A Common NURR1 Polymorphism Associated With Parkinson Disease and Diffuse Lewy Body Disease

Kangni Zheng, MD; Bobak Heydari, BS; David K. Simon, MD, PhD

Background: NURR1 plays a key role in mesencephalic dopaminergic neuron development and survival. A homozygous NURR1 polymorphism (a single base-pair insertion in intron 6) (NI6P) has been reported to be associated with Parkinson disease (PD).

Objective: To assess the association of the NI6P with PD and diffuse Lewy body disease.

Design: Case-control study.

Setting: Movement disorders clinic and tissue provided by brain banks.

Patients: Patients with pathologically proven PD (n=37) or diffuse Lewy body disease (n=35), neuropathologically normal control subjects (n=59), those clinically diagnosed as having PD (n=66), and spousal controls (n=29).

Methods: Determining the frequency of heterozygotes and homozygotes for the NI6P by DNA sequencing and restriction endonuclease analyses.

Results: Overall, 41 (39.8%) of the 103 patients with PD were heterozygotes compared with 22 (25.0%) of the 88 controls (P=.03), with a relative risk (estimated from the odds ratio) for PD of 2.03 (95% confidence interval, 1.08-3.81) for heterozygotes vs wild type subjects. Heterozygotes were more frequent in the subgroup of patients with pathologically confirmed PD (18 [48.6%] of 37) vs controls (14 [23.7%] of 59) (P=.01), with a relative risk for PD of 2.84 (95% confidence interval, 1.17-6.88) for heterozygotes vs wild type subjects. In patients clinically diagnosed as having PD, heterozygotes were more frequent in early-onset cases (onset at ≤45 years) (10 [55.6%] of 18) compared with late-onset cases (onset at >45 years) (10 [23.8%] of 42) (P=.02) or spousal controls (8 [27.6%] of 29) (P=.06), with a relative risk for early-onset PD of 4.17 (95% confidence interval, 1.13-15.33) for heterozygotes vs subjects with 2 wild type alleles. The homozygous NI6P was not associated with PD, but was present in 6 (17.1%) of the 35 patients with diffuse Lewy body disease compared with 3 (5.1%) of the 59 controls (P=.06).

Conclusions: The common heterozygous NI6P is associated with an increased risk of PD. An association of borderline significance was found for the homozygous NI6P and diffuse Lewy body disease.

Arch Neurol. 2003;60:722-725

Evidence is accumulating that genetic factors play an important role in determining the risk of developing Parkinson disease (PD). The identification of specific genes that influence PD risk may reveal clues regarding PD pathogenesis and potential neuroprotective therapies. One such candidate gene is NURR1, a transcription factor that is highly expressed in dopaminergic neurons in the substantia nigra. Nurr1-deficient mice lack mesencephalic dopaminergic neurons. Heterozygote Nurr1-deficient mice, with only 1 functional copy of the Nurr1 gene, develop normal tyrosine hydroxylase-positive neurons in the substantia nigra, but show enhanced susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity. These data raise the possibility that polymorphism-associated variations in NURR1 function in humans could influence the risk of developing PD. A recent report indicated an increased frequency of the homozygous NURR1 intron 6 polymorphism (NI6P) in patients with familial or sporadic PD compared with control subjects (6.7% vs 0.9%), but no difference between heterozygous polymorphism frequencies (21.3% vs 20.4%). A prior study of Swedish patients with PD did not find an association of this polymorphism with PD, but this study involved fewer patients and only allele frequencies were reported, without
consistent with definitions in prior genetic studies. Late-onset PD was defined as onset at 45 years or younger, in accordance with an institutional review board–approved protocol. Genomic DNA was extracted from blood leukocytes or from frozen brain tissue, as previously described. Polymerase chain reaction (PCR) amplifications were performed as previously described. Status for NI6P was determined in all samples by the use of a mismatched reverse primer (Big Dye; Perkin Elmer, Foster City, Calif). In each case, the sequencing data confirmed the results of the BseRI analysis. In most cases, the presence of a heterozygous or homozygous NI6P was further confirmed by the use of a mismatched reverse primer.

### METHODS

#### STUDY SUBJECTS

Five subgroups of subjects are included in this study. Subject characteristics are outlined in Table 1. Blood samples were collected and analyzed, with informed consent obtained in accordance with an institutional review board–approved protocol. Early-onset PD was defined as onset at 45 years or younger, consistent with definitions in prior genetic studies. Late-onset PD was defined as onset after the age of 45 years.

