Neurobiology of Aging 37 (2016) 210.e1–210.e5

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Analysis of the genetic variability in Parkinson's disease from Southern Spain

Sara Bandrés-Ciga [a], Niccolò Emmanuele Mencacci [b], Raquel Durán [a], Francisco Javier Barrero [c], Francisco Escamilla-Sevilla [d], Sarah Morgan [b], Jason Hehir [b], Francisco Vives [a], John Hardy [b], Alan M. Pittman [b,*]

[a] Department of Physiology, Institute of Neurosciences Federico Olóriz, Centro de Investigacion Biomedica (CIBM), University of Granada, Granada, Spain
[b] Department of Molecular Neuroscience, Reta Lila Weston Institute of Neurological Studies, UCL Institute of Neurology, London, UK
[c] Movement Disorders Unit, University Hospital San Cecilio, Granada, Spain
[d] Movement Disorders Unit, Department of Neurology, Instituto de Investigación Biosanitaria (IBS), University Hospital Virgen de las Nieves, Granada, Spain

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form 8 September 2015
Accepted 26 September 2015
Available online 8 October 2015

Keywords:
Parkinson's disease
Genetics
Mutations
Southern Spain population
Next-generation sequencing

ABSTRACT

To date, a large spectrum of genetic variants has been related to familial and sporadic Parkinson's disease (PD) in diverse populations worldwide. However, very little is known about the genetic landscape of PD in Southern Spain, despite its particular genetic landscape coming from multiple historical migrations. We included 134 PD patients in this study, of which 97 individuals were diagnosed with late-onset sporadic PD (LOPD), 28 with early-onset sporadic PD (EOPD), and 9 with familial PD (FPD). Genetic analysis was performed through a next-generation sequencing panel to screen 8 PD-related genes (*LRRK2*, *SNCA*, *PARKIN*, *PINK1*, *DJ-1*, *VPS35*, *GBA*, and *GCH1)* in EOPD and FPD groups and direct Sanger sequencing of *GBA* exons 8–11 and *LRRK2* exons 31 and 41 in the LOPD group. In the EOPD and FPD groups, we identified 11 known pathogenic mutations among 15 patients (40.5 %). *GBA* (E326K, N370S, D409H, L444P) mutations were identified in 7 patients (18.9 %); *LRRK2* (p.R1441G and p.G2019S) in 3 patients (8.1 %); biallelic *PARK2* mutations (p.N52fs, p.V56E, p.C212Y) in 4 cases (10.8%) and *PINK1* homozygous p.G309D in 1 patient (2.7 %). An EOPD patient carried a single *PARK2* heterozygous mutation (p.R402C), and another had a novel heterozygous mutation in *VPS35* (p.R32S), both of unknown significance. Moreover, pathogenic mutations in *GBA* (E326K, T369M, N370S, D409H, L444P) and *LRRK2* (p.R1441G and p.G2019S) were identified in 13 patients (13.4 %) and 4 patients (4.1 %), respectively, in the LOPD group. A large number of known pathogenic mutations related to PD have been identified. In particular, *GBA* and *LRRK2* mutations appear to be considerably frequent in our population, suggesting a strong Jewish influence. Further research is needed to study the contribution of the novel found mutation p.R32S in *VPS35* to the pathogenesis of PD.

1. Introduction

In the last decade, several loci and risk variants have been identified and linked to the pathology of familial and sporadic Parkinson's disease (PD) in diverse populations worldwide (Singleton et al., 2013). Given its geographical location on the southernmost region of Spain, the population from Granada is the result of a particular genetic landscape coming from multiple historical migrations and the settlement of different civilizations. Its complex history over the last millennia has involved the long-term residence of 2 very different populations with distinct geographical origins: North African Muslims and Sephardic Jews. Southern Spain represents a potential migration network and the major cross-link between Europe and Africa. These remarkable interactions across the Mediterranean Sea and the North of Africa have contributed to a genetic enrichment and might have shaped a unique genetic profile.

The long period of coexistence between North Africa and Southern Spain during the 8 centuries of the Islamic invasion suggests a marked genetic relationship. The Jewish presence has also been widespread and long-established in Granada, and admixture analysis indicates a substantial proportion of ancestry from

* Corresponding author at: Department of Molecular Neuroscience, Reta Lila Weston Institute of Neurological Studies, UCL Institute of Neurology, London WC1N 3BG, UK. Tel.: +44 020 7679 2000; fax: +44 705 347 1832.
E-mail address: a.pittman@ucl.ac.uk (A.M. Pittman).

0197-4580/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2015.09.020

Sephardic Jews sources (Adams et al., 2008). Moreover, Southern Spain has been subject to other important influences coming from eastern Mediterranean populations such as the Greek and the Phoenician colonization (Zalloua et al., 2008). However, despite its historical background, the population from Granada has been poorly studied, and it is still unknown which genetic variants contribute significantly to the development of PD.

Our study was to assess the contribution of known genes in a cohort diagnosed with either familial PD (FPD) or early-onset sporadic PD (EOPD) from Southern Spain, predominantly Granada and its area of influence. The genes of interest have been those traditionally associated with autosomal-dominant or -recessive forms and include *LRRK2*, *SNCA* and *VPS35*, *PARKIN*, *PINK1*, and *DJ-1*, respectively. Other risk genes recently linked to the disease such as *GBA* (Sidransky et al., 2009) and *GCH1* (Mencacci et al., 2014) have been also studied. Additionally, we screened for *LRRK2* and *GBA* common pathogenic mutations in a cohort of late-onset sporadic PD (LOPD).

