LRRK2 that previously have been reported to harbor mutations in 98 early-onset and 42 late-onset PD patients. We identified two mutations (c.4321C>T, c.6055G>A) in three early-onset patients. Screening of an additional 220 early-onset PD patients for these mutations revealed another mutation carrier. In conclusion, LRRK2 mutations need to be considered also in early-onset PD. © 2006 Movement Disorder Society

Key words: early-onset Parkinson’s disease; LRRK2; recurrent mutations

Recurrent LRRK2 (Park8) Mutations in Early-Onset Parkinson’s Disease

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Abstract: Mutations in LRRK2 (leucine-rich repeat kinase 2) have been associated with autosomal dominant Parkinson’s disease (PD) and cluster in several 3’ exons of the gene. The majority of mutations have been detected in late-onset cases (age at onset >50 years). We screened 5 of the 51 exons of LRRK2 that previously have been reported to harbor mutations in 98 early-onset and 42 late-onset PD patients. We identified two mutations (c.4321C>T, c.6055G>A) in three early-onset patients. Screening of an additional 220 early-onset PD patients for these mutations revealed another mutation carrier. In conclusion, LRRK2 mutations need to be considered also in early-onset PD. © 2006 Movement Disorder Society

Key words: early-onset Parkinson’s disease; LRRK2; recurrent mutations

LRRK2 (leucine-rich repeat kinase 2) is the latest member of a growing family of Parkinson’s disease (PD) genes and is associated with autosomal dominant PD.1,2 LRRK2 is a large gene with 51 exons encoding a 2,527–amino acid protein.2 To date, more than 40 variants have been described in this gene. Due to reduced penetrance and phenocopies, it is not sure how many of these variants play a role in the pathogenesis of PD; however, approximately 16 variants seem to be pathogenic, including 14 missense, a splice site mutation, and a synonymous mutation near a splice site.1–11 These mutations occur in only 10 of the 51 exons of LRRK2, and 8 of them were found recurrently (c.3342A>G, c.4321C>T, c.4321C>G, c.4322G>A, c.5096A>G, c.5096A>G, c.6055G>A, c.6059T>C). Mutations are clustered in some of the exons, indicating that it may be justified to limit genetic testing to these exons only.9 For the most frequent and well-investigated mutation (c.6055G>A), a common founder has been suggested.3,10,12 This mutation has been detected in approximately 5% of familial cases, in 1% of sporadic PD patients, and only rarely in healthy controls.13 Due to the founder effect, the frequency of this mutation highly depends on the ethnicity of patients and ranges from less than 1% in a German sample11 to 18% in Ashkenazi Jews12 and 41% in sporadic Arab cases.14

Although mutations in other PD genes such as Parkin have been shown to play an important role in early-onset PD forms,15 LRRK2 mutations initially have been reported mostly in patients with a late onset with a mean in the late 5th or 6th decade of life.1,2,4 However, very recent studies also identified mutation carriers with an onset of PD in the 3rd and 4th decade of life.16,17 To further elucidate the role of LRRK2 mutations in both early- (EOPD) and late-onset PD (LOPD), we screened 140 European PD patients for mutations in the most frequent mutation-bearing exons and tested an additional 220 EOPD patients for our recurrent mutations.

PATIENTS AND METHODS

A diagnosis of PD was established as previously described18 and a positive family history was defined as
having at least one first- or second-degree relative with a diagnosis of PD. We included 98 EOPD (age at onset [AAO] < 50 years) and 42 LOPD (AAO ≥ 50 years) cases that were selected based on an early onset and/or a positive family history (Group A). Patients in this group (61% men) had a mean AAO of 43.1 ± 14.4 (range, 15–80) years, and 115 (82%) reported a positive family history. They included 115 German, 14 Serbian, and 11 Italian patients and were tested for the known and additional mutations in the five most relevant reported mutation-bearing exons (Exons 24, 25, 31, 35, 41)² by single-strand conformation polymorphism (SSCP) analysis using positive controls (c.3342A>G, c.3451G>A, c.4321C>T, IVS35+23A>T, IVS40-39G>A, c.6055G>A). Samples with observed band shifts were sequenced. Furthermore, we investigated an additional set of 220 EOPD patients (Group B; 55% men) with a mean AAO of 39.4 ± 6.0 (range, 4–49) years and a positive family history in only 5%. They comprised 108 German, 65 Italian, and 47 Serbian patients. Group B and 100 German controls were tested for the recurrent c.4321C>T mutation by SSCP analysis. In addition, we specifically tested all 360 patients and our controls for the c.6055G>A mutation by restriction fragment length polymorphism analysis with SfeI, because this mutation was not easily detectable by SSCP analysis. Segregation of mutations in available relatives was investigated by direct sequencing. To evaluate whether our carriers of recurrent mutations shared the same haplotype, a set of seven microsatellite markers was tested that spanned 8.7 Mb, including and flanking the LRRK2 gene (Table 1). Transcranial sonography (TCS) was performed in one mutation carrier and her sister with a SONOS 5500 ultrasound system (Philips Medical Systems, Best, The Netherlands) with a 2.0 to 2.5 MHz sector transducer (S4 probe; Philips) in a standardized axial mesencephalic plane with a maximum depth of 12 cm from the temporal bone window on each side.

