

Alpha-synuclein A53T mutation is not frequent on a sample of Brazilian Parkinson's disease patients

Mutação A53T da alfa-sinucleína não é frequente em amostra de população brasileira com doença de Parkinson

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ABSTRACT

Introduction: The pathogenesis of Parkinson's disease (PD) involves both genetic susceptibility and environmental factors, with focus on the mutation in the *alpha-synuclein* gene (SNCA). **Objective:** To analyse the polymorphism SNCA-A53T in patients with familial PD (FPD) and sporadic PD (SPD). **Method:** A total of 294 individuals were studied, regardless of sex and with mixed ethnicity. The study group with 154 patients with PD, and the control group included 140 individuals without PD. The genotyping of SNCA-A53T was performed by PCR/RFLP. Significance level was $p < 0.05$. **Results:** Among all patients, 37 (24%) had FPD and 117 (75.9%) had SPD. The absence of SNCA-A53T mutation was observed in all individuals. **Conclusion:** SPD is notably observed in patients. However, the SNCA-A53T mutation was absent in all individuals, which does not differ controls from patients. This fact should be confirmed in a Brazilian study case with a more numerous and older population.

Keywords: Parkinson's disease, alpha-synuclein, mutation.

RESUMO

Introdução: A patogênese da doença de Parkinson (DP) envolve fatores ambientais e suscetibilidade genética, destacando-se a mutação de alfa-sinucleína (SNCA). **Objetivos:** Analisar a variante genética SNCA-A53T em pacientes com DP familiar (DPF) e DP esporádica (DPE). **Método:** Foram estudados 294 indivíduos, independente de sexo, com etnia miscigenada, sendo 154 com DP e 140 sem a doença (grupo controle). A genotipagem de SNCA-A53T foi realizada por PCR/RFLP. Nível de significância para $p < 0,05$. **Resultados:** Entre os pacientes, 37(24%) tinham DPF e 117 (75,9%) DPE. A ausência da mutação SNCA-A53T em todos os indivíduos. **Conclusão:** DPE é destacada entre os pacientes, no entanto a mutação SNCA-A53T ausente em todos os indivíduos, não diferenciando os grupo controle e pacientes, o que deve ser confirmado em população brasileira, considerando uma ampla casuística, além da ancestralidade.

Palavras-chave: doença de Parkinson, alfa sinucleína, mutação.

Parkinson's disease (PD) is one of the major degenerative disorders, affecting 2% of the population over 65 years and up to 4% in people over 85 years¹. In Brazil, statistics show that the incidence of PD amounts to 150/200 cases per 100,000 inhabitants². Most cases of PD are idiopathic³. However, in approximately 5%-10% of the cases, there is a genetic component with both dominant and recessive

inheritance patterns³. The chromosomal loci linked to the familial forms of PD are termed PARK1-13. These loci include six autosomal dominant genes [PARK 1 or PARK 4 (*alpha synuclein* - SNCA), PARK 3 (Lewy bodies), PARK 5 (*ubiquitin carboxyl-terminal esterase L1* - UCHL1), PARK 8 (*leucine-rich repeat kinase 2* - LRRK2) and PARK13 (*HTRA serine peptidase 2* - HTRA2)], 4 recessive genes [PARK 2

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Conflict of interest: There is no conflict of interest to declare.

Received 02 July 2014; Received in final form 15 January 2015; Accepted 04 February 2015.

(*parkin* - *PRKN*), PARK 6 (*PTEN-induced putative kinase 1* - *PINK1*), PARK 7 (*oncogene DJ1*), PARK 9 (*ATPase type 13A2* - *ATP13A2*) and PARK11 (*GIGYF2* - *Grb10 Interacting GYF Protein-2*), one gene linked to X chromosome (PARK12) and a form with unknown inheritance pattern (PARK10)^{4,5,6}. Mutations identified in five major genes were related with genetic etiology of familial PD, such as *a-synuclein*, which mediates autosomal dominant forms of the disease⁴.

The *SNCA* gene, located on human chromosome 4q21.3-q22, has 7 exons and encodes the α -synuclein protein of 140 amino acids, which is divided into three regions: N-terminal α -helical region (where mutations occur in the event of PD); central region (hydrophobic NAC sequence, known as non β -amyloid component), responsible for the formation of fibrils that cause toxicity; and the acidic C-terminal region⁵. The analysis of the sequence of exon 4 revealed that a single change of G \rightarrow A (Guanine \rightarrow Adenine) in the position 209 of the nucleotide chain causes the amino acid alanine to be replaced by the amino acid threonine at position 53 of the α -synuclein protein⁶.

