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## Genetic and phenotypic basis of autosomal dominant Parkinson's disease in a large multi-center cohort

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1 **Genetic and phenotypic basis of autosomal dominant Parkinson's disease in a**  
 2 **large multi-center cohort**

3  
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12

13 Short running title: Characterization of dominant Parkinson's disease

14

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## 1 Abstract

2 *LRRK2*, *SNCA* and *VPS35* are unequivocally associated with autosomal dominant Parkinson's disease  
3 (PD). We evaluated the prevalence of *LRRK2*, *SNCA* and *VPS35* mutations and associated clinical  
4 features in a large French multi-center cohort of PD patients. Demographic and clinical data were  
5 collected for 1805 index cases (592 with autosomal dominant inheritance and 1213 isolated cases)  
6 since 1990. All probands were screened with TaqMan assays for *LRRK2* Gly2019Ser. In the absence  
7 of this mutation, the coding sequences of the three genes were analyzed by Sanger sequencing and/or  
8 next-generation sequencing. The data for the three genes were analyzed according to age at onset,  
9 family history, ethnic origin and clinical features. We identified 160 index cases (8.9%) with known  
10 pathogenic variants: 138 with pathogenic *LRRK2* variants (7.6%), including 136 with the Gly2019Ser  
11 mutation, 19 with *SNCA* point mutations or genomic rearrangements (1.1%), and three with the  
12 *VPS35* Asp620Asn mutation (0.16%). Mutation frequencies were higher in familial than isolated  
13 cases, consistent with autosomal dominant inheritance (12.0% vs. 7.3%; OR 1.7, 95% CI [1.2-2.4],  $p$   
14 = 0.001). PD patients with *LRRK2* variants were more likely to have higher rates of late-onset PD (>  
15 50 years; OR 1.5, 95% CI [1.0-2.1],  $p$  = 0.03), whereas those with *SNCA* mutations tended to have  
16 earlier age at onset disease ( $\leq$  50 years,  $p$  = 0.06). The clinical features of *LRRK2* carriers and those  
17 without any pathogenic variants in known PD-associated genes were similar. The likelihood of  
18 detecting disease-causing mutations was higher in cases compatible with autosomal dominant  
19 inheritance.

20

## 21 Contribution to the field

22 Two decades after the identification of *SNCA* as the first causal gene of Parkinson's disease (PD) and  
23 the realization that genetic factors play a substantial role in PD pathogenesis, our knowledge of the  
24 genetic architecture underlying this disease has increased considerably, with numerous genes and  
25 genetic loci now known to cause familial forms of PD or to influence the risk of PD. This study of a  
26 large cohort of genotyped and extensively phenotyped patients, including more than 190 mutation  
27 carriers, provides information about the frequency of the most three relevant genes known to cause  
28 autosomal dominant PD, by age at onset, family history, ethnicity, and associated phenotype. These  
29 findings are critical for guiding genetic testing in clinical practice and identification of patients for  
30 gene-targeting therapeutic trials.

31

32 **Keywords:** Parkinson's disease; *LRRK2*; G2019S; *SNCA*, *VPS35*; Autosomal dominant inheritance;  
33 Genotype-Phenotype correlations

34

35 **Abbreviations:** AAO, age at onset; AD, autosomal dominant; *LRRK2*, leucine-rich repeat kinase 2;  
36 MMSE, mini mental state examination; PD, Parkinson's disease; *VPS35*, vacuolar protein sorting 35

37

38

## 1 INTRODUCTION

2 Heterozygous sequence variants of *LRRK2* or *VPS35*, and mutations or genomic rearrangements in  
3 *SNCA* cause monogenic Parkinson's disease (PD) with autosomal dominant (AD) inheritance  
4 [reviewed in (1)]. Only seven of the hundred or so variants of *LRRK2* reported to date (Asn1437His,  
5 Arg1441Gly/Cys/His, Tyr1699Cys, Gly2019Ser, and Ile2020Thr), which appear to be clustered in  
6 functionally important regions highly conserved throughout evolution, have been demonstrated to be  
7 pathogenic on the basis of co-segregation with the disease and absence or rarity in specific control  
8 populations (2). The most common of these mutations, *LRRK2* Gly2019Ser, has a reported frequency  
9 of 0% to above 40%, depending on the population considered (3), mostly due to a common founder  
10 effect (4). *SNCA* mutations are the second most common cause of autosomal dominant inherited PD;  
11 genomic duplications have been detected in ~1-2% of families with AD PD. Other *SNCA* mutations,  
12 such as whole-locus triplications and a few missense mutations (Ala53Thr/Glu/Val, Glu46Lys,  
13 Ala30Pro, Gly51Asp), are extremely rare [reviewed in (1)]. Finally, *VPS35* was the first PD-causing  
14 gene to be identified by next-generation sequencing (NGS) in large multi-incident families (5, 6).  
15 Subsequent studies in multiple ethnic groups, including a large multi-center study, indicated that  
16 Asp620Asn was the only pathogenic variant, with a relative frequency ranging from 0.1% to 1% in  
17 familial PD, depending on population background (7). As the phenotype of AD PD closely resembles  
18 that of idiopathic PD, we assumed that rare variants of genes causing AD PD might also contribute  
19 to the etiology of isolated PD in the French population.

20 In this study, we aimed at determining the relative frequencies of known mutations of three genes,  
21 *LRRK2*, *SNCA*, and *VPS35*, in a large cohort of familial and isolated cases. The high prevalence of  
22 the *LRRK2* Gly2019Ser mutation provided us with a unique opportunity to compare in details clinical  
23 characteristics between carriers of this mutation and patients with no known mutations of PD-  
24 associated genes.

