Research Article

Genetic Analysis of PARK2 and PINK1 Genes in Brazilian Patients with Early-Onset Parkinson’s Disease

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Parkinson's disease is the second most frequent neurodegenerative disorder in the world, affecting 1-2% of individuals over the age of 65. The etiology of Parkinson’s disease is complex, with the involvement of gene-environment interactions. Although it is considered a disease of late manifestation, early-onset forms of parkinsonism contribute to 5–10% of all cases. In the present study, we screened mutations in coding regions of PARK2 and PINK1 genes in 136 unrelated Brazilian patients with early-onset Parkinson’s disease through automatic sequencing. We identified six missense variants in PARK2 gene: one known pathogenic mutation, two variants of uncertain role, and three nonpathogenic changes. No pathogenic mutation was identified in PINK1 gene, only benign polymorphisms. All putative pathogenic variants found in this study were in heterozygous state. Our data show that PARK2 point mutations are more common in Brazilian early-onset Parkinson’s disease patients (2.9%) than PINK1 missense variants (0%), corroborating other studies worldwide.

1. Introduction

Parkinson’s disease (PD) is a complex neurodegenerative disorder that affects different regions of the nervous system, although it is clinically recognized by typical motor manifestations [1]. Even though much of the etiology of PD remains unknown, early-onset PD (EOPD) (onset before 50 years old), which accounts for approximately 5%-10% of all PD cases, can be explained by monogenic causes [2].

Mutations in coding regions of PARK2 gene are often implicated as the most common cause of EOPD, followed by PINK1 gene variants. These two genes are associated with the autosomal recessive forms of parkinsonism and may act in the same pathway, controlling mitochondrial homeostasis [2–4]. PARKIN is an ubiquitin E3 ligase and, therefore, participates in the ubiquitin-mediated proteasomal degradation pathway [5], whose activity seems to be compromised by pathogenic mutations. Since the first description of PARK2 mutations in Japanese patients with juvenile EOPD [6], more than 170 different mutations have been described throughout its sequence, including large deletions or amplifications, small deletions/insertions as well as missense mutations.
As a common molecular consequence, such mutations are known to cause loss of PARKIN function, leading to impaired mitochondrial integrity [7].

PINK1 (PARK6) encodes a mitochondrial serine/threonine kinase, which is expressed ubiquitously in the human brain. Recent evidence suggests that the physiological role of PINK1 comprises the phosphorylation of mitochondrial proteins in response to cellular stress and the protection of mitochondria against various stressors [8, 9]. Up to date, homozygous and compound heterozygous losses of function mutations affecting the kinase domain of PINK1 gene were observed, all of them reducing its enzymatic activity [10, 11]. The frequency of these mutations varies according to the geographic region from 0 to 15% all over the world [7, 12].

PARKIN and PINK1 are thought to participate in the same pathway concerning controlling mitochondrial integrity and function, with PINK1 functioning upstream from PARKIN [13, 14]. In the present study, we investigated the presence of PARK2 and PINK1 sequence mutations in patients with EOPD from the Southeast and Midwest regions of Brazil.

### 2. Materials and Methods

We analyzed 136 unrelated Brazilian patients (86 men and 50 women; mean age 49.8 ± 13.3 years; mean age at onset 39.5 ± 10.3 years) with idiopathic PD manifesting before 51 years old and 200 healthy Brazilian controls. All patients and healthy volunteers were from the same geographic area, both with similar age and socioeconomic status. Amongst the patients included in this study, 31 cases had familial history of PD in relatives of 1st and 2nd degrees and 105 represented isolated cases of the disease. The Ethics Committee of State University of Rio de Janeiro approved this study and a written informed consent was obtained from all subjects.

The DNA of patients was obtained from peripheral blood. The presence of exon rearrangements in PARK2 and PINK1 genes was formerly screened in 102 probands of our sample through the MLPA analysis [15]. The coding regions of PARK2 and PINK1 genes were sequenced using primers previously described [16]. The sequencing reactions were prepared according to the manufacturer, using the Big Dye Terminator v3.1 Kit (Applied Biosystems). Control DNA samples were screened for p.P437L, p.A339V, and p.K220R substitutions. All reactions were processed on an automated sequencer ABI Prism 3130 (Applied Biosystems), and the sequence analysis was performed using the softwares Chromas Lite 2.0 (Technelysium) and BioEdit Sequence Alignment Editor Version 6.0.6 (Isis Pharmaceuticals, Inc.).

