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Lrrk2 mutations in South America:

A study of Chilean Parkinson's disease

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Abstract

Pathogenic substitutions in the leucine-rich repeat kinase 2 protein (Lrrk2), R1441G and G2019S, are a prevalent cause of autosomal dominant and sporadic Parkinson's disease in the Northern Spanish population. In this study we examined the frequency of these two substitutions in 166 Parkinson's disease patients and 153 controls from Chile, a population with Spanish/European-Amerindian admixture. Lrrk2 R1441G was not observed, however Lrrk2 G2019S was detected in one familial and four sporadic Parkinson's disease patients. These findings suggest Lrrk2 G2019S may play an important role in Parkinson's disease on the South American Continent and further studies are now warranted.

Keywords

LRRK2; Parkinson's disease; mutation, Amerindian

Amino acid substitutions in the leucine-rich repeat kinase 2 (Lrrk2) protein are frequent cause of parkinsonism in ethnically-defined populations [13,18,20]. Recently a common variant (Lrrk2 G2385R), observed only on the Asian continent, was identified as a 'risk-factor' for Parkinson's disease (PD)[4,5]. Similarly the Lrrk2 R1441G substitution has only been observed in the Basque region of Northern Spain and appears in 16% of familial and 4% of sporadic patients with PD [8,20]. Lrrk2 G2019S is also present in Spain and Portugal in as high as 6% of familial and 3% of sporadic PD patients [3,6,9,15], and is geographically widespread throughout Europe and North America [12]. Lrrk2 G2019S is most frequent in Northern Africa

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Arabs where it is found in up to 42% of familial and 41% of sporadic patients with PD [10, 11,13].

Given the influence of the Spanish Diaspora in South America we reasoned both Lrrk2 R1441G and G2019S may be prevalent causes of PD in this continent. Spanish conquistadores arrived in Chile in 1549 to an Amerindian population of approximately 3 million people. Today about 0.5 million indigenous Amerindians remain with the majority of the population composed of Spanish/European-Amerindian admixture. In Chile, allele frequencies for genetic markers are known to vary with more alleles of European origin in higher socioeconomic stratum, while native Amerindian alleles are more prevalent in the lower socioeconomic stratum [2,23].

Our sample originates from the Chilean capital, Santiago and consists of a total of 137 sporadic and 29 familial PD patients (93 female and 73 male; 69 ± 9 years of age) and 153 controls (104 female and 49 male; 61 ± 8 years of age). Interestingly, although PD has a higher prevalence in males, a higher proportion of females agreed to participate in this study than males, although no bias was shown towards female inclusion by the authors. Subjects were recruited through a patient's organization, ‘Parkinson's Liga’, and referred by participating neurologists of the Hospital Barros Luco Trudeau. All patients were specifically diagnosed by neurologists and confirmed as having idiopathic PD, presenting at least two of four cardinal signs: bradykinesia, rest tremor, rigidity, and postural reflex impairment group [7]. Exclusion criteria for a diagnosis of idiopathic PD disease were the use of medications (e.g. phenothiazines) during the 12 months preceding symptom onset; MRI or CT evidence of multiple cerebrovascular events prior to symptom onset; or evidence of another known cause of parkinsonism (e.g. history of brain tumor or encephalitis); or atypical PD presentation. Control subjects are healthy volunteers from the same geographic area and were examined by a neurologist to confirm the absence of any neurological disease. All subjects who participate in this study report Spanish-Amerindian ancestry. Spanish-Amerindian is the major ethnic group of the Chilean population and direct descendants of European genetic background were not included in this study.

Ethical approval of the research was obtained from the Ethical Committee of the Faculty of Medicine, University of Chile and all study subjects gave informed consent. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki (http://www.wma.net).

DNA was isolated from peripheral blood lymphocytes for all subjects, using the Genomic Prep DNA Isolation Kit (Amersham Pharmacia Biotech) following the manufacturer's instructions. DNA was genotyped for the exon 31 *LRRK2* 4321C>G (R1441G) and exon 416055G>A (G2019S) mutations using ABI “by-design” probes and analysis was performed using SDS 2.2.2 software on an ABI 7900 (Applied Biosystems, CA). For the assay, 1 μl of DNA was added to 2.5 μl of TaqMan Universal PCR Master Mix, 2.375 μl of water and 0.125 μl of probe; PCR amplification and single nucleotide polymorphism (SNP) genotyping was performed following the manufacturer's protocol (Applied Biosystems, CA). Positive and negative controls where included on all assay plates. Positive or ambiguous results were confirmed/ resolved with direct sequencing using the sense and anti-sense primers on an ABI3100, as previously reported [14]. Haplotype analysis employing both microsatellite and single nucleotide polymorphic markers was performed on the Lrrk2 G2019S carriers as previously reported [12,16].

The Lrrk2 G2019S substitution was found in 5 of 166 (3 %) patients with PD and in no control subjects, a frequency comparable to that observed in Spain and Portugal (3−6%) [3,6,9,15]. Unexpectedly this frequency is higher than observed in sporadic PD patients from Spain's neighboring European countries such as Italy (1%) [21]. It is also interesting that while present in both the North and South America continents, Lrrk2 G2019S appears to be rare/absent in

countries (e.g. India and China) on the Asian continent [19,22]. Epidemiologic studies find ~14% of patients with PD have one or more 1st degree relatives with parkinsonism [17].Twenty-nine of our 166 PD patients (17.5 %) reported a family history of parkinsonism and only one of these harbor a Lrrk2 G2019S substitution. The fact that only one of five Lrrk2 G2019S carriers have a family history of disease probably reflects the age-associated penetrance of the mutation [12]. The Lrrk2 G2019S proband reported has a sister and grandmother suffering with PD.

