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LRRK2 p.G2019S mutation is not common among Alzheimer's disease patients in Brazil[1]

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Abstract. Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene have emerged as a potential common cause for both sporadic and familial Parkinson's Disease (PD) in different populations. The pleomorphic features exhibited by *LRRK2* mutation carriers and the central role of Lrrk2 protein in the proper functioning of central nervous system suggest that mutations in this protein might be involved in multiple cellular processes leading to other neurodegenerative disorders than PD. The location of *LRRK2* gene on chromosome 12, close to a linkage peak for familial late-onset Alzheimer's Disease (AD), highlights that *LRRK2* mutations might be involved in AD pathogenesis. We screened the most common *LRRK2* mutation (p.G2019S) in a series of 180 consecutive patients clinically diagnosed with Alzheimer Disease (AD). We identified the p.G2019S in one AD patient with no PD signs, indicating that this mutation is not a common etiological factor for AD in our population (0.5%), corroborating recent data found in Norwegian, North American, Chinese and Italian populations. Nevertheless, these observations together with new information about the Lrrk2 critical multifunctionality do not rule out the possible influence of other variants within *LRRK2* in AD, so that other screenings focusing in the whole extension of the *LRRK2* using larger sized confirmed AD sample are urgently needed.

Keywords: Alzheimer's disease, Leucine-rich repeat kinase 2, *LRRK2*, p.G2019S mutation, Parkinson's disease

1. Introduction

Leucine-rich repeat kinase 2 (*LRRK2*, *PARK8*, OMIM 609007) is a large gene encoding for a highly conserved and complex protein (Lrrk2), also known as dardarin, which belongs to the Roco family of the Ras/GTPase super family [23]. Although the function of dardarin is yet unclear, its predicted structure suggests that it is probably a cytoplasmic kinase capable of autophosphorylation, but it also associates with the mitochondrial outer membrane [21]. The presence of multiple protein interaction domains points that dardarin might also acts as a scaffold for assembly of a multiprotein signaling complex, although *LRRK2* physiological substrates have not been characterized yet [12].

Mutations in *LRRK2* gene have been identified in familial Parkisonism and sporadic late-onset Parkinson's disease (PD) [10,23]. Among the pathogenic functional domains mutations, the c.6055G>A transition in exon 41, resulting in p.G2019S in the kinase site, has been

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particularly characterized as a common cause of PD in patient cohorts of different ethnic origins [16,20].

Affected individuals with *LRRK2* mutations exhibit strikingly variable pathologic changes with different clinical presentations, including Lewy body PD, diffuse Lewy body disease, progressive supranuclear palsy, nigral degeneration without distinctive histopathology and other tauopathies [23]. The pleomorphic clinical and pathological features exhibited by *LRRK2* mutation carriers and the multifunctional role of Lrrk2 suggest that mutations in this protein might be involved in numerous cellular processes leading to other neurodegenerative disorders than PD. It is particularly relevant for Alzheimer's disease (AD), since *LRRK2* is located on chromosome 12q12, in a cryptic locus close to a linkage peak for familial late-onset AD [15]. Furthermore, concurrent AD-type pathology was detected in several individuals from autosomal-dominant PD kindreds segregating *LRRK2* mutations and one of them fulfilled tau neuropathologic criteria for AD [23]. Likewise, pathogenic *LRRK2* mutations appear to increase the tendency of dardarin to form inclusions and after the expression of the mutant protein neurons and neuronal cell lines undergo death [6]. Together, these findings lead to the hypothesis that an atypical kinase activity caused by *LRRK2* mutations could be the molecular interplay closely linking abnormal phosphorylation of α-synuclein and tau protein, which could contribute to aggregation of these misfolded proteins in degenerating neurons, leading to AD and/or PD, respectively [23].

In this study, we investigated the presence of the most common *LRRK2* mutation (p.G2019S) in patients with sporadic or familial late-onset, with the purpose of evaluating if this alteration is a common etiological factor for AD in our population.

2. Methods

A series of 180 consecutive unrelated patients (360 chromosomes) clinically diagnosed with probable AD according to NINCDS-ADRDA criteria were recruited. Mini Mental State Examination [3] and Clock Drawing Test [2] were used to assess cognitive functions, whereas laboratory blood assays and brain image analysis (CT or MRI) were performed to exclude secondary causes of dementia. Patients were recruited from Institute of Psychiatry of the Federal University of Rio de Janeiro (CDA-IPUB-UFRJ), Nucleus of Senior's Care of the State University of Rio de Janeiro (NAI-UERJ) and Faculty of Medicine of São José do Rio Preto. Ninety four percent of them had late-onset AD and the remaining expressed the disease before 60 years (early-onset). All probands live in Rio de Janeiro and São Paulo cities (Brazil) and the mean age and age at onset of AD were 77.06 ± 8.04 (51–94) and 72.05 ± 7.9 (48–89), respectively, comprising 69% of women. A positive family history of autosomal dominant dementia was reported in 28% of the cases. Except for eight European index cases (four Portuguese, two Spanish, one German and one Rumanian), all patients were Brazilian. Concerning ancestry, proband's families were mostly of Brazilian descent ($n = 151$), followed by Portuguese ($n = 12$), Spanish ($n = 4$), English ($n = 2$), German ($n = 2$), Swish ($n = 1$), Rumanian ($n = 1$), Uruguayan ($n = 1$) and mixed ancestry ($n = 6$). All subjects (or their representatives) completed the written informed consent, according to the research protocols approved by the institutional Ethics Committees.