25

## 26 MATERIAL AND METHODS

### 27 Patients

28 In total, 673 PD patients from 592 families and 1213 isolated cases without known consanguinity  
29 were recruited from 1990 onwards, through the French Parkinson Disease Genetics Network (the  
30 PDG group, **Supplementary Material**). All participants underwent a detailed medical and family  
31 history, and a family tree were drawn. Familial cases compatible with AD inheritance, and referred  
32 to here as AD PD cases, were defined as AD cases with at least one other affected relative in a  
33 different generation, identified by an examination of secondary cases ( $n = 146$ ) or on the basis of  
34 family history ( $n = 446$ ). PD was diagnosed according to the clinical diagnostic criteria of the UK  
35 Parkinson Disease Society Brain Bank (PDSBB) (8). Comprehensive standardized interviews and  
36 neurological examinations were performed by movement disorder experts. Motor and non-motor  
37 symptoms were assessed by evaluating Unified Parkinson Disease Rating Scale (UPDRS) scores,  
38 Hoehn and Yahr staging, autonomic dysfunction, sleep, cognitive [Mini Mental State Examination  
39 (MMSE)], neuropsychological and behavioral scores. Early onset was defined as the onset of  
40 symptoms before the age of 51 years.

41 Informed consent was obtained from all participants, and genetic studies were approved by local  
42 ethics committees.

43 Most index cases were of European ancestry ( $n = 1530$ ; 84.8%), mostly French ( $n = 1202$ ; 78.6%);  
44 the others were North African ( $n = 221$ ; 12.2%) or of other origins, including Asian, Sub-Saharan  
45 African or "mixed" origins ( $n = 42$ ; 2.3%); or of unknown origin ( $n = 12$ ; 0.7%). This study included  
46 226 index cases from families with PD compatible with AD inheritance reported elsewhere (9, 10).

1

## 2 **Methods**

3 Genomic DNA was obtained from peripheral blood lymphocyte or saliva samples (Oragene™ DNA  
4 Self-Collection Kit, DNA Genotek), by standard protocols. Patients with variants of the *GBA* risk  
5 factor ( $n = 153$ ), or with variants of genes for which the causal role in AD PD was uncertain, such as  
6 *GIGYF2* ( $n = 6$ ), *EIF4G1* ( $n = 2$ ), and *c9ORF72* ( $n = 4$ ), were not included in this study. In addition,  
7 we excluded 25 (23 with bi-allelic *PRKN* mutations and two with bi-allelic *PINK1* mutations) of the  
8 1089 PD index cases who have been screened for autosomal recessive (AR)-PD associated genes  
9 [814 by gene panel/exome sequencing (see below) and 275 by direct sequencing of the two most  
10 frequent AR PD genes, *Parkin* and *PINK1*].

11 All index cases were genotyped in duplicate for *LRRK2* Gly2019ser, by the TaqMan allelic  
12 discrimination Assay-By-Design method, in accordance with the manufacturer's instructions, with 8  
13 ng of DNA mixed with the TaqMan Genotyping Master Mix (Thermo Fisher Scientific Inc.) and  
14 custom-produced TaqMan SNP genotyping assays [C\_63498123\_10 (rs34637584), Thermo Fisher  
15 Scientific Inc.] on an Applied Biosystems PRISM 7000 sequence detection system (Thermo Fisher  
16 Scientific Inc.) or LightCycler® 480 machine (Roche, Life Technologies SAS). All patients found not  
17 to carry *LRRK2* Gly2019ser were then screened for pathogenic variants of the coding sequences  
18 of *LRRK2*, *SNCA*, and *VPS35*, by Sanger sequencing ( $n = 855$ ), targeted sequencing of a  
19 customized next-generation sequencing (NGS) gene panel containing the 22 most prevalent PD-  
20 associated genes ( $n = 404$ ) (**Supplementary Table 1**), or available whole-exome sequencing ( $n =$   
21 410), as previously described (11, 12). We considered known pathogenic mutations of the three genes.

22 Sanger sequencing was used to confirm variants and co-segregation analyses were performed, where  
23 possible. *SNCA* rearrangements were detected by semi-quantitative multiplex PCR (13) or by the  
24 SALSA multiplex ligation-dependent probe amplification method (MLPA, MRC Holland,  
25 Amsterdam, the Netherlands; <http://www.mlpa.com>), according to the manufacturer's instructions.

26

## 27 **Statistical analysis**

28 Demographic and clinical characteristics are expressed as means and standard deviations for  
29 continuous variables and as counts and percentages for qualitative variables, separately for each group  
30 (i.e. with and without mutation). These characteristics were compared between patients with the  
31 *LRRK2* Gly2019ser mutation (*LRRK2*+) and those with no mutations of genes known to be  
32 associated with PD (genetically undefined PD), in Welch's *t*-tests for continuous variables and  
33 Fisher's exact tests for qualitative variables.

34 We used generalized linear models (GLMs) to compare clinical features between patients with the  
35 *LRRK2* Gly2019ser mutation and those with genetically undefined PD, with adjustment for sex, age  
36 at onset (AAO), and disease duration. Disease duration was not included in models of clinical features  
37 at onset. We used GLMs with identity links and normal distributions for continuous clinical features,  
38 and GLMs with logit links and Bernoulli distributions for binary clinical features. Fisher type II tests  
39 were performed to test each effect, and effect size was estimated with Cohen's  $f^2$ . We corrected for  
40 multiple testing by the Benjamini-Hochberg method. Residual normality and heteroskedasticity were  
41 checked visually. Influencers and outliers were checked by calculating hat values and Cook distances.  
42 Only patients with no missing data for the covariables included in the models, such as AAO, sex, and  
43 disease duration, were retained for analysis. Statistical analyses were performed with R 3.6.1.

44

## 45 **RESULTS**

## 1 Demographic and clinical data

2 Table 1 shows the baseline demographic and clinical characteristics of the 1805 PD index cases  
3 included in the study. Men ( $n = 1106$ , 61.3%) and early AAO cases (mean 47.4 [ $SD$  12.5]; range 9-  
4 86 years; **Table 1**) were overrepresented, particularly among isolated cases (mean 46.1 [ $SD$  12.5];  
5 range: 9-79 years) as opposed to familial cases (mean 50.0 [ $SD$  12.1]; range 10-86 years).

