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Pooled-DNA target sequencing of Parkinson genes reveals novel phenotypic associations in Spanish population

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ABSTRACT

Eighteen *loci* and several susceptibility genes have been related to Parkinson's disease (PD). However, most studies focus on single genes in small PD series. Our aim was to establish the genetic background of a large Spanish PD sample. Pooled-DNA target sequencing of 7 major PD genes (*SNCA*, *PARK2*, *PINK1*, *DJ-1*, *LRRK2*, *GBA*, and *MAPT*) was performed in 562 PD cases. Forty-four variants were found among 114 individuals (20.28%, $p<0.05$). Among these variants, 30 were found in Mendelian genes (68.18%) and 14 in PD susceptibility genes (31.82%). Seven novel variants were identified. Interestingly, most variants were found in *PARK2* and *PINK1* genes, whereas *SNCA* and *DJ-1* variants were rare. Validated variants were also genotyped in Spanish healthy controls (n = 597). Carriers of heterozygous *PARK2* variants presented earlier disease onset and showed dementia more frequently. PD subjects carrying 2 variants at different genes (1.42%) had an earlier age of onset and a predominantly akinetic-rigid PD phenotype (55.6%, $p < 0.05$), suggesting that the accumulation of genetic risk variants could modify PD phenotype.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder with a prevalence of 1.8% over the age of 65 years. Mendelian forms of PD have been associated with mutations in *α-synuclein* (*SNCA*; MIM# 163890), *parkin* (*PARK2*; MIM# 602544), *leucine-rich repeat kinase 2* (*LRRK2*; MIM# 609007), *PTEN-induced putative kinase 1* (*PINK1*; MIM# 608309), and *DJ-1* genes (*PARK7*; MIM# 602533) (Singleton et al., 2013). Despite the number of mutational studies in PD, very few large multigene PD screening studies are available (Benitez

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et al., 2016). Hence, a systematic approach sequencing major PD genes in a large PD sample would improve the knowledge and estimation of allele frequencies of genetic variants involved in PD. In addition, genome-wide association studies reported the association of *SNCA*, *LRRK2*, *microtubule-associated protein tau* (*MAPT*; MIM#157140), and *glucocerebrosidase beta acid* (*GBA*; MIM# 606463) variants with PD risk (Simon-Sanchez et al., 2009; Nalls et al., 2014). Therefore, we hypothesized that the accumulation of rare variants at genes mapped by genome-wide association studies can explain the increased risk of PD. Our aim was to study the genetic background of a large PD sample (n = 612), among which 562 subjects were analyzed through pooled next-generation sequencing of 7 PD-related genes (*SNCA*, *PARK2*, *PINK1*, *DJ1*, *LRRK2*, *GBA*, and *MAPT*), and search for phenotypic associations.

2. Methods

2.1. Subjects

The sample included 612 PD patients diagnosed according to standard criteria (Table 1; Hughes et al., 1992). Clinical assessment and selection criteria of the candidates are reflected in the Supplementary material. Written informed consent was obtained from participant subjects or relatives, and the study was approved by the Ethics Committee of the University of Navarra, Spain.

2.2. Initial screening and pooled sequencing analysis

Both subjects with sporadic PD and one index case from each PD family were first screened for the p.G2019S, p.R1441G, p.R1441C, and p.S1761R *LRRK2* substitutions (Lorenzo-Betancor et al., 2012). In addition, all cases with an age of onset (AAO) ≤50 years (Samaranch et al., 2010) and patients with single *PARK2* and *PINK1* heterozygous coding mutations underwent analysis of *SNCA*, *PARK2*, and *PINK1* rearrangements. The remaining 562 PD subjects not carrying these mutations underwent pooled-DNA sequencing (Supplementary material).

2.3. Genotyping and statistical analysis

All missense, nonsense, frameshift and splice-site variants discovered in the pooled-DNA sequencing with a minor allele frequency less than 1% and a *p*-value below SPLINTER's cutoff were further genotyped using Sequenom, KASPar, or Taqman in all PD samples from the corresponding pool (Supplementary material). Sequence variants were also genotyped in Spanish healthy controls (n = 597; Supplementary material).

