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ARTICLE

Frequency of the *LRRK2* G2019S mutation in late-onset sporadic patients with Parkinson's disease

Frequência da mutação G2019S do *LRRK2 em pacientes com doença de Parkinson esporádica e início tardio*

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ABSTRACT

Mutations in the *LRRK2* gene, predominantly G2019S, have been reported in individuals with autosomal dominant inheritance and sporadic Parkinson's disease (PD). The G2019S mutation has an age-dependent penetrance and evidence shows common ancestry. The clinical manifestations are indistinguishable from idiopathic PD. Its prevalence varies according to the population studied ranging from less than 0.1% in Asians to 41% in North African Arabs. This study aimed to identify G2019S mutation in Brazilian idiopathic PD patients. **Method:** We sampled 100 PD patients and 100 age- and gender-matched controls. Genetical analysis was accomplished by polymerase chain reaction (PCR). **Results:** No G2019S mutations were found in both patients with sporadic PD and controls. **Conclusions:** Our results may be explained by the relatively small sample size.

Keywords: Parkinson's disease, *LRRK2*, genetics.

RESUMO

Mutação no gene *LRRK2*, predominantemente G2019S, foi descrita em indivíduos com doença de Parkinson (DP) esporádica ou herança autossômica dominante. A penetrância da mutação varia com a idade e há evidências de ancestral comum. As manifestações clínicas são indistinguíveis da DP idiopática. Sua prevalência depende da população estudada e varia de 0,1% em asiáticos a 41% em árabes do norte africano. O objetivo desse estudo foi identificar a mutação G2019S em brasileiros com DP esporádica. **Método:** Foram testados 100 pacientes com DP e 100 controles pareados por idade e sexo. A análise genética foi realizada pela reação em cadeia por polimerização (PCR). **Resultados:** Não foi encontrada a mutação G2019S nem nos pacientes com DP nem nos controles. **Conclusão:** É possível que nossos resultados sejam devidos ao pequeno número de pacientes incluídos.

Palavras-chave: doença de Parkinson, *LRRK2*, genética.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease in individuals over 60 years of age[1]. PD ethiopathogenesis involves progressive neuronal loss in the substantia nigra of the midbrain leading to dopamine deficiency. The primary clinical manifestation of PD is characterized by resting tremor, bradykinesia, rigidity and postural instability[2,3]. The definitive diagnosis of PD is confirmed by the presence of Lewy bodies during post mortem pathological examination of the brain[4].

PD has a complex, multifactorial etiology[5]. Genetic factors play a major role although most cases are sporadic[6]. The familial forms occur in about 10-15% of patients with PD[7] and a small proportion is of Mendelian inheritance[8].

Mutations in the leucine-rich repeat kinase-2 (*LRRK2) gene*, predominantly G2019S mutation, have been reported in individuals with autosomal dominant familial PD[9,10]. The G2019S mutation has an age-dependent variable penetrance[11] and evidence shows common ancestry[12,13]. It is also found in patients with sporadic forms of PD[8] showing clinical manifestations indistinguishable from idiopathic PD.

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The G2019S mutation occurs in 1-2% of sporadic cases of PD in Caucasians[14,15]. However, the prevalence varies according to the population studied ranging from less than 0.1%[16] to 41% in North African Arabs[17,18].

Few studies have assessed specifically the frequency of the G2019S mutation in late-onset sporadic PD patients. Thus, this study aimed to identify the G2019S mutation in a sample of late-onset Brazilian patients with no known family history of PD.

METHOD

We recruited patients with PD from among those followed up at the neurology outpatient clinic of the Movement Disorders Clinic within Hospital das Clínicas of the *Faculdade de Medicina* of the *Universidade de São Paulo* (HC-FMUSP) and at the Brazilian Parkinson Association. We included 100 patients with PD who met the study inclusion criteria.

We recruited 100 age- and gender-matched individuals to the control group. They comprised of patient's spouses and not blood-related caregivers or other patients with no neurological disease attending outpatient clinics of other specialties within the hospital.

Inclusion criteria

The following eligibility criteria were applied to all individuals participating in the study: male or female patients with clinical diagnosis of PD following the diagnostic criteria established by The United Kingdom Parkinson's Disease Society Brain Research Centre[4]; onset of PD signs and/or symptoms after the age of 50; being able to understand the information conveyed in the free informed consent form approved by HC-FMUSP institutional review board (CAPPesq) and signing the consent form before entering the study.

Exclusion criteria

Individuals with known first-degree relatives with PD were ineligible to participate in the study.

Analysis of biological materials

We collected buccal swab samples with a cotton swab for genetic analysis. The samples of biological materials collected from oral mucosa were stored in standard plastic vials and kept refrigerated until they were sent to the Laboratório de Genética Molecular at the Instituto de Biociências of the *Universidade de São Paulo*. The samples were stored at -70 °C after DNA extraction. The G2019S mutation in the DNA extracted was analysed by polymerase chain reaction (PCR) followed by restriction enzyme digestion[19,20].

