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

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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

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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.

Table 2
Genetic PD-related variants identified in PD patients of Southern Spain

Gene	Exon	rsID	Variant	Nucleotide change	Zygosity	Patients		
						FPD	SP	
							EOPD	LOPD
GBA	10	rs1064651	D409H	c.1342G>C	Het	—	2	2
	11	rs35095275	L444P	c.1448T>C	Het	1	—	2
	10	rs76763715	N370S	c.1226A>G	Het	1	2	4
	9	rs2230288	E326K	c.1093G>A	Het	—	1	2
	8	—	T369M	c.1223T>C	Het	—	—	2
					Hom	—	—	1
LRRK2	41	rs34637584	p.G2019S	c.6055G>A	Het	1	1	3
	31	rs33939927	p.R1441G	c.4321C>G	Het	1	—	1
PARK2	2	N/A	p.N52fsX80	c.154delA	Hom	1	1	N/A
					Het[a]	1	—	N/A
	11	rs55830907	p.R402C	c.1204C>T	Het[b]	—	1	N/A
	2	rs137853059	p.V56E	c.167T>A	Het	1	—	N/A
	6	rs137853058	p.C212Y	c.635G>A	Het	1	—	N/A
PINK1	4	rs74315355	p.G309D	c.926G>A	Hom	1	—	N/A
VPS35	2	N/A	p.R32S[c]	c.96A>T	Het	—	1	N/A

Key: EOPD, early-onset Parkinson's disease; FPD, familial Parkinson's disease; Het, Heterozygous; Hom, Homozygous; LOPD, late-onset Parkinson's disease; N/A, not applicable because it has not been studied; PD, Parkinson's disease; SP, sporadic Parkinson's disease.
[a] PARK2 heterozygous deletion of exons 3 and 4.
[b] Single heterozygous state.
[c] Novel mutation.

heterozygous mutation D409H was found in 2 patients, the heterozygous mutations L444P and E326K were identified simultaneously in 2 patients, the heterozygous mutation N370S was detected in 4 patients, and the variant T369M was found in 3 patients (2 heterozygous and 1 in the homozygous state). The remaining 80 patients (82.5 %) did not show any genetic cause. Frequencies of pathogenic mutations in the genes under study are documented for each group (Table 3).

3.2. In silico *analysis of the novel VPS35 mutation*

Because the novel variant *VPS35* p.R32S raises further questions as to the structural effect, protein stability and malfunction on the vacuolar protein sorting-associated protein 35, we performed an *in silico* analysis using the *SIFT* bioinformatics tool (Kumar et al., 2009) and the *HOPE* Web server (Venselaar et al., 2011). The mutant amino acid was predicted to be damaging (Supplementary data).

Table 3
Allele frequency of found mutations in PD patients from Southern Spain and European controls compiled from ExAc database

Gene	EOPD	FPD	LOPD	State	Controls
GBA					
D409H	0.07	—	0.02	Het	1.0×10^{-4}
L444P	—	0.11	0.02	Het	U
N370S	0.07	0.11	0.04	Het	3.0×10^{-3}
E326K + L444P	—	—	0.02	Het	U
E325K	0.03	—	—	Het	1.2×10^{-2}
T369M	—	—	0.03	Hom/Het	9.0×10^{-3}
LRRK2					
p.G2019S	0.03	0.11	0.03	Het	9.0×10^{-3}
p.R1441G	—	0.11	0.01	Het	U
PARK2					
p.N52fsX80	0.03	0.11	N/A	Hom/Het	U
p.R402C	0.04	—	N/A	Het	2.0×10^{-3}
p.V56E	—	0.11	N/A	Het	2.9×10^{-5}
p.C212Y	—	0.11	N/A	Het	1.5×10^{-5}
PINK1					
p.G309D	—	0.11	N/A	Het	U
VPS35					
p.R32S	0.04	—	N/A	Het	—

Key: EOPD, early-onset Parkinson's disease; FPD, familial Parkinson's disease; Het, Heterozygous; Hom, Homozygous; LOPD, late-onset Parkinson's disease; N/A, not applicable because it has not been studied; U, unknown.

4. Discussion

So far, there have been a very limited number of genetic studies on PD in Southern Spain population (Gao et al., 2009; Gómez-Garre et al., 2014). This is the first report aimed to study in depth the genetic contribution of PD genes in familial or EOPD cases, as well as to explore the influence of *GBA* and *LRRK2* mutations on LOPD in the Southern Spanish population. Although we are aware that the sample size is relatively small, it comprised most PD patients from the province of Granada.

As part of our effort to genetically characterize PD in this population, we used the efficiency of NGS to investigate the frequency of potentially pathogenic mutations of 8 known PD genes in EOPD and FPD cases. Our approach produced highly accurate sequence data, and we identified several genetic variants linked to the disease.