Written informed consent was obtained from all patients and controls. The local ethics committee had approved the study.

RESULTS

We identified three mutation carriers among the German EOPD cases of Group A (Table 2). Two had the same mutation (c.4321C>T, p.R1441C) as previously reported in a family from western Nebraska, an additional North American family, and four European families.²,⁶,⁹,¹⁹ In contrast to these families (AAO 48–78 years), our patients had an AAO of only 30 and 45 years, respectively. The third carrier had the previously frequently reported c.6055G>A (p.G2019S) mutation.

Mutational analysis of Group B only revealed one additional German mutation carrier (Case 4) with the c.6055G>A change (Table 2). None of the two mutations was detected in our 200 control chromosomes.

Relatives to test for segregation of the mutation were available only for Case 2, who had no family history of PD. However, both her mother and sister carried the same mutation. On neurological examination in 2005 at the age of 51 years, the sister (Case 2r) showed mild signs of PD (Table 2) but was unaware of her symptoms. Their mother died at age 79 years and was reported to be unaffected.

Genotype analysis in the c.4321C>T mutation carriers revealed shared alleles at three of the seven markers, however, not at the two adjacent markers of the mutation. In contrast, the two c.6055G>A carriers had at least one common allele at the five markers downstream of the mutation. However, the allele size at one marker differed from that in the proposed founder haplotype (Table 1).³,¹⁰ Notably, chromosomal phase could not be set in 3 of our cases.

### Table 1. Results of genotype analysis in the mutation carriers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Position* (Mb)</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Founder haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>D12S2194</td>
<td>2.595</td>
<td>249/253</td>
<td>249</td>
<td>253/249</td>
<td>253/257</td>
<td>265</td>
</tr>
<tr>
<td>D12S2515</td>
<td>2.831</td>
<td>216/224</td>
<td>212</td>
<td>220</td>
<td>220</td>
<td>224</td>
</tr>
<tr>
<td>LRRK2, c.4321C&gt;T</td>
<td>2.848</td>
<td>C/T</td>
<td>C/T</td>
<td>C/C</td>
<td>C/C</td>
<td>C</td>
</tr>
<tr>
<td>LRRK2, c.6055G&gt;A</td>
<td>2.878</td>
<td>G/G</td>
<td>G/G</td>
<td>G/A</td>
<td>G/A</td>
<td>A</td>
</tr>
<tr>
<td>D12S2519</td>
<td>2.974</td>
<td>138/140</td>
<td>132</td>
<td>132/140</td>
<td>132/134</td>
<td>132</td>
</tr>
<tr>
<td>D12S2520</td>
<td>2.977</td>
<td>257/260</td>
<td>257</td>
<td>260</td>
<td>260</td>
<td>260</td>
</tr>
<tr>
<td>D12S2521</td>
<td>2.986</td>
<td>319/327</td>
<td>371</td>
<td>363/319</td>
<td>363/371</td>
<td>359</td>
</tr>
<tr>
<td>D12S1048</td>
<td>3.170</td>
<td>211/217</td>
<td>211</td>
<td>214/220</td>
<td>214/223</td>
<td>214</td>
</tr>
</tbody>
</table>

Both allele sizes are given when chromosomal phase could not be set. Shared alleles at microsatellite markers for each mutation are in boldface.

Also, we detected five polymorphisms: c.3451G>T, IVS25-22C>T, IVS35+23A>T, IVS35+13_27del, IVS40-39G>A. These alterations were considered to be nonpathogenic, because they were either found in controls or are located in the introns, away from the conserved splice sites.