The α -synuclein protein is the major protein component of intracellular neuron deposits observed in PD⁷. Although the function and the underlying dysregulation of *SNCA* mechanisms are not fully understood, the formation and expression of oligomers of this protein play a critical role in the development of the disease⁸. This wild-type α -synuclein protein is the major component of LB, in both familial and sporadic PD, which highlights the role of the *SNCA* gene variant in all presentations of the disease⁹. Functionally, relevant genetic variants in and near the *SNCA* gene are risk factors for sporadic PD (SPD). For example, the A53T mutation promotes greater aggregation and fibrillation in the LBs, and interferes with neurotransmission⁹, which confirms its role in the pathogenesis of the disease.

Therefore, this study aimed to analyse the frequency of the genetic variant *SNCA*-A53T in a Brazilian population with familial PD (FPD) or sporadic PD.

METHOD

A total of 294 individuals divided into two groups were studied: Study Group with 154 PD patients and Control Group formed by 140 subjects, who had not been diagnosed with PD. The study group included PD patients older than 50 years, regardless of ethnicity¹⁰ and sex. The patients were examined at the Outpatient Clinic for Movement Disorders of Hospital de Base, Medical School of São José do Rio Preto.

Diagnosis of PD followed the criteria recommended by Jankovic¹¹, including bradykinesia, rigidity, resting tremor, postural instability, unilateral onset, L-dopa response for

more than five years, levodopa-induced dyskinesia, progressive disorder, persistent asymmetry and clinical course of ten years or more, as well as complementary tests. The Control Group was selected in ambulatory clinics of that institution, namely, orthopedics, gynecology, among others. All subjects were informed about the study and confirmed their willingness to participate by signing an informed consent form. The study was approved by the Ethics Research Committee of institution.

The subjects were studied considering allele and genotypic frequencies for the *SNCA*-A53T mutation. Blood was collected by venepuncture with EDTA, and the genomic DNA was extracted from whole blood (5 mL) following the salting-out method¹². PCR amplification of DNA was performed in a thermocycler (Eppendorf - Mastercycler). Each tube included 0.5 mL of each deoxynucleotide (0.8 mM); 2.5 mL of 10^x PCR buffer; 2.5 mL of 10% dimethyl sulfoxide; 2.5 mL of each primer (2.5 mM); 0.2 mL of Taq polymerase (5 U/mL); 11 mL of Milli Q water; 2 mL of genomic DNA dilution (0.2 mg). For the PCR, the following primer sequences were used: (exon 4): P1- 5' GCT AAT CAG CAA TTT AAG GCT AG 3'; P2- 5' GAT ATG TTC TTA GAT GCT CAG 3'. The initial DNA denaturation was achieved at 94°C for 4 minutes. Next, the reaction mixture was subjected to 35 cycles of 94°C for 20 seconds and of 55 °C for 30 seconds, to an extension at 72°C for 45 seconds, and to a final cycle at 72°C for 10 minutes¹³. The post PCR product was digested with the restriction enzyme *Tsp45I* at 37°C for 4 hours and coloured by GelRed[®] (Uniscience). Electrophoresis was performed in 1% agarose gel at constant current of 100 V during 40 minutes, in order to separate fragments of 88pb, 128pb and 216pb in the presence of mutation (AG); 216pb for homozygous wild-type (GG) as well as 88pb and 128pb for homozygous mutant (AA). A sample of standard DNA (100bp Invitrogen) was used for comparison of the electrophoretic bands (Figure).

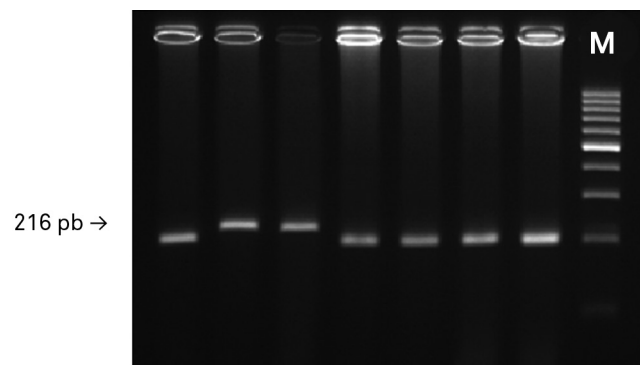


Figure. Diagram presents electrophoretic profile in 1% agarose gel for *alpha-synuclein* mutation, showing wild-type homozygous genotype (GG) on DNA fragments of 216 base pairs (bp). M = Molecular weight marker of 100 bp (Synapse Biotechnology).

The quantitative and qualitative analysis was performed by t-test and Chi-square test, respectively, with the use of the MiniTab14 and GraphPad3 software. The adopted level of significance was $p < 0.05$.

RESULTS

Table shows demographic data and history of PD patients. The mean age was 67.9 ± 11.5 years in the study group, and 51.0 ± 20.4 years in the control group ($p < 0.0001$). The study included only those patients with disease duration longer than 10 years.