## 7 Summary of the mutations identified

8 We identified seven different known mutations of *LRRK2*, *SNCA*, and *VPS35* in 160 of the 1805 PD  
9 index cases (8.9%, 95% confidence interval (CI): [7.6-10.3]). With the exception of rare cases with  
10 the homozygous *LRRK2* Gly2019Ser mutation ( $n = 6$ ), all the known mutations and gene  
11 multiplications identified were heterozygous. We found that 136 of the 138 *LRRK2* mutation carriers  
12 (7.5% of index cases, 95% CI [6.4-8.9]) carried the Gly2019Ser mutation; 13 of these patients had  
13 already been reported elsewhere (9). Two index cases carried the rare *LRRK2* Arg1441His mutation  
14 (9). *SNCA* mutations were found in 19 index cases (1.1%, 95% CI [0.63-1.6]), including 11 families  
15 already reported (13-15): one with whole-gene triplications, 14 with duplications, one with the  
16 Gly51Asp mutation, and three with the Ala53Thr mutation. The three PD index cases with the only  
17 *VPS35* mutation identified, Asp620Asn (0.17%) were described in a previous report (10).

18 The *LRRK2* Gly2019Ser mutation was more common in PD index cases of North-African origin  
19 (100/221, 45.2%; 95% CI [38.6-52.1]) than in Europeans (36/1530, 2.4%; 95% CI [1.7-3.2]) (Fisher's  
20 exact test: odds ratio (OR) = 34.3, 95% CI [22.5-52.4],  $p < 0.0001$ ; **Table 2**). By contrast, *SNCA* point  
21 mutations and locus triplications/duplications were found mostly in PD index cases of European  
22 ancestry, accounting for 1.2% (18/1530) of such patients, whereas only one case of North-African  
23 ancestry (0.45%, 1/221) carried an *SNCA* duplication. *VPS35* mutations were identified exclusively  
24 in patients of European origin, accounting for 0.19% (3/1530) of these individuals.

25 Overall, mutations were more frequently identified in familial (71/592, 12.0%; 95% CI [9.5-14.9])  
26 than in isolated cases (89/1213, 7.3%; 95% CI [5.9-9.0], Fisher's exact test: OR 1.7, 95% CI [1.2-  
27 2.4],  $p = 0.001$ ), particularly for *SNCA* (2.4%, 14/592 vs. 0.41%, 5/1213, Fisher's exact test: OR 5.9,  
28 95% CI [2.1-16.3],  $p = 0.0003$ ; **Table 2**). An analysis of PD cases according to age at onset ( $\leq 50$   
29 years vs.  $> 50$  years) showed that *LRRK2* mutations were more frequent among late-onset PD cases  
30 (61/636, 9.6%; 95% CI [7.4-12.2] vs. 71/1063, 6.7%; 95% CI [5.3-8.4], Fisher's exact test: OR 1.5,  
31 95% CI [1.0-2.1],  $p = 0.03$ ; **Table 2**). By contrast, *SNCA* mutation carriers tended to have an earlier  
32 AAO (16/1063, 1.5% vs. 3/636, 0.5%,  $p = 0.06$ ).

## 34 Clinical characteristics of mutation carriers and comparison of *LRRK2* Gly2019Ser mutation 35 carriers (*LRRK2*+) with individuals with no known PD mutations (genetically undefined PD)

36 Co-segregation analyses identified 193 PD patients as mutation carriers: 151 with *LRRK2*  
37 Gly2019Ser and five with Arg1441His mutations, 29 with *SNCA* (see below) and eight with *VPS35*  
38 Asp620Asn mutations.

## 40 *SNCA*

41 The clinical characteristics of the 29 PD patients carrying either *SNCA* rearrangements (triplications  
42 ( $n = 2$ ) and duplications ( $n = 21$ )) or missense mutations [Ala53Thr ( $n = 3$ ) and Gly51Asp ( $n = 3$ )]  
43 are shown in **Table 3**. All but three of the families concerned originated from France. The remaining

1 three families, originating from Italy, Turkey and Morocco, all had *SNCA* duplications. Within this  
 2 cohort, *SNCA* duplications were the most frequent mutation identified (14/19, 73.7%), followed by  
 3 the Ala53Thr mutation (3/19, 18.8%). Disease onset occurred earliest in patients with the Ala53Thr  
 4 mutation (mean 34.7 [*SD* 7.6], range 26-40 years).

5 Patients carrying the Ala53Thr mutation had an extra-pyramidal parkinsonian syndrome, but with  
 6 heterogeneity between patients with the same mutation: Patient 1172-001, with both *SNCA* Ala53Thr  
 7 and a heterozygous *PRKN* Thr240Met variant, had atypical PD, with a poor response to levodopa,  
 8 early motor fluctuations and cerebellar signs. He rapidly developed impulse control disorders. This  
 9 patient currently displays no cognitive decline. He had a bilateral subthalamic deep brain stimulation  
 10 (STN-DBS). He received clozapine treatment for delusions with a beneficial effect. Patient 1219-001  
 11 presented a parkinsonian syndrome that responded well to levodopa, but developed severe dysarthria.  
 12 She underwent unilateral internal globus pallidus (GPi)-DBS. This patient presented no major  
 13 cognitive and behavioral signs other than an alteration of executive functions. A third PD patient,  
 14 196-016 presented early-onset (26 years) typical PD that responded well to levodopa. Detailed  
 15 clinical data are provided in **Supplementary Table 2**.

16 Both *SNCA* locus triplications and Gly51Asp mutation were associated with early-onset atypical  
 17 parkinsonism (mean AAO: 42.0 years [*SD* 8.5], range: 36-48 years and mean AAO: 42.0 [*SD* 15.7],  
 18 range: 31-60 years, respectively). The patients with *SNCA* triplications were characterized by severe  
 19 cognitive impairment in one of two carriers, dysautonomia, a poor response to levodopa in both  
 20 patients. The three Gly51Asp mutation carriers had shorter disease duration (mean: 3.7 years [*SD*  
 21 3.1], range: 1-7 years), a mild-to-moderate response to levodopa, frequent psychiatric symptoms but  
 22 no dementia or autonomic dysfunction (**Supplementary Table 2**). By contrast, AAO was highest in  
 23 patients with *SNCA* duplications (mean AAO: 45.3 years [*SD* 6.3], range: 36-56 years), consistent  
 24 with typical PD and a good response to levodopa (100%), with more than 50% of these patients  
 25 reporting levodopa-induced motor complications. Non-motor symptoms, including  
 26 depression/psychosis and dysautonomia, were present in about one third of the reported cases, but  
 27 cognitive decline was less frequent (18.8%, 3/16). Detailed clinical characteristics for PD patients  
 28 with *SNCA* multiplications are provided in **Supplementary Table 3**.