TCS in Case 2 showed hyperechogenicity of the substantia nigra (SN) on the left side but not on the right side (area of hyperechogenicity: left: 0.23 cm², right: <0.1 cm²), in keeping with the predominant clinical involvement of the contralateral (right) body half (Figure 1). In her more mildly affected sister, hyperechogenicity was also found on the right side but was less pronounced (area of hyperechogenicity: left: 0.23 cm², right: <0.1 cm²).

**DISCUSSION**

We identified two recurrent mutations in the LRRK2 gene in our EOPD cases but none in the 42 LOPD patients. We found 1.3% (95% confidence interval [CI], 0.1%–2.5%) mutation carriers in our EOPD sample (4/318), although we tested only parts of the gene. This frequency is comparable to the results of two other recent studies which investigated a North American sample.

### TABLE 2. Demographic and clinical description of the examined mutation carriers

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 2r</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset</td>
<td>30, female</td>
<td>45, female</td>
<td>-</td>
<td>42, female</td>
<td>46, male</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>German</td>
<td>German</td>
<td>German</td>
<td>German</td>
<td>German</td>
</tr>
<tr>
<td>Initial signs and symptoms</td>
<td>Rest tremor of her right hand</td>
<td>Micrography and joint pain</td>
<td>General stiffness in the morning</td>
<td>Tremor of her left hand, reduced left arm swing, micrography, sleep disturbances</td>
<td>Gait problems, bradykinesia of the left hand, increased perspiration</td>
</tr>
<tr>
<td>Last neurol. exam.</td>
<td>At age 50 yr</td>
<td>At 54 yr</td>
<td>At 51 yr</td>
<td>At 46 yr</td>
<td>At 49 yr</td>
</tr>
<tr>
<td>Signs</td>
<td>Bilateral rest tremor, bradykinesia, rigidity, postural instability</td>
<td>Bradykinesia (right hand), rigidity (neck, right arm), mild postural instability</td>
<td>Bradykinesia (both hands), mild rigidity (neck, right arm), mild postural instability</td>
<td>Rest tremor (both hands), bradykinesia, rigidity (neck, arms [l&gt;r]), postural instability</td>
<td>Bradykinesia (face, hands), rigidity (neck, arms&gt;legs, l&gt;r), postural instability</td>
</tr>
<tr>
<td>UPDRS III</td>
<td>32</td>
<td>13</td>
<td>13</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>UPDRS IV</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Hoehn &amp; Yahr</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Schwab/England</td>
<td>70</td>
<td>90</td>
<td>100</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>MMSE</td>
<td>27</td>
<td>None reported</td>
<td>29</td>
<td>Not performed</td>
<td>30</td>
</tr>
<tr>
<td>Additional signs</td>
<td>Good (250mg/day)</td>
<td>None reported</td>
<td>Sleep benefit</td>
<td>None reported</td>
<td>Sleep benefit</td>
</tr>
<tr>
<td>l-Dopa response</td>
<td>Good (350 mg/day)</td>
<td>Not treated</td>
<td>Good (375 mg/ day)</td>
<td>Not performed</td>
<td>Good (200 mg/ day)</td>
</tr>
<tr>
<td>Motor complications</td>
<td>After 5 yr: motor fluctuations and dyskinesias</td>
<td>After 6 yr: Mild dyskinesias</td>
<td>-</td>
<td>After 3 yr: motor fluctuations</td>
<td>After 4 months: none</td>
</tr>
<tr>
<td>Psychiatric symptoms</td>
<td>Mild depression</td>
<td>Mild depression</td>
<td>None</td>
<td>None</td>
<td>None reported</td>
</tr>
<tr>
<td>Family history</td>
<td>Positive (mother, AAO 50 yr)</td>
<td>Negative, however, mother and sister are mutation carriers</td>
<td>Not an index</td>
<td>Positive (mother)</td>
<td>Negative</td>
</tr>
</tbody>
</table>

UPDRS, Unified Parkinson’s Disease Rating Scale; MMSE, Mini-Mental State Examination; AAO, age at onset.
(1.6%)\textsuperscript{16} or an Italian sample (3%).\textsuperscript{17} Therefore, mutations in the \textit{LRRK2} gene appear not only to account for a considerable percentage of LOPD cases but also of EOPD.

