Analysis of the PINK1 Gene in a Large Cohort of Cases With Parkinson Disease

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Background: Mutations in the PTEN-induced kinase (PINK1) gene located within the PARK6 locus on chromosome 1p35-p36 have recently been identified in patients with recessive early-onset Parkinson disease.

Objective: To assess the prevalence of PINK1 mutations within a series of early- and late-onset Parkinson disease patients living in North America.

Design: All coding exons of the PINK1 gene were sequenced in a series of 289 Parkinson disease patients and 80 neurologically normal control subjects; the mutation frequencies were evaluated in additional controls (100 white and 50 Filipino subjects).

Results: We identified 27 variants, including the first reported compound heterozygous mutation (Glu240Lys and Leu489Pro) and a homozygous Leu347Pro mutation in 2 unrelated young-onset Parkinson disease patients.

Conclusion: Autosomal recessive mutations in PINK1 are a rare cause of young-onset Parkinson disease.

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PARKINSON DISEASE (PD) IS A neurodegenerative disorder that is characterized by progressive dysfunction of movement due to the predominant degeneration of dopaminergic neurons in the basal ganglia.1 Although familial PD represents less than 10% of all cases, parkinsonism-causing mutations have been defined in at least 4 different genes: SNCA,2 parkin,3 DJ1,4 and possibly UCHL1.5 Several additional PD loci have been identified by genetic linkage methods,6 including the PARK6 locus on chromosome 1p35-p36, which is responsible for a recessive early-onset form of disease.7 Valente and colleagues8 have recently reported that mutations in the PTEN-induced kinase (PINK1) gene cause the PARK6 form of disease. In 3 PD families, they discovered a homozygous nonsense mutation (Trp437stop) and a homozygous Gly309Asp mutation.

PINK1 encodes a putative serine-threonine protein kinase, which is highly conserved in evolution (approximately 75%-85% identity in mammalian orthologues) (Figure 1). PINK1 is transcriptionally transactivated by the phosphates and tensin homologue (PTEN) gene, an oncogene involved in several signal transduction pathways.9-11 PINK1 shows variable levels of expression in different cancer cell types9,10; however, it is not clear whether the connection between PINK1 and PTEN is relevant to PD. Preliminary cell culture investigations using epitope-tagged, overexpressed exogenous PINK1 have suggested that PINK1 is located in mitochondria and may exert a protective effect on the cell, which is abrogated by mutations, resulting in an increased susceptibility to cellular stress.8

The prevalence of PINK1 mutations in PD, however, remains unknown. We now describe the results of the first population study, to our knowledge, of PINK1 within a series of early- and late-onset PD patients living in North America. We report several novel PINK1 variants, including a compound heterozygous mutation (Glu240Lys and Leu489Pro) and a homozygous substitution (Leu347Pro) in 2 PD patients.
Informed written consent was obtained from all individuals involved in the study. Standard neurological clinical examination was performed on all participants recruited from several North American clinics (the diagnosis of PD was based on published criteria). The sample characteristics are summarized in Table 1.

The 209 unrelated subjects collected at the National Institutes of Health (NIH) were diagnosed as having PD by neurologists from the NIH and the University of Florida; 41% of the patients had an early onset (<50 years of age). The second set of 80 unrelated subjects was collected at the Movement Disorders Centre of the Toronto Western Hospital (the subjects were preselected for having an early onset and a positive family history). Age- and sex-matched normal control subjects were recruited from the same population, including 100 individuals from Toronto and 80 from NIH sites. The NIH controls were completely sequenced, while the Toronto controls were used to estimate the frequency of the mutations found in the probands.

In addition, 50 Filipino controls were used to assess the population-specific allele frequency of the 347Pro variant.

### Methods

#### Subjects

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In addition, 50 Filipino controls were used to assess the population-specific allele frequency of the 347Pro variant.

