Prevalence and Phenotype of Patients with PARK2 or PARK8 Gene Mutations in an Early-Onset Parkinsonism Brazilian Cohort

Delcio Bertucci Filho¹, Renato P. Munhoz¹, Suzanne Lesage⁵, Alexis Brice⁵, Salmo Raskin³ and Helio A. G. Teive¹

¹Movement Disorders Unit, Neurology Service, Hospital de Clínicas, Federal University of Paraná, Curitiba, Pr, Brazil
²Inserm, U975, Paris, France
³Genetika Laboratory, Curitiba, Pr, Brazil
*Corresponding author: Dr. Delcio Bertucci Filho, MD, Rua Marechal Deodoro 575, Ponta Grossa PR 84010-030 Brazil, Tel: 55 42 3224-6466; E-mail: dbertucci@uol.com.br
Received: 07-15-2014
Accepted: 07-20-2014
Published: 07-30-2014
Copyright: © 2014 Delcio

Research Article
All authors have contributed to the work and agree with the presented findings. The paper has not been published before nor is being considered for publication in another journal.

Abstract

Objective: To estimate the prevalence of mutations in the PARK 2 or PARK 8 genes and characterize their phenotypes in a Brazilian cohort of early-onset Parkinsonism (EOP).

Methods: A total of sixty-nine unrelated patients with age at onset ≤ 45 were screened.

Results: Mean age of symptoms onset was 35.8 ± 6.8 years. A positive family history of Parkinsonism was found in 8 (11, 59%) patients. Molecular analysis detected five patients (7, 24%) with PARK2 mutations (two homozygous and three compounds heterozygous) and one patient (1.52%) with a heterozygous PARK8 G2019S mutation. The motor phenotype most prevalent in the patients with PARK2 mutations was the rigid-akinetic with postural instability in four (80%); dystonia at disease onset was present in one (20%). The patient with PARK8 mutation had tremor and dystonia at the disease onset but latter developed the rigid-akinetic motor phenotype with severe postural instability.

Conclusions: Although we acknowledge the caveat of examining a limited sample size, our study suggests that PARK2 and PARK8 mutations are uncommon in Brazilian patients with EOP.

Keywords: Early-Onset Parkinsonism, PARK2, Parkin, PARK8, LRRK2, Parkinson’s Disease, Parkinsonism.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease [1]. Although PD was long considered a non-genetic disorder of 'sporadic' origin, 5–10% of patients are now known to harbor monogenic forms. Mutations in seven genes are robustly associated with autosomal dominant (SNCA/PARK1, PARK4, LRRK2/PARK8, EIF4G1, VPS35) or recessive (Parkin/PARK2, PINK1/PARK6, DJ1/PARK7) PD or Parkinsonism [2-5].

The PARK2 (Parkin) gene spans 1, 35 Mb of genomic DNA, contains 12 exons and it encodes the parkin protein, an E3 ubiquitin-protein ligase that targets specific substrates for degradation via the ubiquitin-proteasome pathway [6].
Mutations in PARK2 are the most common known cause of early-onset parkinsonism (EOP), accounting for at least 15% of sporadic cases and 50% of those with recessive inheritance with a clear inverse correlation with age at onset [7,8]. From a phenotypic standpoint patients with PARK2 mutations may have a different clinical profile from those who do not have the mutation including early-onset lower limb dystonia, hyperactive deep tendon reflexes, a more symmetrical motor symptoms onset, a tendency toward a greater response to levodopa despite lower doses [9], a slower disease course and atypical clinical presentation at onset [10].

The PARK2 (leucine-rich repeat kinase 2 - LRRK2) gene was identified in 2004 [11,12] and consists of 51 exons (172,542 bases) that encode a 2,527–amino acid protein called dardarin which it is a multi-domain protein containing enzymatic domains of a GTP-ase and a kinase, along with the protein interaction motifs LRR (leucine-rich repeat) and WD40 [13]. PARK2 mutations are now recognized as the most common cause of genetic Parkinsonism, accounting for 10% of autosomal dominant cases [14] and 3.6% of sporadic cases [15]. Mutations in this gene are mostly associated with a classic PD-like phenotype with age at onset of 50–70 years, variable penetrance and phenotype similarities between patients with homozygous and heterozygous mutations [16] although some studies have also found an association with EOP [17,18].

The aims of this study were to estimate the prevalence of PARK2 or PARK8 gene mutations and characterize their phenotypes in a cohort of Brazilian patients with EOP.