RESULTS

We examined 100 patients with PD (65 males and 35 females) and 100 controls (65 males and 35 females). The mean age of patients and controls was 73.3 (standard deviation [SD]=7.8) and 71.2 years (SD=8.5), respectively. The age at onset of PD symptoms was 64.0 years (SD=8.6). We used Student's *t*-test to compare the groups and verified that age followed a normal distribution. Most patients and controls were Brazilian-born, but Table shows that many had parents who came from other countries.

The genetic analysis of buccal swab samples to assess the frequency of the *LRRK2* G2019S mutation showed no mutations in both patients with PD and controls group.

DISCUSSION

According to Healy et al., 2008[21], the frequency of the mutation G2019S across the world is 4% in familial PD and 1% in sporadic PD cases. However, a higher frequency

Table. General characteristics of patients and controls.

	Patients		Total	Controls		Total
	Male: 65	Female: 35	100	Male: 65	Female: 35	100
Mean age (years)	72.6	74.9	73.3 (SD 7.8)	70.7	72.1	71.2 (SD 8.5)
Mean age at PD onset (years)	63.3	65.3	64.0 (SD 8.6)	N/A	N/A	N/A
Country of birth of each parent			71 Brazil			77 Brazil
			8 Japan			10 Italy
			5 Italy			3 Portugal
			3 Spain			3 Japan
			3 Portugal			2 Lebanon
			2 China			Syria
			1 Lebanon			1 Spain
			1 Africa/Italy			1 Argentina

PD: Parkinson's disease; N/A: not applicable; SD: standard deviation.

has been reported in North African individuals (36% in hereditary and 39% in sporadic PD cases) and Ashkenazi Jews (28% in hereditary and 10% in sporadic PD cases). This mutation rarely occurs in Asians.

In the light of the role of the G2019S mutation in the pathophysiology of PD evidenced in molecular studies, and considering that large waves of Portuguese, Spanish, Arab immigrants have settled in Brazil, epidemiological studies are important as they can help to determine the prevalence of the G2019S mutation in the Brazilian population.

In our study, we selected patients with idiopathic and sporadic PD with symptom onset after the age of 50. It is quite hard to describe their demographic specificities since our sampled patients and controls are reflection of Sao Paulo's population, which is composed of a conglomeration of a large number of intertwined ethnic communities. The fact that no mutations were found in this population is an interesting finding given that our sample comprised mostly of Brazilians-born of Italian, Spanish, Portuguese, Japanese, Arab and African ancestry. A multicenter study conducted by Healy et al.[21] found 4% frequency of the mutation in sporadic PD cases in Portuguese, 3% in Spanish and 2% in Italian individuals.

Earlier Brazilian studies investigating the G2019S mutation have selected familial PD patients with symptom onset before the age of 50 to increase the likelihood of identifying genetic cases. Barsottini et al.[22] examined 119 patients with PD symptom onset before the age of 50 and found a 3.36% frequency of the PARK8 gene. Camargos et al.[23] evaluated a sample of 202 patients with PD symptom onset before the age of 50 and found a familial case with mutation in the *LRRK2* gene. Abdalla-Carvalho at al.[24] studied 204 patients from different nationalities with PD symptom onset at different ages and found the G2019S mutation in three patients, and in all of them symptom onset was before the age of 50. Aguiar at al.[25] examined 72 patients with early onset symptoms and reported an incidence rate of 5.5% of the PARK8 gene. Munhoz et al.[26] studied 83 Brazilians with early PD onset and found an incidence rate of 3.5% of the G2019S mutation.

Our results may be explained by the relatively small sample size and selection bias. Another possibility could be due to a lower frequency of the G2019S mutation in the Brazilian population than the 1% estimated frequency of cases with sporadic PD worldwide. We do not have any comparative studies because the aforementioned studies did not evaluate patients with sporadic PD with symptom onset after the age of 50, they evaluated either familial cases or onset of PD before age of 50.

Another important consideration about our study is the approach of analyzing genetic materials obtained from buccal swab. The advantages of this method are easy sample collection, low sampling risk (no venipuncture required) and the procedure is not painful which increases the willing of the patients to participate in the study. Lastly, mouth lining scraping requires only a small amount of specimen to perform DNA analysis and have being utilized by some authors previously for the same purpose[18,27].

Our study examined a sample of 100 patients with sporadic PD with late symptom onset and 100 age- and gender matched controls for the presence of the G2019S mutation. No mutations were found in both patients and controls. This result can be attributed to the relatively small sample size and selection bias. Further studies with larger samples are needed to assess the frequency of this mutation.

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