### Genetic Analysis

Total RNA and genomic DNA were isolated from blood samples using QIAAGEN extraction kits (Valencia, Calif.). Mutations in parkin and DJ1 had been excluded in all patients as previously described. The entire open reading frame of PINK1 was sequenced in all patients and controls. PINK1 variants with their exon-intron boundaries were amplified using polymerase chain reaction conditions (Table 2). For proband 4685, the reverse transcription–polymerase chain reaction products of PINK1 were amplified with primers placed in exons 1 and 8, they were cloned using a TA cloning kit (Invitrogen, Carlsbad, Calif.), and then 3 randomly selected clones were sequenced. Mutations were detected by direct inspection of the fluorescent chromatographs and analysis using Seqscape software version 1.0 (Applied Biosystems, Foster City, Calif.). The frequencies of the Glu240Lys and Leu489Pro mutations were evaluated in 100 controls using SNaPshot (Applied Biosystems). The frequency of the Leu347Pro in Filipino controls was

(continued)
evaluated using a restriction digest assay. Exon 5 was amplified as already described, it was digested overnight with 0.3 U of HpaII at 37°C, and the resulting restriction fragments were resolved on a 1.5% agarose gel.

RESULTS

GENETIC STUDIES

Genomic DNA sequence analysis of the entire open frame of \textit{PINK1}, the untranslated region, and all 5' and 3' intron-exon boundaries was performed on 289 unrelated subjects with disease and on 80 neurologically normal control subjects. We identified 27 sequence variations (\textbf{Table 3}), including 10 common sequence variations and 8 single heterozygous substitutions with an allele frequency of less than 1% that alter the amino acid sequence (observed in 8 patients and 5 controls).

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We also found 2 cases (from the Toronto set) in whom both alleles of \textit{PINK1} were mutated. In proband 4685 (PD:1 family), we found a compound heterozygous non synonymous mutation (\textbf{Figure 2}): a heterozygous G→A mutation in exon 3 at genomic nucleotide position 6480 (GAG→AAG) and a heterozygous T→C mutation in exon 7 at position 15754 (CTG→CGG) (NT 004610). The G→A 6480 mutation causes the substitution of glutamic acid to lysine at codon 240 (Glu240Lys). The T→C 15754 mutation causes the substitution of leucine to proline at codon 489 (Leu489Pro). The Glu240Lys and Leu489Pro mutations are unique to the PD:1 family and are not present in 360 normal chromosomes.
A second unrelated proband (521-1) of Filipino ancestry was found to have a homozygous T→C substitution in exon 5 at nucleotide position 12186, leading to an amino acid substitution of leucine to proline at codon 347 (Leu347Pro) (Figure 3B). This variant was not identified in any of the sequenced control samples; however, 3 heterozygous carriers of this variation were found among 50 Filipino control subjects.

**CLINICAL FEATURES**

Proband 4685 is 73 years old and developed typical, slowly progressive PD at age 30, with good response to levodopa-carbidopa therapy. The proband’s family history displays an autosomal recessive mode of inheritance of PD (Figure 2A). Both of the proband’s parents, who died after age 70, did not have PD. Of 9 siblings, 3 developed PD (2 affected siblings are not available for study but are reported to have had a similar course). The proband’s offspring (unaffected at age 42) has inherited only the Glu240Lys substitution, and an unaffected sibling of the proband (at age 67) possesses the Leu489Pro mutation (Figure 2B).

Proband 521-1 is 67 years old and first noticed subtle right hand tremor early in the fourth decade of life. Ten years later, the patient developed stiffness, slowness, and pain on the right side, with freezing of gait, and was diagnosed as having PD at age 55 years. The patient initially had an excellent response to levodopa-carbidopa but during the last 10 years has developed dyskinesia, fluctuations, and cramping while not receiving levodopa-carbidopa. Cognition, eye movements, and autonomic function are normal. The proband’s family history displays an autosomal recessive mode of inheritance of PD (2 siblings of the proband, who are not available for study, are reported to have PD) (Figure 3A).

**COMMENT**

Our results support the prior suggestion that mutations in PINK1 are associated with recessive familial PD. In a
data set of 289 patients, we found 2 probands in families with autosomal recessive inheritance: a patient with a compound heterozygous mutation (Glu240Lys and Leu489Pro) and a patient with a homozygous Leu347Pro substitution.