There was a prevalence of males in the study group ($N = 95; 61.6\%$) compared with the control group ($N = 55; 39.2\%$; $p = 0.0002$). In the study group, 37 patients (24.0%) showed history of familial PD versus 117 patients (75.9%) with sporadic PD.

Patients were qualified as familial, when they had at least one relative of first degree diagnosed with PD, regardless of Mendelian pattern of inheritance. Sporadic cases included individuals without any family history of the disease in the past three generations.

The genome analysis of all patients and controls showed homozygous wild-type genotype (GG), which demonstrates the absence of *SNCA*-A53T mutation in both groups.

DISCUSSION

This study has not found any association of A53T mutation in the *SNCA* gene with Parkinson's disease. This corroborates the data presented by Moura et al.¹⁴, who conducted research on Brazilian populations and quantified the *SNCA* gene mutations without finding any alterations. *SNCA* multiplications have been implicated in autosomal dominant forms of PD since 2003¹⁵. However, it became clear that whole-gene multiplications in *SNCA* locus are a rare form of parkinsonism and may account for only a small fraction of the total number of PD patients^{16,17}.

Remarkably, further Brazilian studies found no *SNCA*-A53T mutation in PD patients^{14,18,19}. Furthermore, other genes related to PD, such as *APOE*, *GSTs* and *LRKK2* (*PARK8*), also showed no difference between patients and controls in this population^{20,21,22,23}. On the other hand, mutations in the genes *GIGYF* (*PARK11*), *ATP13A2* (*PARK9*) and *GBA* were associated with PD⁶.

Vaughan et al.¹³ evaluated 7 exons of *SNCA* gene in 30 European and American children with autosomal dominant PD, who did not show either A53T mutation or other mutations. Another study, also with PD patients, did not confirm the presence of this genetic variant in the *SNCA* gene²⁴. Moreover, Athanassiadou et al.²⁵ assessed 19 families with no biological relations, where at least two relatives of first/second degree were affected with PD. The authors

Table. Patients with Parkinson's disease (PD) and Controls, considering sex, age and family history for PD.

Variable	PD (N = 154)		Controls (N = 140)		p
Sex	N	%	N	%	
Female	59	38.3	85	60.7	0.0002*
Male	95	61.7	55	39.3	
Age (years)	M	SD	M	SD	pt
	67.9	11.5	51.0	20.4	< 0.0001†
Family History	N	%	N	%	
Yes	37	24.0	0	0	--
No	117	75.9	0	0	

N: Number of Individuals; M: Mean; SD: Standard Deviation; PD: patients; p: Fisher's Exact Test or Chi-Square Test (X2); pt: t-test; p: Significance level.

observed the presence of the mutation in several members of those 7 families, showing autosomal dominant inheritance pattern. Choi et al.²⁶ identified the A53T mutation in only 1 of 72 patients with early-onset PD, whose family history was consistent with autosomal dominant PD.

In this study, the selected population was of mixed ethnicity¹⁰, unlike the population in which the *SNCA*-A53T mutation was found²⁷. Furthermore, in this study, control subjects, without a family history of PD, were selected from other speciality clinics, which made it difficult to perform matching pair analysis on sex and age with the group of patients. This may be a limitation of this study. Elderly individuals (> 60 years) have been treated in the several speciality clinics of the hospital and showed other neurodegenerative diseases, which were considered as exclusion criteria for this study.

However, these discrepancies seem to have little relevance, considering the absence of said mutation in both groups, which seems to be different in the Brazilian population^{14,18,19}. Additionally, individuals remained in the control group, regardless of the matching pair analysis with patients, which enabled a larger number of individuals to perform comparative analysis.

Mutations in the *SNCA* gene enhance the production of alpha-synuclein protein, which contributes to the pathogenesis of PD²⁸. *SNCA* is expressed throughout the brain, specially in presynaptic nerve terminals, and *SNCA*-A53T rather tends to form aggregates there are critical to Lewy bodies formation, both familial and idiopathic PD. This aggregation of *SNCA* is thought to be a key occurrence in dopaminergic neuronal cell loss^{3,29}. The role of *SNCA* under normal physiological conditions is not yet completely clear, although there is evidence that implicates *SNCA* in neurotransmitter release and vesicle turnover at the presynaptic terminals³⁰.

However, this study conducted in a population of mixed ethnicity showed that both FPD and SPD are not associated with *SNCA*-A53T, which suggests the need for further evaluation and identification of larger and specific risk subgroups. Also, understanding the molecular mechanisms involved in its development will contribute to the application of molecular therapy interventions.

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ERRATUM

Arquivos de Neuro-Psiquiatria 2015;73(6):506-509.

In Page 506, the name of the authors where is written:

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