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Research Article

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

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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]. Mu-

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 PARK8 (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]. PARK8 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 "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 Genetika 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.

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

Age of onset (years)	Patients with PARKIN mutation n (%)	Patients with LRRK2 mutation n (%)	Patients Without mutation n (%)	All patients n (%)
≤ 20	0	0	3 (4.35%)	3 (4.35%)
21-25	0	0	4 (5,80%)	4 (5.80%)
26-30	1 (1,45%)	0	11 (15,94%)	12 (17,39%)
31-35	0	0	10 (14,49%)	10 (14.49%)
36-40	3 (4,35%)	1 (1,45%)	25 (36,23%)	29 (42,03%)
> 40	1 (1,45%)	0	13 (18,84%)	14 (20,29%)
Total	**5**	**1**	**63**	**69**

EOP: Early Onset Parkinsonism.

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 two unrelated patients. We also found polymorphisms of the PARK2 gene in four (5, 8%) other patients: two with Met192Leu, one with A82E and one patient with the rare variant C377R. The only patient with PARK8 mutation was heterozygous for G2019S mutation.

Table 2 – PARK2 mutations in patients with EOP

Patient #	Gender	Gene Exon Mutation	Sequence exon 1-12	Dosage Promoter-exon 1-12	Age at onset	
1	M	2	Asn52fs	----	40	Homozygous
2	F	1 + Prom	----	del prom ex1	28	Homozygous
3	M	2,3	----	Ex 2,3 het dupl	40	Compound heterozygous
4	F	2	Asn52fs	----	40	Compound heterozygous
5	M	7	Arg256Cys	----	43	Compound Heterozygous

EOP: Early Onset Parkinsonism.

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 Asn-52Stop81 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.

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

Patient	1	2	3	4	5
Mutation	Asn52fs	del prom ex1	Ex 2,3 het dupl	Asn52Stop81	Arg256Cys
	Homozygous	Homozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous
Gender	M	F	M	F	M
Age at onset (years)	40	28	40	40	43
Current age (years)	75	58	48	44	51
Duration (years)	35	30	8	4	8
Family history	No	no	no	no	No
Initial symptom	Lower limb tremor	Dystonia lower limb	Bradykinesia upper limb	Bradykinesia upper limb	Tremor upper limb
Motor phenotype	Rigid-acinetic	Rigid-acinetic	Mixed form	Rigid-acinetic	Rigid-acinetic
UPDRS III score(on)	19	39	10	28	13
Hoehn-Yahr score	3	4	2	3	1
Depression	No	no	no	yes	No
MMSE score	29	25	30	30	30
Schwab England	80	80	90	70	90
Levodopa treatment					
Daily LED*	625	1200	850	1300	1100
Duration (years)	29	no information	7,5	7,5	0,5
Time for l-dopa use	6	no information	0,5	1,5	7,5
L-dopa use until complications (years)	21	no information	4	7	
Dyskinesias	No	a,b	a,b	a	No
Fluctuations	C	c,d,e	c	c,e	No
Stereotactic surgery	No	Left talamotomy/ right palidotomy	Right palidotomy	no	No
Imaging study	Normal CT scan	Assimetry of lateral ventricles (R>L)	Normal CT scan	Normal CT scan	Normal CT scan

EOP: Early Onset Parkinsonism
* Levodopa Equivalent Dose
a. peak dose
b. morning dystonia
c. wearing off
d.on-off
e. no-effect dose

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

	PARK2 positive (n=5)	PARK2 negative (n=63)	LRRK2 positive (n=1)
Male/female ratio	3/2	40/23	0/1
Current age (years)	55,2 ± 12,2 (44-75)	46 ± 7,9 (21-65)	59
Age at onset (years)	38,2 ± 5,8 (28-43)	35.5 ± 7 (19-45)	37
Disease duration (years)	17 ± 14,3 (4-35)	10.3 ± 5,3 (2-21)	22
Family history	0	7 (12,7%)	No
Clinical signs at onset			
Rigid-akinetic	2 (40%)	32 (50,8%)	No
Dominant tremor	2 (40%)	27 (42,86%)	yes
Dystonia	1 (20%)	4 (6.06%)	yes
Asymmetry	5 (100%)	66 (100%)	yes
Clinical signs on examination			
Bradykinesia	5 (100%)	63 (100%)	yes
Rigidity	5 (100%)	63 (95.45%)	yes
Resting trem	1 (20%)	26 (41,3%)	no
instability or	4 (80%)	20 (31.7%)	yes
Postural	20 ± 8,9 (10-30)	22.5 ± 8,2 (7-47)	44
UPDRS [a]	2.6 ± 1,1 (1-4)	2.5 ± 0.6 (1-4)	5
Hoehn-Yahr	1 (20%)	12 (18.18%)	no
Depression			
Levodopa treatment			
Daily LED [b] (mg)	1015 ± 275 (625-1300)	744 ± 491 (0 ± 2115)	800
Duration (years)	11,1 ± 7,2 (0.5-29)	6.5 ± 4.8 (1.5 - 17)	20
Time to L-dopa use (years)	3,9 ± 3,4 (0.5-7.5)	4 ± 3,4 (0.5-8)	7
L-dopa use until complications (years)	10,7	4,6	5
Dyskinesias/fluctuations	4 (80%)	32 (50,8%)	yes

a. Unified Parkinson's Disease Rating Scale
b. Levodopa Equivalent Dose

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).

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 family history of Parkinsonism. The finding of none PARK2 mutation 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 assessment 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 described as part of the phenotype of Parkin disease [31]. The rigid-akinetic motor phenotype was prevalent in four of our five patients with PARK2 mutations including the two patients 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 mutation 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 previously 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 mutations are uncommon in this southern of Brazil series of patients with predominantly sporadic EOP.

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Conflict of Interest

The authors have no conflict of interest to report.

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