These substitutions were not found in homozygous or heterozygous states in the initial series of 180 healthy controls. The Leu347Pro, which was identified as a homozygous change in a Filipino patient with parkinsonism, was found as a heterozygous change in 3 of 50 Filipino control subjects. In the absence of segregation data, the pathogenicity of these variants remains unclear. In support of a role for these variants in disease, all 3 are within the kinase domain, and all change evolutionarily conserved amino acids (Figure 2). Biological plausibility may be strengthened by the availability of a kinase assay for this protein. The identification of the 347Pro variant in 3% of Filipino control chromosomes suggests 2 possibilities: this change is a benign rare variant present in the Filipino population, or this alteration is pathogenic when homozygous. If the latter were true, a Filipino allele frequency of 3% would suggest a population prevalence of approximately 1 in 4000 for disease caused by a homozygous 347Pro substitution.

Our survey also uncovered several intronic and exonic sequence variants, including 2 common nonsynonymous polymorphisms that affect nonconserved residues and that are present as low-frequency (<30%) alleles in PD-affected and non–PD-affected subjects (Table 3). The present study does not have sufficient statistical power to discern whether some or all of these alleles are enriched in the PD-affected subjects and therefore might represent weaker-risk alleles for increased susceptibility to “less penetrant” late-onset forms of disease. Future studies in larger case-control data sets will be required to address this issue.

In support of the notion that heterozygous mutations in genes causing recessive forms of early-onset PD can also cause late-onset PD, there have been several late-onset PD families with a single parkin mutation. However, the evidence linking these variants to disease has been equivocal, and a study has shown a high frequency (approximately 3%) of heterozygous pathogenic parkin mutations in neurologically healthy controls. It will be interesting to evaluate the nigrostriatal dopamine system in the subjects with heterozygous PINK1 variants to assess the potential subclinical alterations in striatal dopamine function. Such alterations have been found in patients heterozygous for parkin mutations, suggesting that partial loss of function of the gene can cause subclinical reduction in dopaminergic function.

It is conceivable that there may be further mutations in the PINK1 gene within the series of patients exam-

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**Table 3. Summary of Sequence Variations Found in PINK1 During Sequencing Analysis**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Substitution</th>
<th>Genomic Nucleotide Position</th>
<th>Codon</th>
<th>PD</th>
<th>Controls</th>
<th>Comments</th>
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<td>1</td>
<td>C→T</td>
<td>283</td>
<td>Leu63Leu†</td>
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<td>Thr236Thr</td>
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<td>Ala340Thr†</td>
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<td>12186</td>
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<td>Pro425Ser</td>
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<td>15715</td>
<td>Glu476Lys</td>
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<td>1</td>
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<tr>
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<td>15754</td>
<td>Leu488Pro</td>
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<tr>
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<td>Asn521Thr†</td>
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<td>0</td>
<td>Novel</td>
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<tr>
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<td>A→G</td>
<td>17219</td>
<td>Ser576Ser</td>
<td>0</td>
<td>1</td>
<td>Novel</td>
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<td>UTR†</td>
<td>Common</td>
<td>rs686658</td>
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<tr>
<td>3' UTR</td>
<td>G→A</td>
<td>17277</td>
<td>UTR†</td>
<td>Common</td>
<td></td>
<td>Novel</td>
</tr>
</tbody>
</table>

Abbreviations: PD, Parkinson disease; UTR, untranslated region.
*Homozygous or compound heterozygous mutations are in boldface.
†Allele frequency is greater than 5% in patients and controls.
‡This variant was identified in 3 of 100 Filipino control chromosomes and was not present in 160 white control chromosomes.
ined herein (the next step in the analysis of \textit{PINK1} should include a quantitative polymerase chain reaction to evaluate the presence of a heterozygous deletion or insertion). The fact that mutations in \textit{PINK1} constitute a rare cause for early-onset parkinsonism does not negate a role for this gene in the pathogenesis of PD. The localization of \textit{PINK1} in mitochondria provides a potential link with prior theories of mitochondrial deficits in PD; however, further studies are needed to establish the subcellular, cellular, and tissue distribution of \textit{PINK1} protein in healthy tissues and in brain tissue affected by PD.

Several lines of evidence suggest that \textit{PINK1} is likely to be a functional kinase: bioinformatic analysis suggests that residues Gly193 to Leu507 comprise the catalytic kinase domain, and biochemical results suggest that \textit{PINK1} is capable of autophosphorylation. Finally, all reported \textit{PINK1} mutations affect the kinase domain. Because kinases are attractive therapeutic targets, this genetic discovery may identify novel therapeutic opportunities.

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