DNA samples which showed sequence variations underwent PCR-RFLP analysis or Taqman SNP genotyping to confirm the alteration found. The prediction analysis of the effects of changes which cause amino acid substitutions in proteins was performed using electronic tools PolyPhen and Pmut. To exclude the possible effect of silent mutations on splicing, we have used SpliceView and NNsplice softwares.

### 3. Results

We identified twelve sequence variants in PARK2 gene: three silent variants and nine missense mutations (Table 1). Between the alterations found, five are nonpathogenic polymorphisms: c.500G > A (p.S167N), c.1138G > C (p.V380L), c.783A > G (p.L261L), c.1180G > A (p.D394N), and c.111G > A (p.P377P) [7]. The already-known c.245C > A (p.A82E) and c.719C > T (p.T240M) variants were found in one patient each, both in heterozygous state. The p.A82E substitution had also been found in control subjects worldwide [17, 18].

We also identified the heterozygous c.1310C > T (p.P437L) variant in two probands and one healthy individual. This well-known alteration had already been reported in PD patients and controls in some populations [18, 19]. The patient found with the heterozygous c.434G > A (p.S145N) substitution also harbors the p.S167N polymorphism in PARK2 exon 4. This variant has an uncertain pathogenic nature, and it has never been identified in controls worldwide.

Three changes found in this study have never been described: c.659A > G (p.K220R), c.1016C > T (p.A339V), and c.1021C > T (p.L341L) (Table 1). The new variant recognized in exon 9 of PARK2 gene, c.1021C > T, was identified in two patients and resulted in a silent mutation (p.L341L).

Among the 200 healthy controls, the c.1310C > T (p.P437L) substitution in PARK2 gene was found just in one individual in heterozygous state. Besides, we investigated the presence of c.1016C > T (p.A339V) and c.659A > G (p.K220R) variants in the same population, and we were unable to detect these mutations in any of the control individuals analyzed.

In PINK1 gene we found only known benign polymorphisms, including the exon 5 variant c.1018G > A (p.A340T) in seven patients, the silent variant c.1173T > C (p.D391D) in one patient (exon 6), the exon 7 variant c.1426G > A (p.E476K) in two patients, and exon 8 variant c.1562A > C (p.N52I) that was found in half of our patients (66/132) (Table 2).

The quantitative analysis, previously realized by our group, identified 4 patients with dosage mutations: one proband with exon 1 heterozygous deletion of PINK1 gene; an index case with heterozygous deletion of PARK2 exon 4; one patient with heterozygous duplication of PARK2 exon 4, and a compound heterozygous patient that harbors two PARK2 mutations, a deletion of exons 5-6, and a duplication of exon 3 [15]. Among PD patients with heterozygous pathogenic or probably pathogenic PARK2 variants, the quantitative analysis with MLPA has not revealed dosage alterations.

### 4. Discussion

So far, PARK2 and PINK1 are the genes most frequently associated with autosomal recessive EOPD, and both coding products participate in the same metabolic pathway centered on maintenance of the morphological integrity of mitochondria. Studies of PARK2 and PINK1 genes in Latin American populations are very scarce. In our study, we found twelve substitutions in PARK2 gene, from which nine are nonpathogenic variants and three putative pathogenic
mutations. Among the heterozygous variants identified, three are silent mutations, including one that is novel (p.L341L).

The exon 3 c.245C > A (p.A82E) variant was identified in one proband and was previously described by other authors in PD patients and controls [17, 18], suggesting that the p.A82E variant is probably nonpathogenic. Besides, the in silico analysis through the PolyPhen and Pmut softwares showed that this alteration is benign. We also identified the c.719C > T (p.T240M) substitution in one patient. This change had already been described, and it had been previously classified as a known pathogenic variant [18, 20]. Our in silico analysis corroborated these data and considered that this alteration affects the PARKIN function, being probably pathogenic.

