Published in final edited form as:
Parkinsonism Relat Disord. 2011 September ; 17(8): 629–631. doi:10.1016/j.parkreldis.2011.05.003.

Lrrk2 p.Q1111H substitution and Parkinson's disease in Latin America

Ignacio F. Mata[1,2,*], **Greggory J. Wilhoite**[3], **Dora Yearout**[1,2], **Justin A. Bacon**[3], **Mario Cornejo**[4], **Pilar Mazzetti**[4], **Victoria Marca**[4], **Olimpio Ortega**[4], **Oscar Acosta**[5], **Carlos Cosentino**[6], **Luis Torres**[6], **Angel C. Medina**[7], **Carolina Perez-Pastene**[8], **Fernando Díaz-Grez**[8], **Carles Vilariño-Güell**[3,9], **Pablo Venegas**[10], **Marcelo Miranda**[10,11], **Osvaldo Trujillo-Godoy**[10], **Luis Layson**[12], **Rodrigo Avello**[13], **Elena Dieguez**[14], **Victor Raggio**[15], **Federico Micheli**[16], **Claudia Perandones**[16], **Victoria Alvarez**[17], **Juan Segura-Aguilar**[4], **Matthew J. Farrer**[3,9], **Cyrus P. Zabetian**[1,2], and **Owen A. Ross**[3]

[1]Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA

[2]Department of Neurology, University of Washington School of Medicine, Seattle, Washington, USA

[3]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, Florida, USA

[4]Neurogenetics Unit, Instituto Nacional de Ciencias Neurologicas, Lima, Peru

[5]School of Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru

[6]Movement disorders, Instituto Nacional de Ciencias Neurologicas, Lima, Peru

[7]Universidad Nacional del Altiplano, Puno, Peru

[8]Molecular and Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile

[9]Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada.

[10]Liga del Parkinson de Chile

[11]Clinica Las Condes, Santiago, Chile

[12]Hospital Barros Luco Trudeau, Chile

[13]Hospital Regional de Concepcion, Chile

[14]Department of Neurology, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay

[15]Department of Genetics, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay

[16]Department of Neurology, Hospital de Clinicas, Buenos Aires, Argentina

[*]Corresponding author: Geriatric Research Education and Clinical Center S-182 VA Puget Sound Health Care System 1660 South Columbian Way, Seattle, Washington 98108 Tel: (206)-277-6590, Fax: (206)- 764-2569 nachofm@u.washington.edu .

[17]Laboratorio de Genetica Molecular, Instituto de Investigacion Nefrologica (IRSINFRIAT), Hospital Universitario Central de Asturias, Oviedo, Spain

Abstract

Mutations in the *LRRK2* gene are the most common genetic cause of Parkinson's disease, with frequencies displaying a high degree of population-specificity. Although more than 100 coding substitutions have been identified, only seven have been proven to be highly penetrant pathogenic mutations. Studies however are lacking in non-white populations. Recently, Lrrk2 p.Q1111H (rs78365431) was identified in two affected Hispanic brothers and absent in 386 non-Hispanic white healthy controls. We therefore screened this variant in 1460 individuals (1150 PD patients and 310 healthy controls) from 4 Latin American countries (Peru, Chile, Uruguay and Argentina).

In our case-control series from Peru and Chile we observed an increased frequency of Lrrk2 p.Q1111H in patients (7.9%) compared to controls (5.4%) although the difference did not reach significance (OR 1.38; p=0.10).

In addition, the frequency of Lrrk2 p.Q1111H varied greatly between populations and further screening in a set of pure Amerindian and pure Spanish controls suggested that this variant likely originated in an Amerindian population. Further studies in other Latin American populations are warranted to assess its role as a risk factor for Parkinson's disease. Screening in Parkinson's disease patients from under-represented populations will increase our understanding of the role of *LRRK2* variants in disease risk worldwide.

Keywords

Parkinson Disease; Lrrk2; Latin America

Introduction

Genetic forms of Parkinson's disease (PD) have revolutionized the way we view this late-onset sporadic disorder. Mutations in five genes (*Parkin, Pink1, DJ-1, SNCA* and *LRRK2*) are sufficient to cause parkinsonism, however most are rare and found in patients with a family history or early-onset symptomatic presentation. The complexity of the more frequent sporadic late-onset PD suggests the combined effect of a number of common low penetrant genetic variations which increase the risk of developing the disease. To date, most *LRRK2* studies have examined populations of European or Asian origin, and have helped to determine the role of variants in PD susceptibility in these populations. Through these studies, it has become clear that the frequency of *LRRK2* variants may be population specific. For example, the prevalence of Lrrk2 p.G2019S varies greatly, accounting for approximately 1% of PD cases in populations of European origin and less than 1% in Asian populations, but increasing up to 41% in North Africans [1-2]. Also, the identification of PD risk factors Lrrk2 p.R1628P and p.G2385R in Asia shows the power of good ethnically-matched case-control studies [3-4].