2. Methods

2.1. Patients

We included a group of 134 PD patients, of which 97 were diagnosed with LOPD, 28 with EOPD, and 9 unrelated FPD, all treated at the Movement Disorders Unit of the Service of Neurology in the both Hospital Clínico San Cecilio and Hospital Virgen de las Nieves of Granada (Spain). PD was diagnosed at least by 2 experienced neurologists in the field of movement disorders following the criteria of the UK PD Society Brain Bank (Gibb and Lees, 1988). EOPD defined by an age of onset $\leq$50 years. Patients who had at least 1 first-degree PD affected relative were classified as familial. The study was approved by the local ethic committee, and written informed consent was taken from each participant.

2.2. Genetic analysis

Genomic DNA was isolated from peripheral blood leucocytes or saliva as manufacturer's protocols (QIAamp DNA Blood Midi Kit, QIAGEN; Oragene Kit, DNA Genotek). EOPD and FPD patients were screened for potential mutations using next-generation sequencing (NGS). We used an Illumina's Miseq with a polymerase chain reaction (PCR) amplicon-based (TruSeq Custom amplicon) target enrichment to screen for variants across the coding exons of the 8 PD genes listed previously. Probes were designed using Ilumina Truseq custom amplicon assay Design Studio v1.6 online (http://www.illumina.com/applications/designstudio.ilmn). The assay was performed according to the manufacturer's recommended protocol. Targeted exons with a coverage of less than 10 reads were subsequently screened by Sanger Sequencing. We excluded from the analysis variants with a minor allele frequency $\geq$1 % in general population according to 1000 Genome Project (http://www.1000genomes.org/).

LOPD patients were sequenced for *GBA* exons 8–11 and *LRRK2* exons 31, 41 and their flanking intronic sequences by Sanger sequencing because most pathogenic mutations are within these exons (Duran et al., 2013; Paisán-Ruiz et al., 2013). *GBA* allele names refer to the processed protein, excluding the 39-residue signal peptide.

The primers and PCR conditions we used are available on request. PCR products were bidirectional sequenced using the BigDye Terminator version 3.1 sequencing chemistry and then loaded on the ABI3730xl genetic analyzer (Applied Biosystems, Foster City, CA, USA).

PD cases that carried point mutations in *PARK2* and *PINK1* were screened for exon rearrangements through multiplex ligation-dependent probe amplification (MLPA) using the P051-C3 Salsa MLPA Parkinson probe set (MRC Holland, Amsterdam, the Netherlands). This set includes probes that detect exonic rearrangements in *PARK1* ([SNCA]; exons 2–7), *PARK2* ([PRKN]: exons 1–12), *PARK6* ([PINK1]; exons 1–8), *PARK7* ([DJ-1]; exons 1b, 3, 5, and 7) and *PARK9* ([ATP13A2]: exons 2 and 9), *PARK8* ([LRRK2]: exon 41). Data analysis was performed using Genemarker, version 2.6.2, software.

3. Results

3.1. Mutational screening

Demographic and clinical characteristics of the groups under study are summarized in Table 1. For the genes assessed by NGS in the FPD and EOPD group, a total of 11 known PD-related mutations were identified among 15 patients (40.5%, see Table 2). Interestingly, 1 EOPD patient carried a novel mutation in *VPS35* (p.R32S). Four different heterozygous *GBA* mutations were detected among 6 patients. Two sporadic (SP) cases with EOPD carried the mutation D409H and 1 familial case carried the L444P. The mutation N370S was found in 2 SP cases with EOPD and in 1 FPD case, and the E326K was identified in 1 SP case with EOPD. In *LRRK2*, we found 2 heterozygous pathogenic mutations in 3 patients. One familial and 1 SP case with EOPD carried the mutation p.G2019S. The mutation p.R1441G was identified in 1 FPD case. Moreover, 4 mutations were identified in *PARK2* among 5 patients. Three independent cases carried the frameshift deletion p.N52fs, 2 homozygous and 1 heterozygous. MLPA analysis revealed the presence of a *PARK2* deletion of exons 3 and 4 in the latter case. The heterozygous mutation p.R402C of uncertain significance was identified in 1 SP case with EOPD. However, no rearrangements were found in the single heterozygous *PARK2* mutation carrier. One FPD case carried the heterozygous point mutations p.V56E and p.C212Y simultaneously. The *PINK1* homozygous mutation p.G309D was identified in 1 FPD case.

The remaining 22 patients (59.5%) did not have any identifiable genetic risk variant. We found genetic variants related to the disease in 28.6 % of EOPD and 88 % of FPD cases. Four additional variants of unknown significance were detected in *LRRK2 (*p.I1371V, p.N2081D, p.M1646T, p.R1514Q) in 2 EOPD cases and 2 FPD cases, as well as 1 *DJ-1* variant (p.R98Q) in a familial PD. These variants are most likely benign as they are all reported at relatively high frequencies in the ExAc database (minor allele frequency >0.001). No mutation carriers were found for *SNCA* and *GCH1.*

In the LOPD group, we identified 6 variants in *GBA* and 2 in *LRRK2* among an overall of 17 patients (17.5%) (Table 2). The *GBA*

Table 1
Demographic and clinical characteristics of the groups under study

	SP		
	FPD	LOPD	EOPD
Age at onset (y)	46.3	65.8	49.0
Disease duration (mo)	150.8	74.0	97.8
Female:male	3:6	35:62	11:17
Disease severity (H&Y score) %			
I	26.7	29.3	11.2
II	26.7	47.6	50.4
III	26.7	19.5	30.1
IV	20.0	3.7	8.5

Key: EOPD, early-onset Parkinson's disease; FPD, familial Parkinson's disease; H&Y score, Hoehn & Yahr score; LOPD, late-onset Parkinson's disease; SP, sporadic Parkinson's disease.