#### POLYMORPHISM ANALYSIS

Genomic DNA was extracted from blood leukocytes or from frozen brain tissue, as previously described. Polymerase chain reaction (PCR) amplifications were performed as previously described. Status for NI6P was determined in all samples by the elimination of a BseRI cleavage site, resulting in a single 604-bp base pair (bp) product, rather than 2 bands (395 and 209 bp) seen for wild type (WT) samples; all 3 of these bands are seen in heterozygotes. The PCR primers for this assay were as follows: 5′-AGTGGAGATGACACCCAGCATATC-3′ (forward) and 5′-TTGCAAAAGATGAGTTTACCCTTCCAC-3′ (reverse). The presence or absence of the NI6P was confirmed by sequencing the PCR product using the forward PCR primer as the sequencing primer in 13 cases, including 4 WT, 6 heterozygous, and 3 homozygous cases. Additional sequencing using the reverse PCR primer as the sequencing primer was performed in 4 cases (2 WT and 2 heterozygous cases). Sequencing reactions were performed using a sequencing reaction premix (Big Dye; Perkin Elmer, Foster City, Calif). In each case, the sequencing data confirmed the results of the BseRI analysis. In most cases, the presence of a heterozygous or homozygous NI6P was further confirmed by the use of a mismatched reverse primer.

#### STATISTICAL ANALYSIS

The frequencies of NI6P were compared using χ² analyses. In addition, relative risks (RRs) were estimated from the odds ratios.

### RESULTS

Analysis of all patients with PD compared with all controls indicates a significantly increased frequency of NI6P heterozygotes among the patients with PD (41 [39.8%] of 103) vs the controls (22 [25.0%] of 88) (P = .03) (Table 2). The RR for PD was 2.03 (95% confidence interval [CI], 1.08-3.81), for heterozygotes vs WT subjects. The frequencies of homozygotes were not significantly different between patients with PD (5 [4.9%] of 103) and controls (4 [4.5%] of 88) (P = .92).

Among the neuropathologically normal control group, 14 (23.7%) were heterozygous and 3 (5.1%) were homozygous for the NI6P. The frequency of heterozygotes was significantly higher among those with patho-

### Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No.</th>
<th>Age, Mean ± SD, y†</th>
<th>Gender‡§</th>
<th>Source†</th>
<th>Ethnicity‡§</th>
<th>Sex§§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con-path</td>
<td>59</td>
<td>73.9 ± 9.8</td>
<td>M</td>
<td>MGH</td>
<td>Af</td>
<td>F</td>
</tr>
<tr>
<td>Con-clin</td>
<td>29</td>
<td>62.3 ± 9.7</td>
<td>M</td>
<td>MGH</td>
<td>Ar</td>
<td>F</td>
</tr>
<tr>
<td>PD-path</td>
<td>37</td>
<td>77.9 ± 8.8</td>
<td>M</td>
<td>NA</td>
<td>As</td>
<td>M</td>
</tr>
<tr>
<td>PD-clin</td>
<td>66</td>
<td>52.8 ± 10.6</td>
<td>M</td>
<td>NA</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>DLB</td>
<td>35</td>
<td>76.1 ± 7.5</td>
<td>M</td>
<td>NA</td>
<td>F</td>
<td>M</td>
</tr>
</tbody>
</table>

Abbreviations: Af, African American; Ar, Arabian; As, Asian; Con-clin, clinically healthy (by history) spouses of a subset of the PD-clin patients; Con-path, neuropathologically normal controls; DLB, subjects with pathologically proven Lewy body disease; HBTRC, Harvard-McLean Brain Tissue Resource Center (Belmont, Mass); MGH, Massachusetts General Hospital Alzheimer’s Disease Research Center Brain Bank (Boston, Mass); NA, data not applicable; PD-clin, subjects who were recruited from movement disorders centers in the Boston area and who were diagnosed as having Parkinson disease by a neurologist with expertise in movement disorders; PD-path, subjects with pathologically proven Parkinson disease; W, white.

*None of the Af, Ar, or As subjects had a heteroplasmic NURR1 intron 6 polymorphism.
†Refers to age at the time of death for the Con-path, PD-path, and DLB groups, age at sample collection for the Con-clin group, and age of onset for the PD-clin group (available for 60 subjects).
‡Data are given as number of subjects.
§Data are shown for those samples for which these data were available.
| Each of the Con-clin subjects lacked a family history of Parkinson disease.