In a comprehensive *LRRK2* sequencing study of all 51 exons, Nichols and colleagues found several putative pathogenic variants in patients from 88 multiplex families, mostly of European-origin [5]. A Glutamine to Histidine substitution (p.Q1111H; rs78365431), was identified in 2 Hispanic siblings; DNA was not available for any other family members and thus segregation analysis could not be performed. This Glutamine in position 1111 is highly conserved in most species, thus a change from this uncharged amino acid, to a positively charged Histidine could modify the leucine-rich repeat domain and have an impact in Lrrk2 activity. Since this variant was absent in 368 white healthy controls, the authors nominated

Lrrk2 p.Q1111H as potentially pathogenic. However, this finding has not been confirmed as several subsequent studies that screened the entire *LRRK2* coding region in PD case-control samples of European and Asian origin failed to detect this variant [2, 6-8].

Given that Lrrk2 p.Q1111H was identified in Hispanics siblings, and that this amino acid is highly conserved across most species, we sought to determine the frequency and pathogeneicity of this variant by screening samples from four countries in Latin America.

Samples and Methods

For this study, we screened a total of 1150 patients with PD from four different countries in Latin America (Table 1). The samples from Peru, Uruguay and Argentina were collected through the Latin American Research Consortium on the Genetics of PD (LARGE-PD). The Chilean sample originated from the capital, Santiago and was recruited independently. All patients met UK PD Society Brain Bank clinical diagnostic criteria. Healthy controls came from Peru (n=197) and Chile (N=162). Since admixture is very different in these populations, both patients and controls were asked about maternal and paternal ancestry. All subjects from Chile and almost all from Peru defined themselves as “mestizo”, a term that indicates a mixture of European (mostly Spanish) and Amerindian ancestry. On the other hand, the vast majority of subjects from Argentina and Uruguay reported exclusively European ancestry.

We also screened 49 controls of pure Amerindian ancestry, based on origin of both parents and all 4 grandparents, from Peru (Aymara, n=16; Quechua, n=25; Aymara and Quechua, n=8), and 250 controls from Spain to estimate the frequency of p.Q1111H in the parental populations.

Seventeen patients carrying known pathogenic genetic PD-variants in the *LRRK2* gene were excluded in this study (15 with p.G2019S and 2 with p.R1441G).

Ethical approval for the research was obtained from the Ethical Committee of each institution 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. DNA was genotyped for *LRRK2* c.3333G>T (p.Q1111H; rs78365431) by direct sequencing of exon 24 using the Applied Biosystems Big-Dye terminator v3.1 Cycle Sequencing Kit as previously reported or ABI TaqMan probes “by-design” (Applied Biosystems, Foster City, CA) [7]. Analysis was performed using Phred/Phrap (University of Washington Genome Sciences) or SeqScape (Applied Biosystems) for sequencing and SDS 2.2.2 software on an ABI 7900 for TaqMan. Positive and negative controls where included on all TaqMan assay plates. Positive and ambiguous results in the TaqMan assay were also confirmed with direct sequencing. Association of PD with p.Q1111H alleles was assessed by logistic regression analysis under an additive model, adjusting for gender, age and site.

Results

We screened a total of 1150 patients and identified 130 carriers, including 10 homozygotes, for the p.Q1111H variant. However the allele frequency distribution varied greatly across sites, from more than 10% in Peru to approximately 1% in Uruguay and Argentina (Table 2). The frequency in controls from Peru (7.4%) and Chile (3.4%) was slightly lower than that observed in patients from the same sites (10.3% and 4.6%); however this difference did not reach significance in either the Peruvian or Chilean series alone, or in the combined dataset (Table 2). In the combined Chilean and Peruvian sample, the odds ratio (OR) in

favor of PD under an additive model adjusted by age, sex and site was 1.38 (95% confidence interval [CI], 0.94-2.03; p =0.10).

Screening of controls from the parental populations showed that the allele frequency of p.Q1111H in pure Amerindians (18.4%) was nearly double that seen in the Peruvian case-control sample, while the variant was absent in controls from Spain (Table 2). The latter observation is consistent with our on-going studies of coding *LRRK2* variants in over 14,000 subjects from Caucasian and Asian populations that did not identify any carriers of p.Q1111H in these populations (data not shown).

Discussion

In the present study we have screened for the Lrrk2 p.Q1111H substitution in PD cohorts from four Latin American countries: Peru, Chile, Uruguay and Argentina. In contrast to the findings of Nichols and colleagues [9], our results suggest that p.Q1111H is not a pathogenic mutation, but rather a polymorphism that occurs primarily in populations with Amerindian admixture. Whether p.Q1111H is a risk variant for PD is not entirely clear. Though we did observe a higher frequency of the “H” allele in PD patients in both the Peruvian and Chilean samples, the difference did not reach significance. These findings highlight the need for ethnically-matched control subjects when nominating a variant identified through sequencing as putatively pathogenic.

