleep benefit</td>
<td>112</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Dystonia at onset</td>
<td>53</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Hyperreflexia</td>
<td>51</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Dementia</td>
<td>41</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Depression</td>
<td>NA</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>62</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Other psychosis</td>
<td>26</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hoehn-Yahr stage, mean (SD)</td>
<td>2.5 (1.0)</td>
<td>2.3 (0.7)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: ADPD, autosomal dominant Parkinson disease; ARPD, autosomal recessive Parkinson disease; NA, not applicable; PD, Parkinson disease.

a Thirteen of the patients with ARPD were reported previously by our group.13,15,17

b n=22.

c n=19.

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806

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conditions such as essential tremor, progressive supranuclear palsy, and multiple system atrophy.25,26 In this regard, some patients with 2 allele parkin mutations without Lewy bodies were reported to have normal 123I-MIBG uptake.2729 Another study demonstrated markedly low heart to mediastinum ratios in patients with classic PD with Lewy bodies and in incidental Lewy body disease, suggesting that Lewy body pathology itself may be responsible for low 123I-MIBG uptake.30 Although a single case with a homozygous PINK1 mutation was reported to have a very mild decrease in 123I-MIBG uptake,31 our data showed that 2 patients with homozygous PINK1 mutations (patient B with disease duration of 10 years and patient C with disease duration of 2 years) had normal myocardial 123I-MIBG uptake. In contrast, 3 patients with single heterozygous PINK1 mutations (patients E, H, and J) had low myocardial 123I-MIBG uptake. These findings suggest that patients with a single heterozygous mutation are more likely to have cardiac sympathetic denervation than those with homozygous PINK1 mutations, which accounts for the low 123I-MIBG uptake. One can further speculate that patients with heterozygous PINK1 mutations may have Lewy body pathology, whereas those with homozygous PINK1 mutations have no Lewy body pathology, similar to patients with parkin mutations,1032 though no pathologic study of patients with 2 allele PINK1 mutations has been reported to date. Additional studies of cardiac scintigraphy in a larger number of PINK1-positive patients with PD are required to clarify these points.

In summary, we assume that homozygous PINK1 mutations may manifest in an early-onset autosomal recessive form of PD. We can also speculate that single heterozygous mutations may be 1 of the risk factors in developing the sporadic or autosomal dominant form of PD. Additional studies are necessary to clarify the etiopathogenic roles of 1 allele PINK1 mutation in developing various forms of PD.

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REFERENCES