### Table 2. NURR1 Intron 6 Polymorphism Status

<table>
<thead>
<tr>
<th>Group</th>
<th>Wild Type</th>
<th>Heterozygotes</th>
<th>Homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 88)</td>
<td>62 (70.5)</td>
<td>22 (25.0)</td>
<td>4 (4.5)</td>
</tr>
<tr>
<td>Con-path (n = 59)</td>
<td>42 (71.2)</td>
<td>14 (23.7)</td>
<td>3 (5.1)</td>
</tr>
<tr>
<td>Con-clin (n = 29)</td>
<td>20 (69.0)</td>
<td>8 (27.6)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>PD (N = 103)</td>
<td>57 (55.3)</td>
<td>41 (39.8)</td>
<td>5 (4.9)</td>
</tr>
<tr>
<td>PD-path (n = 37)</td>
<td>19 (51.4)</td>
<td>18 (48.6)</td>
<td>0</td>
</tr>
<tr>
<td>PD-clin (n = 66)</td>
<td>38 (57.6)</td>
<td>23 (34.8)</td>
<td>5 (7.6)</td>
</tr>
<tr>
<td>DLB (N = 35)</td>
<td>20 (57.1)</td>
<td>9 (25.7)</td>
<td>6 (17.1)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) of subjects in each group. Percentages may not total 100 because of rounding. Abbreviations are explained in the first footnote to Table 1.
The NI6P was homozygous in 6 of 35 subjects with pathologically proven DLB, representing an increased frequency compared with the neuropathologically normal control group (3 of 59 subjects) that did not quite reach significance (P = .06) (RR, 3.86; 95% CI, 0.90-16.57). The frequency of heterozygotes was not significantly different between those with early-onset PD at ≤45 years (P = .83) compared with those with late-onset PD at >45 years. Thus, few patients with an early age of PD compared with the frequency in the control group (4.5%) among our controls (4.5%) compared with the frequency in the control group of clinically healthy spouses was also not significant (7.6% vs 3.4%; P = .45). The trend toward an increased frequency of homozygotes in the PD-clin group compared with the control group of clinically healthy spouses was not statistically different from the frequency of homozygotes in the neuropathologically normal control group (P = .16).

The frequency of heterozygotes for the NI6P among the entire group of patients with clinically defined PD (PD-clin) was not significantly different from the frequency in the clinically defined controls (34.8% vs 27.6%; P = .49). The trend toward an increased frequency of homozygotes in the PD-clin group compared with the control group of clinically healthy spouses was also not significant (7.6% vs 3.4%; P = .45). Of the 12 subjects in the PD-clin group with a parent, sibling, or offspring with PD, 5 (41.7%) harbored a heterozygous polymorphism and 7 (58.3%) were WT.

Age-of-onset data for the PD-clin group are shown in Table 1. The average age of onset was not significantly different between patients in the WT, heterozygous, and homozygous groups (unpaired t test, P >.10 for each comparison). However, the frequency of the heterozygous NI6P in those with early-onset PD (onset at ≤45 years) was higher compared with the frequency in those with late-onset PD (onset at >45 years) or compared with the frequency in the control group of clinically healthy spouses (Table 3). Male subjects comprised more of the PD-clin group (49 [74.2%] of 66) compared with the control group of clinically healthy spouses (10 [34.5%] of 29). However, NI6P frequencies were similar in men and women in each subgroup.

The NI6P was homozygous in 6 of 35 subjects with pathologically proven DLB, representing an increased frequency compared with the neuropathologically normal control group (3 of 59 subjects) that did not quite reach significance (P = .06) (RR, 3.86; 95% CI, 0.90-16.57). The frequency of heterozygotes was not significantly different (P = .83) different between these 2 groups. Among the patients with pathologically proven DLB, 1 of 8 women and 5 of 27 men were homozygous for the NI6P.

**Table 3. NURR1 Intron 6 Polymorphism Status in the PD-Clin Group According to Age of Onset**

<table>
<thead>
<tr>
<th>Group</th>
<th>Wild Type</th>
<th>Heterozygotes</th>
<th>Homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-onset PD</td>
<td>6 (33.3%)</td>
<td>10 (55.6)†</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>(onset ≤45 y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late-onset PD</td>
<td>29 (69.0%)</td>
<td>10 (23.8)</td>
<td>3 (7.1)</td>
</tr>
<tr>
<td>(onset &gt;45 y)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are explained in the first footnote to Table 1. †P = .02 for the increased frequency of heterozygotes among early-onset vs late-onset PD. P = .06 for increased frequency of heterozygotes among early-onset PD compared with the control group of clinically healthy spouses. The relative risk of early-onset PD among heterozygotes (compared with wild type) is 4.17 (95% confidence interval, 1.13-15.33).

The data presented herein indicate an association between the heterozygous NI6P and PD. The implications of this association are significant, because the heterozygous NI6P is common; it was present in 25.0% of controls and in 39.8% of patients with PD, including 55.6% of those with early-onset PD in the PD-clin group. This association is particularly intriguing given the well-established role of NURR1 in the development and survival of dopaminergic neurons. The mean age of onset of patients with sporadic PD in the prior study was older than 60 years, with the youngest age of onset reported as 42 years. In contrast, the average age of onset in our PD-clin group was 52.8 years (among the 60 patients in this group for whom these data were available), with 18 patients having an age of onset of 45 years or younger (the youngest age of onset was 31 years). Thus, few patients with an early age of onset may account for the lack of an association for PD with the heterozygous polymorphism in this prior study. An earlier age of onset was reported by Xu et al for patients with sporadic PD with the homozygous NI6P, but age-of-onset data were not reported for patients with PD with a heterozygous polymorphism. Data on age of onset also were not reported in a prior study in which similar allele frequencies were found for the NI6P in Swedish patients with PD compared with controls. Age-of-onset data were unavailable for the group with pathologically proven PD in the present study and, therefore, an association between early-onset cases and the NI6P could not be assessed in this subgroup. A preferential impact of the NI6P on early-onset PD risk is consistent with prior studies suggesting that genetic factors play a relatively greater role in early-onset PD compared with late-onset PD.