29

### 30 ***VPS35***

31 Five of the eight PD patients carrying *VPS35* Asp620Asn mutations have been described before (10).  
 32 The three newly genotyped patients were relatives of patient 838-006 (**Supplementary Table 4**).  
 33 Briefly, patients carrying *VPS35* mutations had features similar to those with idiopathic PD, with a  
 34 mean AAO of ~57 years (range: 38-71 years, **Table 3**): all patients presented the classical triad, with  
 35 akinesia as the predominant symptom at onset (100%), but a much lower frequency of tremor as an  
 36 initial symptom (37.5%), a good response to levodopa (100%), with less than 37% of those treated  
 37 developing dyskinesias and motor fluctuations, and a low rate of dysautonomia (2/8, 25%), with no  
 38 cognitive or neuropsychiatric symptoms or atypical signs.

39

### 40 ***LRRK2***

41 We compared the clinical features of the *LRRK2* G2019S+ PD patient (*LRRK2*+) group with those  
 42 of PD patients with no mutations of known PD-associated genes, excluding subjects with missing  
 43 data for the covariables included in the models, such as sex, AAO and disease duration: 135/151  
 44 *LRRK2*+ and 1552/1693 PD patients without mutations were included in the final analysis (**Table 4**).  
 45 The proportion of men was greater in the genetically undefined PD patient group than in the *LRRK2* +  
 46 group (61.1% vs. 51.9%,  $p = 0.04$ ). The mean AAO of the *LRRK2* Gly2019Ser carriers was four years



1 higher than that of non-carriers ( $p < 0.001$ ). The Gly2019Ser carriers were more likely to be of North-  
 2 African ancestry ( $p < 0.001$ ) and to report a family history of PD ( $p < 0.02$ ). They had a higher  
 3 UPDRS III score during the “OFF” state and a higher Hoehn and Yahr score during the “ON” state  
 4 than non-carriers, but these results were no longer significant after adjustment for AAO and disease  
 5 duration. The frequencies of signs at onset and at examination, the degree of response to treatment,  
 6 motor complications and non-motor signs, including cognitive impairment and autonomic  
 7 dysfunction were similar in both groups.

8 Clinical comparisons between heterozygous ( $n = 144$ ) and homozygous ( $n = 7$ ) Gly2019Ser mutation  
 9 carriers revealed no significant differences in sex (men: 52.1% vs. 42.9%,  $OR$  1.4,  $CI$  [0.31-6.7],  $p =$   
 10 0.71), AAO (mean 51.4 [ $SD$  12.1] vs. mean 53.7 [ $SD$  11.2] years,  $p = 0.62$ ), disease duration (mean  
 11 8.7 [ $SD$  6.7] vs. 9.1 [ $SD$  4.5] years,  $p = 0.87$ ) or clinical presentation, but the number of homozygous  
 12 carriers was small.

13 Unlike *LRRK2* Gly2019Ser carriers, all patients with the Arg1441His mutation were French and all  
 14 reported a family history of PD. They had a shorter disease duration (mean: 5.2 years [ $SD$  5.1], range:  
 15 2-14 years vs. mean 9.0 years [ $SD$  8.0], range: 0.5-63 years), were more likely to develop an akinetic-  
 16 rigid motor phenotype (80% vs. 53%), had a slightly better response to levodopa (100% vs. 90%),  
 17 and an absence of cognitive and neuropsychiatric symptoms, but a similar mean age at onset (52.6  
 18 years [ $SD$  9.6], range: 39-64 years vs. 51.4 years [ $SD$  12.1], range: 29-86 years).

19

## 20 DISCUSSION

21 This is one of the largest national multi-center studies to investigate the frequency of variants of the  
 22 three major genes unequivocally linked to AD PD — *LRRK2*, *SNCA*, and *VPS35* — and their  
 23 associated phenotypes in a large cohort of >1800 French and North African index PD cases. We  
 24 report an overall mutation frequency of 8.9% across both populations, the *LRRK2* G2019S mutation  
 25 being the most frequently identified variant (7.5%), particularly in familial rather than isolated cases.  
 26 However, the frequency of mutations differed considerably between populations. We confirm here  
 27 that the *LRRK2* Gly2019Ser mutation is the principal genetic cause of PD in our cases of North-  
 28 African ancestry, reaching an overall frequency of 45% (100/221) and 62% (34/55) in familial cases.  
 29 By contrast, this mutation was present at a much lower rate of 2.4% (36/1530) in our native French  
 30 PD cases. Our findings are consistent with those of previous multi-center studies (16). *SNCA*  
 31 duplications were the second most common type of mutations, identified in 14 index cases (0.78%).  
 32 *SNCA* duplication carriers were mostly of European ancestry, particularly French (93%), tended to  
 33 be predominantly females, probably due to random or recruitment bias, and had a higher frequency  
 34 of a family history of PD. We also identified three unrelated PD patients carrying the *SNCA* Ala53Thr  
 35 mutation. Although generally rare, this mutation appears to be particularly common in the Italian and  
 36 Greek populations, due to a founder effect (17, 18). Only four individuals without Greek or Italian  
 37 ancestry have been reported to carry this mutation, in haplotypes different from those reported in  
 38 Greek and Italian families (19-22). Additional haplotype analysis would determine the ancestral  
 39 origin of our three French mutation carriers. Other rare known mutations were also identified in our  
 40 study: *VPS35* Asp620Asn and *LRRK2* Arg1441His in three and two AD PD families, respectively.  
 41 At least 25 *LRRK2* Arg1441His carriers, including those described here, have been reported to date  
 42 [(3); www.mdsgene.org]. Most were Caucasian, and all but one case reported a family history of the  
 43 disease. This pathogenic variant was not found in more than 6,000 healthy controls tested (23) and is  
 44 very rare in the Genome Aggregation Database (GnomAD) (1/31,298 alleles); it is therefore very  
 45 likely to be pathogenic. Consistent with this conclusion, the Arg1441His variant affects the same  
 46 amino-acid residue as two other recurrent PD-causing mutations (Arg1441Cys and Arg1441Gly).  
 47 Finally, previous haplotype analyses did not support the hypothesis of a common founder for the  
 48 Arg1441His variant, instead suggesting that there might be a mutational hotspot. Following initial