Another mutation, c.1310C > T (p.P437L), was found in two patients and one healthy subject. Other groups have reported this variant in a similar frequency between cases and controls in North American and European populations [18, 19]. Although our in silico predictions have classified this variant as probably pathogenic, this mutation was present in a Brazilian control individual (59 years old), which leads us to believe that it is a nonpathogenic polymorphism. However, the evaluation of pathogenicity of this variant must be done with caution. We have identified this mutation in three asymptomatic daughters (35, 33, and 31 years old) and a granddaughter (12 years old) of a carrier patient. Functional analyses should help to clarify if the p.P437L substitution is a risk factor for PD.

The c.434G > A (p.S145N) variant was found in a patient that also harbors the polymorphism c.500G > A (p.S167N) in the same exon of PARK2 gene. The in silico analysis had contradictory results, and this mutation has its pathogenic nature unclear in the literature [7]. Although the pathogenicity is not yet confirmed, we believe that functional analyses are important to establish if the p.S145N variant is related with a high risk of developing PD.

Three changes identified in this study have never been described: c.659A > G (p.K220R), c.1016C > T (p.A339V),
and c.1021C > T (p.L341L). The first one (p.K220R) was found in only one patient, in heterozygous state. We were unable to detect this mutation in any of the 200 healthy subjects. All in silico evidence points this variant as nondeleterious for PARKIN protein. In addition, this mutation does not encode any part of the catalytic domain of the protein. We believe that it might be a rare polymorphism, but functional studies and segregation analysis would be valuable tools to determine the pathogenicity of this substitution.

Two novel mutations were found in exon 9 of PARK2: p.A339V in one patient with familial history of PD and p.L341L in two sporadic cases. We did not observe the p.A339V variant in 400 control chromosomes, reinforcing the rarity of this variant or its potential association with the patient’s phenotype. On the other hand, the in silico analysis showed contradictory results, which did not help to clarify the pathogenicity of this mutation. Another known missense mutation in the same amino acid residue had already been identified and does not cause phenotypic changes [7]. We cannot classify the pathogenicity of this variant until functional studies are performed.

The second new alteration found in PARK2 exon 9 (c.1021C > T) results in a silent mutation, p.L341L. The in silico analysis using SpliceView and NNsplice programs showed that this variant does not affect the recognition of donor and receptor sites of splicing, and, because of its silent nature, this change is considered nonpathogenic.

No pathogenic mutation was identified in PINK1 gene. We found the variant c.1426G > A (p.E476K) in two patients. As this substitution has already been found in some healthy control subjects by other studies [1, 25], it was predicted to be benign by bioinformatic programs and is poorly conserved even within mammals [25], and we suggest it is a nonpathogenic variant.

All putative pathogenic point mutations identified in this study in PARK2 gene were in heterozygous state (Table 1). Until now, the role of homozygous and compound heterozygous variants was already established as a cause of autosomal recessively inherited EOPD, but the pathogenic significance of heterozygous mutations is still uncertain, particularly for missense substitutions in the context of recessive inheritance. Different mechanisms have been suggested to explain the effects of single heterozygous variants like loss-of-function mutations by lowering the biological activity of the encoded protein (haploinsufficiency), dominant-negative property, or gain-of-function dominant mutations [26]. So, increasing evidence indicates that heterozygous variants are noncausative mutations but rather genetic susceptibility factors which may contribute to the risk of developing PD [26].

5. Conclusions

Our findings showed that PARK2 point mutations are more frequent than PINK1 pathogenic variants in our sample of Brazilian EOPD patients. The absence of pathogenic mutations in PINK1 gene in our population is consistent with other studies [19, 23, 27], supporting the hypothesis that mutations in PINK1 may not be a relevant cause of EOPD among Brazilian sporadic and familial patients. Our results of PARK2 gene are in agreement with studies worldwide that have found similar frequencies of PARK2 point mutations in different populations [18–20, 23, 24] (Table 3), although they differ from the frequency identified by other Brazilian groups (5.5% and 11.1%) [21, 22]. One possible explanation for these different results would be the reduced sample sizes tested by them (72 and 45 patients, resp.) (Table 3).

In conclusion, we strengthen that the functional analyses of the missense variants found by us are still missing and would help us to clarify the real pathogenic value of these mutations in our population. Besides, whether heterozygous mutations in recessive genes act as susceptibility factors or as causal agents in the PD process remains to be determined.

Conflict of Interests

The authors have no conflict of interests to declare.

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