Notably, the frequencies of the two recurrent mutations in our entire sample were the same, indicating that one should not focus on the detection of only one of them. The c.6055G\textsuperscript{16}/H11022\textsuperscript{18}A (p.G2019S) mutation, detected at a frequency of 0.6% (2 of 360; 95% CI, 0.2%–1.3%), seems to be underrepresented in our mostly German cases compared to the published frequency of approximately 1.5% (95% CI, 1.2%–1.8%) in mixed European and North American populations as revealed by a literature review. This finding is explained by a founder effect,\textsuperscript{3,10} leading to population-specific frequencies. Of interest, an even lower frequency of p.G2019S was detected in another German sample,\textsuperscript{11} whereas a very high frequency was reported recently among Ashkenazi Jews\textsuperscript{12} and Arabs.\textsuperscript{14} Alternatively, it might be related to the age-dependent penetrance of this mutation,\textsuperscript{3} because the majority of our cases had an early disease onset. In keeping with an age-dependent penetrance, the younger sister of Case 2 was not yet aware of her mild PD signs, stressing the necessity to personally examine relatives with mutations to assess the rate of penetrance.

Except for the early AAO, the clinical presentation of \textit{LRRK2} mutations in our patients was similar to that of idiopathic PD. However, 2 of our cases were notable for depression, and 2 carriers had reported sleep benefit.

TCS in Case 2 but also in the asymptomatic Case 2r showed a distinct hyperechogenic pattern in the SN as found in approximately 90% of patients with idiopathic PD but only in 9% of healthy controls.\textsuperscript{20} This finding is notable because high echogenicity of SN has been published recently for asymptomatic carriers of \textit{Parkin} mutations and suggested this method as a useful tool to detect subclinical parkinsonism. Surprisingly, another recent study demonstrated only moderate hyperechogenicity (area of hyperechogenicity of >0.19 and ≤0.24 cm\textsuperscript{2}) in 3 of 4 patients with \textit{LRRK2} mutations.\textsuperscript{11} It remains to be investigated in a larger samples size whether these differences are related to the mutation type and to the underlying pathogenic mechanism leading to PD.

Our 4 \textit{LRRK2} mutation carriers belong to a sample of 111 EOPD patients that were initially collected for \textit{Parkin} testing.\textsuperscript{18} In that group, a total of 15 mutation carriers were identified, including 10 patients with \textit{Parkin},\textsuperscript{18} 4 with \textit{LRRK2}, and 1 with a \textit{PINK1} mutation. This finding underlines that mutations in \textit{LRRK2} need to be added to the differential diagnosis of other genetic forms of EOPD, such as \textit{Parkin}-, \textit{PINK1}-, and \textit{DJ-1}-associated PD. However, one distinguishing factor between \textit{Parkin}- and \textit{LRRK2}-associated PD might be an overall younger mean age at onset in \textit{Parkin} mutation carriers. However, more early and young onset cases need to be investigated for \textit{LRRK2} mutations before drawing any definite conclusions. Of interest, only 2 of our 4 cases had a family history consistent with autosomal dominant PD, confirming the notion that \textit{LRRK2} mutations may occur (pseudo)sporadically due to reduced penetrance as shown in the family of Case 2.

The position c.4321 in \textit{LRRK2} seems to represent a mutational hot spot for two reasons: (1) our 2 patients with this mutation appear not to have a common founder, because they do not share an allele at the two markers

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Findings of transcranial sonography (TCS). A: The mesencephalic brainstem (dark gray) contains the (1) crus cerebri, (2) substantia nigra (SN), and (3) tectum cerebri. B–C: Transtemporal axial image in Case 2 of the mesencephalic brainstem that appears in a butterfly shape and was used as a landmark. Echogenicity of the SN was markedly increased on the left side (B) but not on the right side (C), in keeping with the predominant clinical involvement of the contralateral (right hand) body half.}
\end{figure}

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flanking the mutation, and (2) a different substitution at the same base pair has been described. However, it can also not be excluded that they have a very old founder and that recombination events in LRRK2 obscured the common haplotype. In contrast, the 2 carriers of the c.6055G>A mutation might have the same founder as previously suggested. In conclusion, our result of c.6055G
LRRK2
mutations will have implications for specific genetic testing of this large gene in patients with clinically typical PD as well as in early onset cases.

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REFERENCES

Dystonia, Mental Deterioration, and Dyschromatosis Symmetrica Hereditaria in a Family With ADAR1 Mutation

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Abstract: A family with dystonia associated with dyschromatosis symmetrica hereditaria (DSH), mental deterioration, and tissue calcification is described. The proband possessed an adenosine deaminase acting on the RNA 1 gene (ADAR1) mutation Gly1007Arg. This ADAR1 mutation could disturb RNA editing at Q/R sites of glutamate receptor in the brain and increase Ca2+ influx into neurons, which is thought to induce dystonia and mental deterioration. The observations in our family raise the possibility that the ADAR1 mutation might be a direct cause or a

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