1 reports of the existence of several *VPS35* variants (5, 6), pathogenicity has been confirmed only for  
2 the Asp620Asn variant. Consistent with our findings, this recurrent mutation has been identified  
3 predominantly in families of Caucasian descent affected by AD PD. A meta-analysis of 21,824 PD  
4 patients from 15 case-control studies performed worldwide from 2011 to 2016 identified an overall  
5 mutation frequency of 0.12% (0.29% in familial cases and 0.023% in isolated cases) [reviewed in  
6 (24)], and an absence of this mutation from healthy controls and the GnomAD public database.

7 The clinical features of our *LRRK2* mutation carriers, whether heterozygous or homozygous, were  
8 indistinguishable from those of patients with no mutations in known PD-associated genes. These  
9 features overlapped those of typical, idiopathic PD. In our study, patients with the G2019S mutation  
10 had a mean AAO of ~52 years, a high proportion of patients with late AAO (> 50 years), a good  
11 response to levodopa, a predominance of tremor as a first symptom of PD, about a quarter had  
12 cognitive impairment, about 10% had dysautonomia, but no other atypical signs after a mean disease  
13 duration of approximately 10 years. Although the clinical features of the *LRRK2* Gly2019Ser carriers  
14 compared with patients with idiopathic PD in literature are conflicting [meta-analysis in (25)], even  
15 for the same ethnic PD population [i.e. of North-African origin, (26-31)], our data are consistent with  
16 those of 724 *LRRK2* mutation carriers listed in the MDSGene database. Like *LRRK2* mutation  
17 carriers, *VPS35* Asp620Asn carriers had a phenotype very similar overall to that of idiopathic PD:  
18 absence of atypical signs, excellent levodopa response, normal cognition and absence of  
19 neuropsychiatric features. However, the mean AAO of our patients appeared to be later (57 years)  
20 than that reported in a recent meta-analysis (51 years) (32), due to the presence of multiple affected  
21 relatives with a late onset of disease within the same family (see **Supplementary Table 4**). Lastly,  
22 *SNCA* mutation carriers had motor features similar to those of idiopathic PD, but an overall earlier  
23 AAO, a shorter disease course, a higher frequency of motor complications, a higher frequency of non-  
24 motor signs and symptoms (cognitive decline in 17%, autonomic dysfunction in 39%, and psychotic  
25 symptoms and depression in 32%). Atypical signs have also been observed in rare PD patients  
26 carrying the *SNCA* Gly51Asp mutation (14). In this study, we also identified a rare known variant of  
27 *SNCA*, His50Gln, in the homozygous state. This variant has been described as a causal variant  
28 associated with late-onset PD, dementia and dystonia (33, 34), but a reevaluation in larger datasets of  
29 PD patients and controls, including the GnomAD database (23/282,808 alleles), provided no evidence  
30 of pathogenicity for this variant (35). However, interestingly, the 42 year-old female patient carrying  
31 the His50Gln variant in our cohort had clinical features similar to those observed in carriers of other  
32 types of *SNCA* mutation carriers. She presented an early AAO (32 years), an excellent response to  
33 levodopa, motor complications, akinetic-rigid parkinsonism and dystonia, an absence of cognitive  
34 decline and neuropsychiatric symptoms, but the presence of autonomic dysfunction and atypical  
35 neurological signs, such as postural instability, REM sleep behavior disorder (RBD) and impulse  
36 control disorders (See **Supplementary Table 2**). However, in this study, the *SNCA* His50Gln was  
37 found using our customized gene panel and in absence of whole exome/genome sequencing to detect  
38 other possible pathogenic mutations, its pathogenicity remains inconclusive.

39 The principal strength of this national multi-center study is the large group of well-phenotyped and  
40 genotyped patients and family members recruited at the 16 different PDG centers, and the use of a  
41 standardized protocol, ensuring comparable and consistent clinical data reporting and diagnoses at  
42 each center. This enabled us to refine the estimated prevalence of mutations in genes causing AD PD  
43 in France. We show that our population, although mixed, has a relatively high frequency of *SNCA*,  
44 *LRRK2* and *VPS35* mutations. However, the clinical data were cross-sectional, most patients were  
45 European or North African, and our populations were biased towards EO cases.

46 In most instances, the phenotypes of cases due to AD PD mutations are indistinguishable from those  
47 of cases without mutations, demonstrating the need for genetic analysis for their identification. Gene-  
48 specific disease-modifying therapies are currently being developed and tested. More generalized

- 1 genetic testing is therefore required in PD patients, to identify those most likely to benefit from
- 2 personalized care (36).
- 3

## 1 **DATA AVAILABILITY STATEMENT**

2 The raw data supporting the conclusions of this article will be made available by the authors, without  
3 undue reservation.

4

## 5 **ETHICS STATEMENT**

6 The studies involving human participants were reviewed and approved by INSERM, CCPPRB du  
7 Groupe Hospitalier Pitié-Salpêtrière, Paris, France. Written informed consent to participate in this  
8 study was provided by the participant's legal guardian/next of kin.