3. Results and discussion

We identified the p.G2019S in a 65-years-old female (L.M.A.M.) with AD and no history of PD, who attended school for 12 years and experienced a first episode of depression in 2003. At the same occasion, she presented with mild aphasia symptoms, not being able to find the proper words to name the objects in front of her. She came to a first outpatient consultation at the Center for Alzheimer's disease of the Institute of Psychiatry /Federal University of Rio de Janeiro five years later presenting with a prominent aphasia together with mild apathy and apraxia. The clinical neurological examination showed persistent glabellar reflex and osteotendinous hyperreflexia. Her global cognitive status was found to be slightly impaired, with a Mini Mental State Exam [3] score of 25. A Computerized Brain Tomography in November/2007 was normal, although with a mild atrophy of the left parietal lobe. In April/2008, a Magnetic Resonance showed a diffuse volumetric decrease. The spectroscopy showed decreased levels of N-Acetyl-Aspartate in the hippocampi and higher levels of mio-inositol at the frontal lobes and at the posterior cingulate. Another neurological examination was performed in April/2009, again showing no changes regarding the previous ones, except for her MMSE score that was 21. The patient is now taking donepezil 10 mg/day, risperidone 0.5 mg bid, and sertraline 50 mg/day. The absence of symptons consistent to PD in the proband could be explained by usual incomplete penetrance of *LRRK2* mutations and variabil-

ity of onset ages, both within and between families [4]. Although the proband's relatives revealed no familial history of PD, two maternal aunts of her also suffered from AD. Segregation analysis of the p.G2019S mutation showed that the healthy patient's mother (85 years-old) didn't carry the mutation, which probably came from her Portuguese father, who died at age 78 years-old from esophageal cancer without signs of parkinsonism, although he didn't reach the maximum age of PD onset [5]. Therefore, as the proband is the only daughter of the couple, no additional segregation tests could be performed.

Our results indicate that the mutation p.G2019S does not appear to be frequent among AD patients in our population (0.5%), being consistent with data recently found in Norwegian, North American, Chinese and Italian populations [7,11,18,19,22]. Amongst PD patients, the frequency of p.G2019S mutation varies from 0 (Chinese) to 41% (North African Arabs) in different populations across the world [17]. In Brazil, our group recently showed an overall p.G2019S mutation frequency of $\sim$2% among sporadic and familial PD cases and no carriers of this mutation were found among 250 control chromosomes from healthy elderly individuals [16]. Gly2019 is contained in the N-terminus of the kinase activation segment located in the MAPKKK-related domain, in which phosphorylation of some conserved residues regulates kinase activity [14]. Nevertheless, the predicted effect of p.G2019S on Lrrk2 function is yet controversial. *In vitro* biochemical studies showed that p.G2019S did not have an effect on protein steady-state levels, localization or turnover, but kinase activity appears to be enhanced, consistent with a possible gain-of-function model [21]. On the other hand, structural analysis of Lrrk2 presumably supports that p.G2019S affects the proper positioning of the relevant C-helix of kinases and its role in contacting magnesium binding loop, impairing kinase activity [1].

Over the last twenty years, the identification of a number of genes related to neurodegeneration has provided great advances in understanding the role of molecular pathways involved in aging diseases. For AD, three highly penetrant genes linked to rare early-onset forms of familial cases (*APP*, *PSEN1*, *PSEN2*) and one susceptibility allele (*APOE* $\varepsilon 4$) involved in both early and late-onset forms were identified. Nevertheless, the *APOE* ε4 allele is neither necessary nor sufficient for the occurrence of the disease and mutations in the other three *loci* only explains a small fraction of familial AD cases, so that the genetic etiology of the most common AD form, representing sporadic late-onset cases, remains to be elucidated.

Although it is not completely obvious how mutations in dardarin could lead to neurodegeneration, recent evidences suggest that *LRRK2* gene is constitutively expressed in all central nervous system cell types, in addition to widespread tissues of the body [13] and toxic effects of the mutant protein might guide to formation of intraneuronal aggregations and cell death [6]. Furthermore, using immunocytochemistry techniques, Miklossy and colleagues [13] also found that Lrrk2 labels a subset of tau neurofibrillary tangles in AD and appears to be strongly associated with other pathological inclusions typical of several neurodegenerative disorders, such as those which occur in Parkinson disease, Pick disease, multiple system atrophy, Huntington disease and in amyotrophic lateral sclerosis. Finally, a recent study using a cellular model of neurodegeneration [9] demonstrated that *LRRK2* transduces cell death signals via Fas-associated protein with death domain and caspase-8, establishing a molecular link between *LRRK2* mutations and programmed cell death, that could be shared among different neurodegenerative diseases.

In conclusion, our data showed that, in Brazil, *LRRK2* p.G2019S mutation is not a common etiological factor among AD patients, albeit been prevalent among PD patients [16]. However, in light of recent findings, these results do not rule out the possible influence of other variants within *LRRK2* in AD. By this way, we consider of great relevance proceeding functional characterization of Lrrk2 mutations and further screening research in the whole extension of the *LRRK2* gene using clinically well-documented autopsied AD cases and larger sized sample.

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