All Lrrk2 G2019S carriers presented with typical L-Dopa responsive PD with relatively mild symptoms and a slow progression; the Hoehn and Yahr staging remained between 2−3 even with relatively long disease duration (~20 years), which is consistent with past reports [1,11] (Table 1). One of the sporadic Spanish-Amerindian patients is homozygous for Lrrk2 G2019S possibly reflecting the inter-marriage of closely related families, and suggests the mutation may be more frequent in specific Spanish-Amerindian groups. As in past studies, heterozygous and homozygous carriers have comparable ages at onset and clinical presentations [11](Table 1). All the Lrrk2 G2019S carriers in this study were found to carry the common reported haplotype, designated by SNPs rs28903073-A and rs10878245-C alleles, that is proposed to have arisen in Tunisia (Table 2) [12,24].

The Lrrk2 R1441G substitution was not present in this sample from the Chilean population and this may indicate that this variant is restricted to the northern regions of Spain due to a more recent mutational event, or that the majority of conquistadores in Chile were from the south of Spain. Geographically Chile is located on the South-West coast of the South American Continent, for which our sample may not be representative (Figure 1). Santiago is in the center of Chile, a city of about 5−6 million inhabitants with a heterogeneous population and areas of marked European influence. However, all subjects in the present study were drawn from areas with low economic and educational attainment with considerable Spanish-Amerindian admixture [23].

The evidence of the common founder haplotype, as observed throughout North Africa, Europe (including Spain) and North America supports the hypothesis that this mutation was brought to the Chilean population by Spanish settlers. Given the frequency of Lrrk2 G2019S discovered, and the European/African founder haplotype, we recommend further studies of Lrrk2 variants in community-based samples from Chile and South America. Given population specificity of Lrrk2 substitutions complete gene sequence analysis in Amerindian patients with familial parkinsonism is warranted.

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Figure 1.
The continent of South America

Table 1

Clinical characteristics of Parkinson's disease patients with Lrrk2 G2019S

Patient	fPD-225	sPD-173	sPD-270	sPD-283	sPD-251
Mutation (Heterozygous)	G2019S	G2019S	G2019S	G2019S	
Mutation (Homozygous)					G2019S
Gender	Male	Female	Female	Female	Female
Ethnic background	Chilean/Amerindian	Chilean/Amerindian	Chilean/Amerindian	Chilean/Amerindian	Chilean/Amerindian
Family history	Yes	No	No	No	No
Age-at-onset (year)	54	50	73	65	65
Last examination age (year)	72	69	75	67	77
Disease duration	18	20	2	4	12
L-Dopa response	Yes	Yes	Yes	Yes	Yes
Drug induced dyskinesia	Yes	Yes	Yes	Yes	Yes
Hoehn and Yahr staging	3	3	2	3	3
UPDRS part III motor	18	15	10	29	25
Typical PD symptoms	Yes	Yes	Yes	Yes	Yes
Sign of dementia	No	No	No	No	No

Table 2

Chromosome 12q12 haplotype analysis of Chilean Parkinson's disease patients with Lrrk2 G2019S Chromosome 12 genotypes are shown for five Lrrk2 G2019S PD patients. *LRRK2* 6055 G>A (G2019S) is within exon 41 of the gene. The most parsimonious haplotype associated with disease, consistent with the consensus haplotype previously identified is in the right-hand column in bold. Marker positions (in base pairs) are from the UCSC database (March 2006; http://www.genome.ucsc.edu/). The haplotype is flanked by markers D12S2194 (Chr12q12: 38,738,008b; Allele '257' not shared by fPD-225) and D12S1701 (Chr12q12: 46,208,212b; homozygosity lost for sPD-251).

	Marker	Genomic Position	fPD-225	sPD-173	sPD-270	sPD-283	sPD-251 (homozygous)	Consensus sharing
	D12S2194	38,738,008	249/261	257/261	249/257	249/257	257/257	
	D12S2514	38,873,791	285/291	291/291	291/297	291/297	291/291	**291**
ex5	**rs10878245**	38,918,058	C/C	T/C	T/C	T/C	C/C	**C**
in t13	**rs28903073**	38,939,777	G/A	G/A	G/A	G/A	A/A	**A**
in t30	**D12S2516**	38,989,235	254/254	254/254	254/254	254/254	254/254	**254**
in t34	**rs11564205**	39,000,276	A/G	A/A	A/A	A/A	AA	**A**
ex41	**G2019S**	39,020,469	G/A	G/A	G/A	G/A	A/A	**A**
ex43	**rs10878405**	39,028,521	A/A	G/A	G/A	G/A	A/A	**A**
in t43	**rs11176143**	39,028,630	G/G	G/G	G/G	G/G	G/G	**G**
in t45	**D12S2518**	39,034,806	154/154	154/154	154/170	154/170	154/154	**154**
ex49	**rs3761863**	39,044,919	C/C	C/C	T/C	T/C	CC	**C**
	D12S2519	39,116,760	132/140	132/132	132/132	132/132	132/132	**132**
	D12S2520	39,120,028	260/260	257/260	251/260	251/260	260/260	**260**
	D12S2521	39,128,575	327/359	359/359	359/367	359/371	359/359	**359**
	D12S2522	39,132,267	287/297	297/297	297/299	297/299	297/297	**297**
	D12S2517	39,282,898	192/202	186/192	192/192	190/192	192/192	**192**
	D12S1048	39,312,654	211/214	211/214	214/223	214/226	214/214	**214**
	D12S1701	46,208,212	95/101	95/101	95/97	89/95	95/103	