Materials and Methods

Patients and Families: The study was carried out at the Movement Disorders Unit, Neurology Service, Hospital de Clínicas, Federal University of Paraná, and Curitiba, Brazil. Cases were recruited at this center and at the State of Paraná Parkinson Association. Sixty-nine unrelated patients with age at onset ≤ 45 years were selected according to the Queen Square Brain Bank Criteria [19]. Eight patients (11.59%) had recessive inheritance of Parkinsonism. There were no cases of known consanguinity. One patient had two daughters with lower limb dystonia; however both were unavailable for examination.

The motor phenotype was divided into three forms: tremor-dominant when resting tremor was the main feature, with non-debilitating rigidity and/or bradykinesia; rigid-akinetic when tremor was not present, and mixed form [20]. DSM-V criteria were used to diagnose depression and the Mini-Mental State Examination (MMSE) to diagnose dementia. MMSE cut-off levels for diagnosing dementia were 13 for illiterate patients, 18 for patients with 1 to 8 years of schooling and 26 for patients with more than 8 years, as previously established for Brazilian patients [21]. The overall levodopa equivalent daily dose (LEDD) was obtained using the formula described by Tomlinson et al. [22]. Disease stage was determined according to the Hoehn and Yahr scale (H&Y). The severity of the motor signs was quantified using the Unified Parkinson’s disease rating scale (UPDRS) part III and the limitations on daily life activities was assessed by the Schwab and England scale. Patients were assessed in the “off” and “on” levodopa period.

Molecular Analysis: Blood samples were taken from all 69 index cases. Genomic DNA was extracted from peripheral blood leukocytes using standard procedures at the Genetics Laboratory, Curitiba, Brazil. The genetic analysis was carried out at Hôpital de la Salpêtrière, Paris, France, using methods described by Lesage et al. [23]. All 69 patients were tested for mutations in the PARK2 gene, and 66 for mutations in the LRRK2 gene. The study was approved by the Ethics Committee at the Hospital de Clínicas, Federal University of Paraná.

Results

Prevalence and Molecular Analysis: Among the 69 patients included, six (8, 69%) were found to have a mutation in at least one allele: five (7, 25%) had a mutation in the PARK2 gene and one (1.45%) in the PARK8 gene. Of the five cases with PARK2 gene mutations, two were homozygous (2, 9%), and the remainder (4, 35%) compound heterozygous. The Asn52sf mutation was present in one compound heterozygous. The Met192Leu, one with A82E and one patient with the rare compound heterozygous. The Asn52sf mutation was present in one compound heterozygous. The Met192Leu, one with A82E and one patient with the rare compound heterozygous.

Table 1 – Prevalence of PARK2 and LRRK2 gene mutations in patients with EOP stratified according to age of onset

<table>
<thead>
<tr>
<th>Age of onset (years)</th>
<th>Patients with PARK2 mutation n (%)</th>
<th>Patients with LRRK2 mutation n (%)</th>
<th>Patients without mutations n (%)</th>
<th>All patients n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>0 (0%)</td>
<td>1 (1.45%)</td>
<td>63 (89.80%)</td>
<td>64 (95.51%)</td>
</tr>
<tr>
<td>21-30</td>
<td>0 (0%)</td>
<td>1 (1.45%)</td>
<td>65 (94.20%)</td>
<td>66 (97.10%)</td>
</tr>
<tr>
<td>31-40</td>
<td>1 (1.45%)</td>
<td>1 (1.45%)</td>
<td>62 (89.80%)</td>
<td>64 (95.51%)</td>
</tr>
<tr>
<td>41-50</td>
<td>1 (1.45%)</td>
<td>1 (1.45%)</td>
<td>62 (89.80%)</td>
<td>64 (95.51%)</td>
</tr>
<tr>
<td>51-60</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>69 (100%)</td>
<td>69 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (2.90%)</td>
<td>2 (2.90%)</td>
<td>67 (98.70%)</td>
<td>69 (100%)</td>
</tr>
</tbody>
</table>

Table 2 – PARK2 mutations in patients with EOP

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Gender</th>
<th>Gene Exon Mutation</th>
<th>Sequence exon 1-12</th>
<th>Allele promoters exon 1-12</th>
<th>Age at onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2</td>
<td>A53T</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homozygous</td>
</tr>
<tr>
<td>2</td>
<td>P</td>
<td>3 + Point</td>
<td>del gene mRNA 20</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homozygous</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>2,3</td>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homozygous</td>
</tr>
<tr>
<td>4</td>
<td>P</td>
<td>2</td>
<td>A53T</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homozygous</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>7</td>
<td>Arg356Gys</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homozygous</td>
</tr>
</tbody>
</table>
Clinical Findings: Forty three patients were males (62.3%). The clinical features and the treatment profile of the patients with PARK2 gene mutations are shown in Table 3. In both patients with either heterozygous or homozygous Asn52Stop81 mutation, age at onset was the same (40 years). Both patients with Met192Leu polymorphisms had atypical findings: bulging eyes in one and facial muscle atrophy in the other; one had a positive family history for PD (a female maternal cousin). The clinical profile of the groups with and without PARK2 mutations are shown in Table 4.