3. Results

Initial screening for *LRRK2* substitutions revealed 26 p.G2019S carriers (4.25%), 13 p.R1441G carriers (2.12%), and 3 p.S1761R carriers (0.49%). *PARK2* exon rearrangements were found in 8 individuals (1.31%). Pooled-DNA sequencing of *SNCA*, *PARK2*, *PINK1*, *DJ1*, *LRRK2*, *GBA*, and *MAPT* genes was performed in the remaining 562 patients. Forty-four variants were validated with direct genotyping. These 44 variants were found in 114 of 562 PD individuals (20.28%). Among these 114 individuals, 100 carried at least 1 variant (87.72%), 13 carried 2 variants (11.40%), and only 1 carried 3 variants (0.88%). Among the 44 variants, 7 were novel (15.91%) and 37 had been previously identified (84.09%), either as pathogenic (n = 18, 40.91%), pathogenicity unclear (n = 11, 25%), or with unknown clinical significance (n = 8, 18.18%) (Table 2). Thirty variants (68.18%) were found in Mendelian PD genes (Singleton et al., 2013). Among these, 5 were novel (16.67%) and 25 known (83.33%). The remaining 14 variants (31.82%) were found in PD susceptibility genes (Simon-Sanchez et al., 2009; Nalls et al., 2014). Among these, 2 were novel (14.29%) and the other 12 had been previously described (85.71%).

Among the 6 *PINK1* missense variants with unknown or unclear significance, 3 were novel variants (p.C166R, p.G411C, and p.R501L) and the family with compound *PINK1* mutations already described (Samaranch et al., 2010) (Table 2). All *PINK1* variant carriers (n = 12, 2.14%) were heterozygous. There were no significant differences in the frequency of *PINK1* variants between our PD cohort and the Spanish control population (Table 2) or clinical differences between *PINK1* carriers and patients without mutations (n = 446) (Table 3).

Thirteen *PARK2* variants were validated including 8 pathogenic and 1 novel heterozygous variant of unknown pathogenicity (p.Y147C) (Table 2). Homozygous (n = 4, 17.39%), heterozygous (n = 11, 47.83%) and compound heterozygous (n = 8, 34.78%) PARKIN mutations were found in 23 patients. Three of them showed gene rearrangements (Supplementary Table 3). Allele frequencies of *PARK2* variants did not differ significantly between patients and control subjects (Table 2).

Patients with heterozygous *PARK2* variants had an earlier disease onset and more frequently dementia than individuals with no mutations ($p < 0.05$). In addition, all PD patients with compound heterozygous variants in *PARK2* (n = 8) presented an earlier AAO and more often had a family history and motor complications than the nonmutation subgroup ($p < 0.05$; Table 3).

Table 1
Demographic data of the PD cohort and healthy subjects

Parameter/PD phenotype	Whole-PD group (n = 612)	TD-PD (n = 46)	AR-PD (n = 143)	C-PD (n = 341)	Unknown[a] (n = 82)	Controls (n = 597)
Female (%)	40.2	30.43	44.06	37.24	51.22	62.81
Age [mean ± SD (range)] (y)	67.54 ± 10.75 (22–100)	67.48 ± 9.57 (39–86)	66.40 ± 11.17 (22–88)	67.96 ± 9.87 (29–90)	67.89 ± 14.20 (29–100)	65.68 ± 11.21 (23–92)
AAO [mean ± SD (range)] (y)	57.87 ± 12.24 (16–84)	59.13 ± 10.68 (25–78)	57.43 ± 12.04 (17–84)	58.59 ± 10.95 (17–84)	54.81 ± 17.41 (16–83)	-
Disease duration [mean ± SD (range)] (y)	9.47 ± 7.33 (0–51)	8.35 ± 6.19 (1–25)	9.01 ± 6.71 (0–29)	9.36 ± 6.83 (0–41)	11.68 ± 10.64 (0–51)	-
Diagnosis						
sLOPD (%)	69.28	63.64	71.11	73.48	40.00	-
fLOPD (%)	23.37	34.09	22.22	21.65	40.00	-
sEOPD (%)	3.76	0	5.19	1.83	6.15	-
fEOPD (%)	3.59	2.27	1.48	3.05	13.85	-
Dementia (%)	24.10	29.73	27.86	22.33	NA	-

Key: AAO, age at onset; AR-PD, akinetic-rigid PD; C-PD, classical PD phenotype; fEOPD, familial early-onset PD; fLOPD, familial late-onset PD; NA, data not available; PD, Parkinson's disease; SD, standard deviation; sEOPD, sporadic early-onset PD; sLOPD, sporadic late-onset PD; TD-PD, tremor-dominant PD.
[a] PD individuals with unknown phenotype.