9

## 10 **AUTHOR CONTRIBUTIONS**

11 SL conceived, designed and organized the study, wrote the first draft, reviewed, and critically revised  
12 the manuscript. JCC and AB conceived the project, reviewed, and critically revised the manuscript.  
13 MH contributed to the statistical analysis and critically revised the manuscript. GM, CT, HB, SF,  
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15 contributed to the execution of the research project and critically revised the manuscript. All authors  
16 contributed to the article and approved the submitted version.

17

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28

## 29 **SUPPLEMENTARY MATERIAL**

30 The Supplementary Material for this article can be found online at:  
31 <https://www.frontiersin.org/articles/10.3389/fneur.2020.00682/full#supplementary-material>

32

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1 **Table 1. Baseline demographics for our study population**

2

	All index cases <i>n</i> = 1805	AD PD index cases <i>n</i> = 592	Isolated cases <i>n</i> = 1213
<b>Sex, <i>n</i> (%)</b>			
Male	1106 (61.3)	342 (57.8)	764 (63)
Female	699 (38.7)	250 (42.2)	449 (37)
<b>Ancestry, <i>n</i> (%)</b>			
Europeans	1530 (84.8)	525 (88.6)	1005 (82.8)
North-Africans	221 (12.2)	55 (9.3)	166 (13.7)
Other/mixed origins	42 (2.3)	11 (1.9)	31 (2.6)
Unknown origins	12 (0.7)	1 (0.2)	11 (0.9)
Age at onset, ( <i>SD</i> ),	47.4 (12.5)	50.0 (12.1)	46.1 (12.5)
Range, years	9-86	10-86	9-79
Age at examination, ( <i>SD</i> )	55.8 (13.3)	57.9 (12.5)	54.9 (13.5)
Range, years	12-88	16-88	12-85
Early-onset ( $\leq 50$ years), <i>n</i> (%)	1065 (62.7)	311 (54.6)	754 (66.8)
Late-onset ( $> 50$ years), <i>n</i> (%)	634 (37.3)	259 (45.4)	375 (33.2)
Disease duration, ( <i>SD</i> )	8.4 (6.8)	7.8 (6.7)	8.8 (7.4)
Range, years	0-52	0-52	0-46

3

4 Frequencies were compared in Fisher's exact tests for qualitative traits and means were compared in  
5 Welch's *t*-tests for continuous variables. Age-at-onset was missing for 106 index cases.

6 AD, autosomal dominant; PD, Parkinson's disease; *SD*, standard deviation

7



**Table 2. Overall frequencies of *LRRK2*, *SNCA* and *VPS35* mutations according to index case ethnicity, family history of Parkinson's disease and age at onset**

	Europeans	North-Africans	North-Africans vs. Europeans OR, 95% CI, <i>p</i>	AD PD	Isolated cases	AD PD vs. Isolated cases OR, 95% CI, <i>p</i>	EO-PD	LO-PD	LO-PD vs. EO-PD OR, 95% CI, <i>p</i>
	<i>n</i> = 1530	<i>n</i> = 221		<i>n</i> = 592	<i>n</i> = 1213		<i>n</i> = 1063	<i>n</i> = 636	
<b>All <i>LRRK2</i>, <i>n</i></b> (%, 95% CI)	38 (2.5%, [1.8-3.4])	100 (45.2%, [38.6-52.0])	OR = 32.5, [21.4-49.2], <i>p</i> < 0.0001	54 (9.1%, [6.9-11.7])	84 (6.9%, [5.6-8.5])	OR = 1.3, [0.9-1.9], <i>p</i> = 0.11	71 (6.7%, [5.3-8.4])	61 (9.6%, [7.4-12.2])	OR = 1.5, [1.0-2.1], <i>p</i> = 0.03
Gly2019Ser <i>n</i> (%)	36 (2.4%)	100 (45.2%)		52 (8.8%)	84 (6.9%)		71 (6.7%)	59 (9.3%)	
Arg1441His <i>n</i> (%)	2 (0.13%)	0 (0%)		2 (0.34%)	0 (0%)		0 (0%)	2 (0.31%)	
<b>All <i>SNCA</i>, <i>n</i></b> (%, 95% CI)	18 (1.2%, [0.7-1.9])	1 (0.45%, [0.01-2.5])	OR = 0.38, [0.05-2.9], <i>p</i> = 0.50	14 (2.4%, [1.3-3.9])	5 (0.41%, [0.1-1.0])	OR = 5.9, [2.1-16.3], <i>p</i> = 0.0003	16 (1.5%, [0.9-2.4])	3 (0.47%, [0.1-1.4])	OR = 0.31, [0.09-1.1], <i>p</i> = 0.06
Triplications <i>n</i> (%)	1 (0.065%)	0 (0%)		1 (0.17%)	0 (0%)		1 (0.094%)	0 (0%)	
Duplications <i>n</i> (%)	13 (0.85%)	1 (0.5%)		11 (1.9%)	3 (0.25%)		11 (1.0%)	3 (0.47%)	
Ala53Thr <i>n</i> (%)	3 (0.20%)	0 (0%)		1 (0.17%)	2 (0.16%)		3 (0.28%)	0 (0%)	
Gly51Asp <i>n</i> (%)	1 (0.065%)	0 (0%)		1 (0.17%)	0 (0%)		1 (0.094%)	0 (0%)	

<b>VPS35</b> Asp620Asn, <i>n</i> (%, 95% CI)	3 (0.20%, [0.04-0.57])	0 (0%)	$p = 1$	3 (0.51%, [0.1- 1.5])	0 (0%)	$p = 0.04$	2 (0.19%, [0.02-0.7])	1 (0.16%, [0.0-0.9])	OR = 0.84, [0.08-9.2], $p =$ 1
Total mutations, <i>n</i> (%, 95% CI)	59 (3.9%, [2.9- 4.9])	101 (45.7%, [39.0-52.5])	OR = 21.0, [14.5-30.4], $p <$ 0.0001	71 (12.0%, [9.5-14.9])	89 (7.3%, [5.9- 9.0])	OR = 1.7, [1.2- 2.4], $p = 0.001$	89 (8.4%, [6.8 -10.2])	65 (10.2%, [8.0-12.8])	OR = 1.3, [0.9-1.8], $p =$ 0.22

Age at onset was missing for 106 cases, including 6 with the *LRRK2* G2019S mutation. We also considered patients with missing AAO data examined at an age  $\leq 50$  years ( $n = 26$ ) to have early-onset PD.