Not surprisingly, we found that p.G2019S is a common *LRRK2* mutation amongst PD patients from Granada, with a frequency of 3.7 %. Although p.G2019S is responsible of 0.5%–4% of idiopathic PD cases amongst Caucasians, its prevalence has been found much higher amongst Ashkenazi Jews and North African Arabs (Healy et al., 2008; Lesage et al., 2006). However, the frequency of p.G2019S-carriers in PD cases from Granada suggests that there is not a strong influence from North Africa to our genetic landscape, as previously reported in a neighboring province (Gao et al., 2009).

Additionally, the *LRRK2* mutation p.R1441G found in our PD cohort with a frequency of 11.1% in the familial group and 1 % in the LOPD group was originally identified in both autosomal-dominant familial (46%) and idiopathic (2.5%) cases of PD, in the Basque region of northern Spain(Gorostidi et al., 2009; Paisán-Ruíz et al., 2004). The p.R1441G mutation is also identified at lower frequencies in patients from other Spanish provinces (between 0.7% in the East and 2.2% in the North), but it is very rare outside Northern Spain. In addition, we identified several *LRRK2* variants of unknown significance or not considered as disease causing variants.

The most prevalent genetic risk factor in PD identified up to date is heterozygous loss-of-function variants in *GBA1* (Sidransky et al., 2009). Interestingly, our PD cohort yielded 17 carriers (12.7%) of previously described pathogenic mutations. The frequency rate of *GBA* mutations varies considerably depending on the population ethnicity, with a remarkably high frequency in individuals of Ashkenazi Jewish descent. Our results show a significant higher frequency of *GBA* mutations in Southern Spain population in comparison to another study carried out in the Spanish population which found *GBA* mutations with a frequency of 9.8 % (Setó-Salvia et al., 2012). Previous studies in other European populations reported lower frequencies too; 6.4 % in Greeks (Kalinderi et al., 2009), 4.2 % in British (Neumann et al., 2009), and 8.3 % in Portuguese population (Bras et al., 2009).

The most common *GBA* variant found was N370S, encountered in 7 patients (5.2%). However, this is believed to be a rare risk factor for PD in North African Berber population (Nishioka et al., 2010). D409H, our second most prevalent *GBA* mutation, was detected in 4 patients. Additionally, we found the E326K mutation in 3 patients, 2 of which carried simultaneously the L444P variant (mutation phase unknown).

The present study ascertains that mutations in *GBA* contribute substantially to FPD and SPD in Andalusian population. As we screened in LOPD cases only selected *LRRK2* and *GBA* exons (mutation hotspots), some mutant alleles may have been missed.

Of particular interest is *PARK2*, which is the most frequently mutated gene in autosomal-recessive EOPD, with mutations found in 10%–20% of early-onset familial cases (Periquet et al., 2003). However, their prevalence and involvement in the modulation of PD risk have a wide variation depending on the studied population and the age of the subjects under study (Bardien et al., 2009; Bras et al., 2008; Schlitter et al., 2006). Our *PARK2* mutational spectrum included the homozygous deletion p.N52fs responsible for a recessive EOPD and an FPD case with a frequency of 5.4 %. The heterozygous deletion p.N52fs was found to cause FPD in 2.7 % of our cohort. Moreover, MLPA revealed heterozygous deletions in exons 3 and 4 of *PARK2*, which were also present in the affected brother. Furthermore, we found the previously reported homozygous variant p.G309D in *PINK1* in 1 FPD case(Valente et al., 2004).

Mutations in *VPS35* have been described as responsible of autosomal-dominant PD in late-onset familial cases. One of our most interesting findings is the presence of a novel mutation (p.R32S) which has not been reported in public single nucleotide polymorphism databases including ExAc, EVS, and 1000 Genome project. It was found in 1 EOPD case without a positive familial history for PD. Although it has been predicted as damaging, we cannot assume this mutation may be causative because we could not screen it in matched controls and we could not test segregation of the mutation in other family members.

5. Conclusions

We identified pathogenic mutations in 22.4 % of our PD population. *GBA* mutations appear to be considerably frequent, and it might reveal a strong influence from the Jewish population. In concordance with a previous study, *LRRK2* genetic variants in Andalusian individuals are common causes of PD. Further studies should be necessary to evaluate the possible pathogenicity of the novel found mutation p.R32S in *VPS35*. We found *SNCA* and *GCH1* mutations to be rare causes of the disease in our cases. Finally, we suggest that taken as a whole, these findings have clinical implications, showing that genetic screening may aid the diagnosis of PD in this population.

Disclosure statement

The authors have no financial disclosure to report. No pharmaceutical entity has collaborated in this study, and no financial purpose exists.

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Appendix A. Supplementary data

Supplementary data related to this article can be found in the online version at http://dx.doi.org/10.1016/j.neurobiolaging.2015.09.020.

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