We found that the frequency of p.Q1111H in controls was highest in “pure” Amerindians (18%), intermediate in “mestizos” from Peru and Chile (3-7%), and absent in controls from Spain. These data, combined with our unpublished results in which we failed to detect the variant in thousands of subjects of European origin strongly suggest that p.Q1111H originated in an Amerindian population. This is consistent with the low frequencies seen in the case series from Uruguay and Argentina where historically much less Amerindian admixture has occurred.

Differences in admixture proportion are also likely the source of variation reported in the frequency of another Lrrk2 variant (p.G2019S) across South America. Haplotype analyses indicate that G2019S was introduced into South American populations via European settlers [10-11]. Accordingly, G2019S mutation frequency and European admixture proportion parallel one another: both are higher in Uruguay and Argentina and lower in Peru and Chile [12].

We observed a trend toward a higher frequency of p.Q1111H in PD patients in our Peruvian and Chilean samples. This, together with the fact that this variant results in substitution of an uncharged for a charged amino acid in a highly conserved position within Lrrk2 suggests that the role of p.Q1111H as a PD risk factor merits further study in Latin America. Given the findings of the present study, future analyses will require larger case-control samples and careful matching for ancestry. Use of ancestry informative markers to test and account for population structure would also be useful.

To date, studies on the genetics of PD have largely focused on individuals of European origin. However, as demonstrated by the work presented here, a great deal of knowledge stands to be gained from analyses of non-European populations.

Establishing consortiums like LARGE-PD will help getting access to those understudied populations in Latin America.

Acknowledgments

This work was supported by the National Institutes of Health (R01NS065070, P50 NS062684, P50 NS072187), Department of Veterans Affairs (1I01BX000531), FONDECYT (# 1061083), Parkinson's Disease Foundation, Michael J. Fox Foundation, and the family of Carl and Susan Bolch. Dr Cornejo-Olivas is the recipient of an NIH Fogarty International Clinical Research Fellowship at Vanderbilt University (R24 TW007988).

References

[1]. Lesage S, Condroyer C, Lannuzel A, Lohmann E, Troiano A, Tison F, et al. Molecular analyses of the LRRK2 gene in European and North African autosomal dominant Parkinson's disease. J Med Genet. 2009; 46(7):458–64. [PubMed: 19357115]

[2]. Zabetian CP, Yamamoto M, Lopez AN, Ujike H, Mata IF, Izumi Y, et al. LRRK2 mutations and risk variants in Japanese patients with Parkinson's disease. Mov Disord. 2009; 24(7):1034–41. [PubMed: 19343804]

[3]. Ross OA, Wu YR, Lee MC, Funayama M, Chen ML, Soto AI, et al. Analysis of Lrrk2 R1628P as a risk factor for Parkinson's disease. Ann Neurol. 2008; 64(1):88–92. [PubMed: 18412265]

[4]. Di Fonzo A, Wu-Chou YH, Lu CS, van Doeselaar M, Simons EJ, Rohe CF, et al. A common missense variant in the LRRK2 gene, Gly2385Arg, associated with Parkinson's disease risk in Taiwan. Neurogenetics. 2006; 7(3):133–8. [PubMed: 16633828]

[5]. Nichols WC, Elsaesser VE, Pankratz N, Pauciulo MW, Marek DK, Halter CA, et al. LRRK2 mutation analysis in Parkinson disease families with evidence of linkage to PARK8. Neurology. 2007; 69(18):1737–44. [PubMed: 17804834]

[6]. Paisan-Ruiz C, Nath P, Washecka N, Gibbs JR, Singleton AB. Comprehensive analysis of LRRK2 in publicly available Parkinson's disease cases and neurologically normal controls. Hum Mutat. 2008; 29(4):485–90. [PubMed: 18213618]

[7]. Mata IF, Kachergus JM, Taylor JP, Lincoln S, Aasly J, Lynch T, et al. Lrrk2 pathogenic substitutions in Parkinson's disease. Neurogenetics. 2005; 6(4):171–7. [PubMed: 16172858]

[8]. Di Fonzo A, Tassorelli C, De Mari M, Chien HF, Ferreira J, Rohe CF, et al. Comprehensive analysis of the LRRK2 gene in sixty families with Parkinson's disease. Eur J Hum Genet. 2006; 14(3):322–31. [PubMed: 16333314]

[9]. Nichols WC, Pankratz N, Hernandez D, Paisan-Ruiz C, Jain S, Halter CA, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet. 2005; 365(9457): 410–2. [PubMed: 15680455]