The presence of only one patient with a mutation in the PARK8 gene, limits our possibility to present clinical correlations; her clinical features are shown in Table 4. There was no family history of Parkinsonism. This patient’s initial clinical sign was right upper limb tremor; later developing the full blown manifestations of PD. She reported dystonia in her left foot from disease onset. At the time of clinical assessment, she did not have signs that fulfilled criteria for dementia (MMSE score of 23, cut-off of 18) and responded well to levodopa with typical motor complications (peak dose dyskinesias; wearing off and no-effect doses fluctuations).

Table 4 - Clinical characteristics of EOP patients with and without PARK2 mutations with and without LRRK2 mutation

<table>
<thead>
<tr>
<th>Patient</th>
<th>PARK2 positive (n=5)</th>
<th>PARK2 negative (n=63)</th>
<th>LRRK2 positive (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female ratio</td>
<td>3/2</td>
<td>47/15</td>
<td>3/2</td>
</tr>
<tr>
<td>Current age (years)</td>
<td>55.2 ± 1.2 (44-71)</td>
<td>65.9 ± 1.8 (45-85)</td>
<td>65.7 ± 1.9 (45-84)</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>43.2 ± 1.5 (29-58)</td>
<td>46.0 ± 1.7 (25-65)</td>
<td>47.2 ± 1.7 (30-65)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>45.3 ± 1.9 (20-45)</td>
<td>32.7 ± 2.1 (20-55)</td>
<td>37</td>
</tr>
<tr>
<td>Family history</td>
<td>6 (60%)</td>
<td>2 (6.06%)</td>
<td>No</td>
</tr>
<tr>
<td>Clinical signs at onset</td>
<td>Rigid-dystonic</td>
<td>2 (40%)</td>
<td>32 (50.8%)</td>
</tr>
<tr>
<td>Motor phenotype</td>
<td>2 (40%)</td>
<td>27 (42.8%)</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>1 (20%)</td>
<td>4 (6.06%)</td>
<td></td>
</tr>
<tr>
<td>Asymmetry</td>
<td>5 (100%)</td>
<td>46 (100%)</td>
<td>10 (18.18%)</td>
</tr>
</tbody>
</table>

Table 3 - Clinical features of patients with EOP and PARK2 mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Asn52Stop81</td>
<td>Asn52Stop81</td>
<td>Arg256Cys</td>
<td>Arg256Cys</td>
<td>Asn52Stop81</td>
</tr>
<tr>
<td>Gender</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>40</td>
<td>44</td>
<td>40</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Current age (years)</td>
<td>40</td>
<td>44</td>
<td>40</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>35</td>
<td>30</td>
<td>35</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Family history</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial symptoms</td>
<td>Lower limb tremor</td>
<td>Lower limb tremor</td>
<td>Lower limb tremor</td>
<td>Lower limb tremor</td>
<td>Lower limb tremor</td>
</tr>
<tr>
<td>Motor phenotype</td>
<td>Rigid-dystonic</td>
<td>Rigid-dystonic</td>
<td>Mixed form</td>
<td>Rigid-dystonic</td>
<td>Rigid-dystonic</td>
</tr>
<tr>
<td>UPDRS II score (on)</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Hoehn &amp; Yahr</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Depression</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hasegawa score</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Schwab &amp; England</td>
<td>74</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Levodopa treatment</td>
<td>Daily LED (mg)</td>
<td>744 ± 491 (91-2130)</td>
<td>744 ± 491 (91-2130)</td>
<td>744 ± 491 (91-2130)</td>
<td></td>
</tr>
<tr>
<td>Time to Levodopa use (years)</td>
<td>11.1 ± 2.9 (5.5-21)</td>
<td>6.5 ± 4.8 (1.5-17)</td>
<td>6.5 ± 4.8 (1.5-17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Dopa use until complications (years)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Dyskinesias/fluctuations</td>
<td>4 (80%)</td>
<td>4 (80%)</td>
<td>4 (80%)</td>
<td>4 (80%)</td>
<td>4 (80%)</td>
</tr>
</tbody>
</table>

Discussion

We found a low frequency of PARK2 mutations in our cohort of EOP. Our frequencies of PARK2 mutations were lower than those reported in Europe [8] and almost half than in other studies from Brazil [24,25]. Periquet et al in 2003 studied 146 patients of various geographical origins with EOP, without family history, including cases from Brazil, and concluded that at least 15% of patients had PARK2 mutations [8]. Camargos et al, studying 45 patients with EOP, found five with mutations (11.1%) in the PARK2 gene; heterozygous mutations in this gene accounted for 4.4% of
their patients, and 6.6% were compound heterozygous
mutations [25]. Aguiar et al. described 72 patients with
EOP and nine (12.5%) had a mutation in the PARK2 gene,
two of these were homozygous and seven heterozygous
for the mutation [24]. Other published studies including
Brazilian patients with EOP confirm the frequent
involvement of PARK2 gene [26-28].