AD, autosomal dominant; CI, confidence intervals; EO, early-onset; LO, late-onset; OR, odds ratio; PD, Parkinson's disease

**Table 3. Summary of the clinical data for patients carrying *SNCA*, *VPS35* and *LRRK2* Arg1441His mutations identified in this study**

	<i>SNCA</i> rearrangements		<i>SNCA</i> missense mutations		<i>VPS35</i>	<i>LRRK2</i>
	Triplications <sup>a</sup>	Duplications <sup>a</sup>	Ala53Thr	Gly51Asp <sup>b</sup>	Asp620Asn <sup>c</sup>	Arg1441His <sup>d</sup>
No. of carriers (index cases)	2 (1)	21 (14)	3 (3)	3 (1)	8 (3)	5 (2)
No. of index cases with family history of PD	1/1	11/14 (78.6%)	1/3 (33.3%)	1/1	3/3 (100%)	2/2 (100%)
Sex (M:F)	2:0	7:14	1:2	1:2	5:3	2:3
Mean age at onset ( <i>SD</i> ) [range], years	42 (8.5)[36-48]	45.3 (6.3) [36-56]	34.7 (7.6) [26-40]	42.0 (15.7) [31-60]	57.1 (10.7) [38-71]	52.6 (9.6) [39-64]
Mean age at examination ( <i>SD</i> ) [range], years	50 (11.3) [42-58]	52.2 (6.0) [43-64]	42.7 (17.5) [28-62]	45.7 (18.7) [32-67]	65.0 (10.1) [52-80]	57.9 (10.0) [42-66]
Mean disease duration ( <i>SD</i> ) [range], year	8 (2.8) [6-10]	6.3 (3.9) [1-16]	8.2 (12.0) [0.5-22]	3.7 (3.1) [1-7]	7.9 (5.4) [1-17]	5.2 (5.1) [2-14]
<b>Signs at onset</b>						
Akinesia	1/2 (50%)	14/18 (77.8%)	3/3 (100%)	2/2 (100%)	7/7 (100%)	4/5 (80%)
Tremor	2/2 (100%)	6/18 (33.3%)	1/3 (33.3%)	0/2 (0%)	3/8 (37.5%)	1/4 (25%)
Micrographia	0/2 (0%)	8/18 (44.4%)	1/2 (50%)	1/2 (50%)	3/6 (50%)	2/5 (40%)
Dystonia	0/2 (0%)	0/17 (0%)	0/3 (0%)	0/2 (0%)	0/8 (0%)	1/4 (25%)
<b>Clinical signs at examination</b>						
Tremor	2/2 (100%)	11/19 (57.9%)	2/3 (66.7%)	1/3 (33.3%)	6/8 (75%)	4/4 (100%)
Bradykinesia	2/2 (100%)	19/19 (100%)	3/3 (100%)	3/3 (100%)	8/8 (100%)	5/5 (100%)
Rigidity	2/2 (100%)	19/19 (100%)	3/3 (100%)	3/3 (100%)	8/8 (100%)	5/5 (100%)
Asymmetry	1/2 (50%)	16/17 (94.1%)	3/3 (100%)	3/3 (100%)	8/8 (100%)	5/5 (100%)

Apraxia	NA	0/16 (0%)	0/3 (0%)	0/2 (0%)	0/8 (0%)	0/5 (0%)
Dysarthria	NA	3/15 (20%)	1/3 (33.3%)	0/2 (0%)	0/8 (0%)	0/3 (0%)
Mean (or value) UPDRS III OFF (/108) (SD) [range], year	51 (7.1) [47, 56]	40.7 (23.7) [5- 86]	47.7 (20.4) [30-70]	13	25 (9.9) [18- 32]	23.8 (23.3) [2- 56]
Mean (or value) UPDRS III ON (/108) (SD) [range], year	44.5 (2.1) [43- 46]	17.7 (13.2) [4- 48]	34 (1.4) [33-35]	7	21.9 (8.1) [8- 33]	10.5 (9.5) [0- 19]
Mean (or value) Hoehn & Yahr ON (/5) (SD) [range], year	NA	1.9 (0.64) [1- 3]	3	1.5	2.3 (0.5) [2- 3]	1.3 (0.3) [1- 1.5]
<b>Treatment and its complications</b>						
Levodopa responsiveness <sup>#</sup>	0/2 (0%)	15/15 (100%)	2/3 (66.7%)	Mild to moderate	5/5 (100%)	5/5 (100%)
Dyskinesias	NA	9/15 (60%)	2/3 (66.7%)	2/3 (66.7%)	2/8 (25%)	1/4 (25%)
Motor fluctuations	NA	8/15 (53.3%)	2/3 (66.7%)	2/3 (66.7%)	3/8 (37.5%)	3/4 (75%)
Dystonia	NA	3/15 (20%)	0/3 (0%)	1/3 (33.3%)	0/8 (0%)	2/4 (50%)
<b>Non-motor signs</b>						
Cognitive impairment (MMSE < 24/30)	1/2 (50%)	3/16 (18.8%)	0/3 (0%)	0/2 (0%)	0/8 (0%)	0/5 (0%)
Dysautonomia*	2/2 (100%)	6/16 (37.5%)	1/3 (33.3%)	0/2 (0%)	2/8 (25%)	2/5 (40%)
Depression/neur opsychiatric disorders	NA	4/16 (25%)	1/3 (33.3%)	2/3 (66.7%)	0/8 (0%)	0/5 (0%)

Patients previously reported by

<sup>a</sup>Ibanez *et al.* (13) and Book *et al.* (15)

<sup>b</sup>Lesage *et al.* (14)

<sup>c</sup>Lesage *et al.* (10)

<sup>d</sup>Lesage *et al.* (9)