[10]. Mata IF, Cosentino C, Marca V, Torres L, Mazzetti P, Ortega O, et al. LRRK2 mutations in patients with Parkinson's disease from Peru and Uruguay. Parkinsonism Relat Disord. 2009; 15(5):370–3. [PubMed: 18980856]

[11]. Zabetian CP, Hutter CM, Yearout D, Lopez AN, Factor SA, Griffith A, et al. LRRK2 G2019S in families with Parkinson disease who originated from Europe and the Middle East: evidence of two distinct founding events beginning two millennia ago. Am J Hum Genet. 2006; 79(4):752–8. [PubMed: 16960813]

[12]. Perez-Pastene C, Cobb SA, Diaz-Grez F, Hulihan MM, Miranda M, Venegas P, et al. Lrrk2 mutations in South America: A study of Chilean Parkinson's disease. Neurosci Lett. 2007; 422(3):193–7. [PubMed: 17614198]

Table 1

Patient characteristics for each series

Series	PD cases	Healthy controls
Peru	n=492	n=148
Age	62.4 ±11.6 (27-89)	53.5 ±12.4 (24-85)
Age at onset	62.0 ±12 (18-77)	---
Early-Onset (≤50 years old)	124 (25.2%)	---
Family History of PD	103 (20.9%)	---
Gender		
Male	270 (54.9%)	47 (31.8%)
Mestizo	486 (98.8%)	147 (99.3%)
Caucasian	2 (0.4%)	0
Other	4 (0.8%)	1 (0.7%)
Chilean	n=358	n=162
Age	71.8 ±8.8 (50-94)	67.6 ±8.8 (48-91)
Age at onset *	63.0 ±8.9 (46-86)	---
Early-Onset (≤50 years old)	32 (17.0%)	---
Family History of PD	82 (22.9%)	---
Gender		
Male	211 (58.9%)	84 (51.8%)
Mestizo	358 (100%)	162 (100%)
Caucasian	0	0
Other	0	0
Uruguay	n=205	---
Age	63.1 ±12.8 (18-85)	
Age at onset	56.0 ±13.4 (18-83)	
Early-Onset (≤50 years old)	63 (30.7%)	
Family History of PD	50 (24.4%)	
Gender		
Male	97 (47.3%)	
Mestizo	2 (1.0%)	
Caucasian	197 (96.1%)	
Other	6 (2.9%)	
Argentina	n=95	---
Age	62.2 ±11.2 (32-85)	
Age at onset	55.8 ±10.9 (29-83)	
Early-Onset (≤50 years old)	33 (34.7%)	
Family History of PD	25 (26.3%)	
Gender		
Male	56 (58.9%)	
Mestizo	0	
Caucasian	94 (98.9%)	

Series	PD cases	Healthy controls
Other	1 (1.1%)	

- The sample mean ± SD (minimum – maximum) is given for age and age at onset.

* Data missing for 170 individuals

Table 2

Allele and genotype frequencies of Lrrk2 p.Q1111H (rs78365431)

Series	Affection status	Samples No.	Genotype GG No. (%)	Genotype GT No. (%)	Genotype TT No. (%)	G allele No. (%)	T allele No. (%)	Odds ratio (95% CI)	p-value
Peru	Patient	492	400 (81.3)	83 (16.9)	9 (1.8)	883 (89.7)	101 (10.3)	1.38 (0.87-2.2)	0.17
	Control	148	128 (86.5)	18 (12.2)	2 (1.3)	274 (92.6)	22 (7.4)		
Chile	Patient	358	326 (91.0)	31 (8.7)	1 (0.3)	683 (95.4)	33 (4.6)	1.37 (0.69-2.76)	0.46
	Control	162	151 (93.2)	11 (6.8)	0	313 (96.6)	11 (3.4)		
Combined	Patient	850	726 (85.4)	114 (13.4)	10 (1.2)	1566 (92.1)	134 (7.9)	1.38 (0.94-2.03)	0.10
	Control	310	279 (90.0)	29 (9.4)	2 (0.6)	587 (94.6)	33 (5.4)		
Uruguay	Patient	205	201 (98.0)	4 (2.0)	0	406 (99.0)	4 (1.0)	-	-
Argentina	Patient	95	93 (97.9)	2 (2.1)	0	188 (98.9)	2 (1.1)	-	-
Spain	Controls	250	250 (100)	0	0	500 (100)	0	-	-
Peru (Amerindian)	Controls	49	34 (69.4)	12 (24.5)	3 (6.1)	80 (81.6)	18 (18.4)	-	-

Estimated odds ratios and p-values result from logistic regression models adjusted for age, sex, and series (combined series only). Associations between PD with p.Q1111H alleles were measured by chi-square statistics with Yates probability estimates and corresponding odds ratios (ORs) with confidence intervals (CIs). Total sample sizes given for each series do not account for genotyping failure, which occurred in <5% of samples.