We were unable to confirm the reported association of PD with the homozygous NI6P. The frequency of the homozygous polymorphism among PD cases was similar in our study and the study of Xu et al (6.7%) (P = .52). In contrast, we identified a relatively high number of heterozygotes among our controls (4.5%) compared with the 0.9% reported by Xu et al (P = .04), suggesting genetic differences between the control groups in these 2 studies. Because of the relatively low numbers of homozygotes compared with heterozygotes, our study was insufficiently powered to exclude an association of the homozygous NI6P with PD.

It has been speculated, based on its proximity to the exon 6/intron 6 junction (18 bp downstream), that the NI6P may be pathogenic through alteration of intron splicing. If the polymorphism is associated with a NURR1 splice variant that has reduced transcriptional activity, then this may lead to enhanced susceptibility to substantia nigra neuronal death in a manner analogous to the enhanced susceptibility to MPTP among the heterozygous Nurr1-deficient mice. There are numerous precedents for the pathogenicity of intronic mutations in human diseases. Most are located at the invariant acceptor and donor splice sites at the exon/intron boundaries. Al-
though the NI6P is 18 bp downstream from the exon 6/intron 6 boundary, many similarly positioned pathogenic intronic mutations that lead to altered splicing have been well documented. An example is a report14 of 2 TSC2 gene mutations located within an intron 15 or 18 bp upstream from the splice acceptor site, resulting in a splice variant lacking a portion of the subsequent exon. Similar intronic mutations that alter tau messenger RNA splicing have been reported in association with progressive supranuclear palsy.15 Alternatively, the NI6P could be a marker for a different mutation at a nearby site. However, in a prior study,8 sequencing of the entire coding region of the NURR1 gene in 20 patients with PD did not reveal any alternative candidate mutations.

We also identified an association of the homozygous NI6P with DLB, raising the possibility that the NI6P represents a common genetic risk factor for PD and DLB. However, this association was of borderline significance. Thus, it will be important to test additional patients with DLB. The association of the homozygous NI6P with DLB also raises the possibility that patients with DLB misdiagnosed as having PD could account for the previously reported apparent association of the homozygous NI6P with PD. Inadvertent inclusion of patients with DLB in the PD-clin subgroup in the present study does not account for the observed increase in the heterozygous NI6P in patients with PD, because there was no association of the heterozygous NI6P with DLB.

In conclusion, the data presented herein suggest that the heterozygous NI6P is associated with an increased risk of PD. This polymorphism may preferentially increase the risk of early-onset PD. An increased frequency, which approached statistical significance, was found for the homozygous polymorphism and DLB, raising the possibility of a common genetic risk factor for PD and DLB. Further studies are needed to verify these associations and to clarify the role of NURR1 in PD and DLB.

Accepted for publication December 18, 2002.

Author contributions: Study concept and design (Dr Simon); acquisition of data (Drs Zheng and Simon and Mr Heydari); analysis and interpretation of data (Drs Zheng and Simon and Mr Heydari); drafting of the manuscript (Mr Heydari and Dr Simon); critical revision of the manuscript for important intellectual content (Drs Zheng and Simon and Mr Heydari); statistical expertise (Mr Heydari and Dr Simon); obtained funding (Dr Simon); administrative, technical, and material support (Drs Zheng and Simon and Mr Heydari); study supervision (Drs Zheng and Simon).

This study was supported in part by an anonymous donor award for Parkinson’s Disease Research; grant KO2 NS54311-01 from the National Institute of Neurological Disorders and Stroke, Bethesda, MD (Dr Simon); and a Cotzias Fellowship from the American Parkinson Disease Foundation, Inc, Staten Island, NY (Dr Simon).

We thank Alberto Ascherio, MD, DrPH, Sandra L. Dabola, MD, PhD, and Donald R. Johns, MD, for helpful discussions and/or comments on the manuscript; and Michael T. Lin, MD, for technical assistance with sequencing.

Corresponding author and reprints: David K. Simon, MD, PhD, Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, 4 Blackfan Cir, Room 856, Boston, MA 02115 (e-mail: dsimon1@bidmc.harvard.edu).

REFERENCES