<sup>#</sup>Levodopa responsiveness was defined as a > 30% improvement in subjective perceived motor symptoms

\*Dysautonomia included at least one of the following three signs: orthostatic hypotension, erectile dysfunction and/or urinary problems

AD, autosomal dominant; MMSE, Mini Mental State Examination; NA, not available; PD, Parkinson's disease; UPDRS III, the motor subsection of the Unified Parkinson's Disease Rating Scale

**Table 4. Comparison of demographic and clinical characteristics of patients with Parkinson's disease by *LRRK2* mutation status (*LRRK2* carriers versus patients without mutations in known Parkinson's disease-associated genes)**

	<i>LRRK2</i> G2019S+ and patients without mutations; unadjusted comparisons			<i>LRRK2</i> G2019S + and patients without mutations; adjusted comparisons		
	<i>LRRK2</i> + <i>n</i> = 135 (8%)	Genetically undefined PD <i>n</i> = 1552 (92%)	<i>p</i> -value	Coefficient or OR (CIs) (reference: non-mutation carriers)	<i>p</i> -value	<i>p</i> -value adjusted †
<b>Demographic characteristics</b>						
Sex (% male)	70/135 (51.9%)	949/1552 (61.1%)	0.04*			
Age at onset ( <i>SD</i> ), years	51.6 (12.8)	47.3 (12.8)	<0.001*			
Age at examination ( <i>SD</i> ), years	60.6 (13.3)	55.7 (13.4)	<0.001*			
Disease duration ( <i>SD</i> ), years	9.0 (8.0)	8.4 (7.0)	0.32			
<b>Ancestry</b>						
European	43/135 (31.9%)	1390/1543 (90.0%)				<0.001*
North-African	92/135 (68.1%)	110/1543 (7.1%)				
Other/Mixed origins	0/135 (0.00%)	43/1543 (2.8%)				
<b>Family history of PD</b>						
AD PD	61/135 (45.2%)	536/1552 (34.5%)				0.02*
Isolated cases	74/135 (54.8%)	1016/1552 (65.5%)				
<b>Clinical characteristics</b>						

Levodopa responsiveness <sup>#</sup>	69/77 (89.6%)	788/958 (82.3%)	0.12	1.76 [0.81;3.80]	0.13	0.44
<b>Symptoms at onset</b>						
Dystonia	8/101 (7.9%)	125/1306 (9.6%)	0.72	0.88 [0.41;1.87]	0.74	0.78
Akinesia	57/108 (52.8%)	805/1332 (60.4%)	0.13	0.75 [0.50;1.11]	0.15	0.44
Tremor	72/107 (67.3%)	809/1350 (59.9%)	0.15	1.32 [0.87;2.01]	0.19	0.44
Micrographia	22/102 (21.6%)	454/1314 (34.6%)	0.009*	0.50 [0.31;0.81]	0.003*	0.06
<b>Symptoms at examination</b>						
Bradykinesia	109/111 (98.2%)	1369/1411 (97.0%)	0.77	1.73 [0.41;7.26]	0.42	0.63
Rigidity	106/111 (95.5%)	1333/1406 (94.8%)	1.00	1.12 [0.44;2.85]	0.80	0.80
Tremor	89/111 (80.2%)	1040/1397 (74.4%)	0.21	1.32 [0.81;2.15]	0.25	0.50
Asymmetry	105/108 (97.2%)	1313/1366 (96.1%)	0.79	1.64 [0.50;5.40]	0.38	0.62
<b>Motor features</b>						
UPDRS III ON (/108) (SD)	19.5 (13.4)	18.7 (13.2)	0.57	-0.73 [-3.62;2.16]	0.62	0.76
UPDRS III OFF (/108) (SD)	38.9 (18.2)	32.5 (17.6)	0.02*	4.91 [-0.33;10.14]	0.07	0.44
Hoehn & Yahr ON (/5) (SD)	2.2 (1.0)	2.0 (0.9)	0.03*	0.13 [-0.06;0.31]	0.17	0.44
Hoehn & Yahr OFF (/5) (SD)	2.7 (1.1)	2.4 (1.0)	0.21	0.18 [-0.18;0.54]	0.33	0.59
<b>Motor complications</b>						
Dyskinesias	52/98 (53.1%)	537/1164 (46.1%)	0.21	1.42 [0.90;2.25]	0.13	0.44
Motor fluctuations	58/98 (59.18%)	640/1164 (55.0%)	0.46	1.18 [0.75;1.86]	0.48	0.66
Dystonia	27/98 (27.6%)	303/1164 (26.0%)	0.72	1.11 [0.68;1.80]	0.68	0.77
<b>Non-motor features</b>						
Dysautonomia*	9/94 (9.6%)	111/1214 (9.1%)	0.85	0.84 [0.40;1.75]	0.64	0.76
MMSE score (/30) (SD)	27.4 (3.7)	28.2 (3.2)	0.07	-0.56 [-1.41;0.28]	0.19	0.44

Data are given as the mean  $\pm$  standard deviation for continuous variables and as counts (percentages) for qualitative variables.

Welch's *t*-test was used to compare the groups for continuous variables and Fisher's exact test was used for binary variables. Coefficients for continuous clinical features and odds ratios (ORs) for binary clinical features, confidence intervals (CIs) and *P*-values were calculated from GLMs with mutation status, sex, age, and disease duration, for all variables except for onset variables, for which only mutation status, sex and age at onset were added. Linear models were used for continuous variables; GLMs with logit link and Bernoulli distributions were used for binary variables

#Levodopa responsiveness was defined as a >30% improvement in subjective perceived motor symptoms

\*Dysautonomia included at least one of the following three signs: orthostatic hypotension, erectile dysfunction and/or urinary problems

AD, autosomal dominant; MMSE, CI, Confidence Intervals; Mini Mental State Examination; OR, Odds Ratio; PD, Parkinson's disease; UPDRS III, the motor subsection of the Unified Parkinson's Disease Rating Scale

¥ *P* corrected for multiple testing by the Benjamini-Hochberg procedure

\*  $p < 0.05$