The frequency of PARK2 mutations is estimated to be 49%
in cases of EOP with a positive family history [7,8,29]. In our
Brazilian series of 69 cases of EOP, only 11, 59 % had a fam-
ily history of Parkinsonism. The finding of none PARK2 mu-
tation in familial cases in our study may be due to the small
sample size and also to different genetic background of the
patients involved in others Brazilian series.

Some heterozygous PARK2 variants (polymorphisms) have
been observed in healthy control individuals, making asses-
sement of pathogenicity for these variants quite complex.
It has been suggested a role of these heterozygous recessive
mutations as risk factors for disease [30]. Atypical findings
were found only in two of our patients and they both had
Met192Leu polymorphisms.

The small number of patients with PARK2 mutations made
comparative analysis unsuitable. But postural instability
was the only clinical feature that tended to be more common
in our patients with PARK2 mutations which had been de-
scribed as part of the phenotype of Parkin disease [31]. The
rigid-akineti c motor phenotype was prevalent in four of our
five patients with PARK2 mutations including the two pa-
tients with homozygous mutations. One of the patients with
homozygous mutations had dystonia as the initial symptom.

Only one of our patients had a mutation in the PARK8 gene,
which can be explained by the fact that there is a higher
prevalence of these mutations in patients in whom the
disease has a late onset [15]. At disease onset the patient
presented with tremor in her right hand, in agreement with
a study by Marras et al. that found tremor to be the most
frequent initial symptom [32]. The PARK8 G2019S mu-
tation in our patient (heterozygous) is reported to be the
most common one worldwide [14,33] and in Brazil [34,35].
The PARK8 G2019S mutation causes 4-5% of familial and
1-2% of sporadic PD in populations of European descent,
30-40% of both familial and sporadic PD in Arab patients
from North Africa and 10-30% in Ashkenazi Jews [36]. In
South America PARK8 G2019S mutation is estimated to be
present in 3% of familial and 2% in sporadic cases of PD
[34,37]. The prevalence of PARK8 mutations in a previ-
ously published Brazilian sample of EOP patients was 3,5 %
[33]. Therefore PARK8 mutation is rare in sporadic EOP.

Although we acknowledge the caveat of examining a limited
sample size, our study suggests that PARK2 and PARK8 mu-
tations are uncommon in this southern of Brazil series of
patients with predominantly sporadic EOP.

Acknowledgment
We wish to thank Dr. André Troiano (INSERM) for his help
with the data.

Conflict of Interest
The authors have no conflict of interest to report.

References
1. Poewe W. Non-motor symptoms in Parkinson’s disease.

2. Puschmann A. Monogenic Parkinson’s disease and Par-
kinsonism: Clinical phenotypes and frequencies of known

3. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Lincoln
SJ, Léprêtre F et al. Translation Initiator EIF4G1 Muta-
tions in Familial Parkinson Disease. Am J Hum Genet. 2011, 89:
398–406.

JM et al. VPS35 mutations in Parkinson disease. Am J Hum

5. Zimprich A, Benet-Pagès A, Struhal W, Graf E, Eck SH et
al. A mutation in VPS35, encoding a subunit of the retromer
complex, causes late onset Parkinson disease. Am J Hum

al. Familial Parkinson disease gene product, parkin, is an

7. Lücking CB, Dürr A, Bonifati V, Vaughan J, De Michele G
et al. Association between early-onset Parkinson’s disease
1560–1567.

Michele G et al. Parkin mutations are frequent in patients
with isolated early-onset parkinsonism. Brain. 2003, 126:
1271–1278.

9. Lohmann E, Periquet M, Bonifati V, Wood NW, De Michele
G et al. How much phenotype variation can be attributed to

10. Rawal N, Periquet M, Lohmann E, Lücking CB, Teive
HA et al. New parkin mutations and atypical pheno-
types in families with autosomal recessive parkinsonism.

al. Mutations in LRRK2 cause autosomal-dominant parkin-
sonism with pleomorphic pathology. Neuron. 2004, 44:
601–607.

Cite this article: Bertucci Filho D. Prevalence and Phenotype of Patients with PARK2 or PARK8 Gene Mutations in an Early-Onset Parkinsonism Brazilian Cohort. J J Neur Neurosci. 2014, 1(1): 003.


