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Abstract

Studies of the phenotype and population distribution of rare genetic forms of parkinsonism are required, now that gene-targeting approaches for Parkinson's disease have reached the clinical trial stage. We evaluated the frequencies of *PRKN*, *PINK1*, and *DJ-1* mutations in a cohort of 1587 cases. Mutations were found in 14.1% of patients: 27.6% were familial and 8% were isolated. *PRKN* was the gene most frequently mutated in Caucasians whereas *PINK1* mutations predominated in Arab-Berber individuals. Patients with *PRKN* mutations had an earlier age at onset, and less asymmetry, levodopa-induced motor complications, dysautonomia, and dementia than those without mutations.

Keywords: Parkinson's disease; *PRKN*; *PINK1*; autosomal recessive inheritance; genotype-phenotype correlations

Abbreviations: AAO, age-at-onset; AR, autosomal recessive; EO, early-onset; GLM, generalised linear model; PD, Parkinson's disease; LRRK2, leucine-rich repeat kinase 2; PINK1, PTEN-induced putative kinase 1

Our understanding of the genetic basis of Parkinson's disease (PD) has been improved with the identification of several disease-causing genes.[1] Trials targeting these genes are underway and the development of cohorts ready for precision clinical trials that target genetic forms of PD are now required.[2,3]

PRKN, *PINK1*, and *DJ-1* mutations are the most frequent cause of early-onset (EO) autosomal recessive (AR) typical PD. We investigated the frequency and nature of pathogenic variants of these three genes in a cohort of 1587 PD probands, comparing clinical characteristics between patients with *PRKN* mutations (*PRKN*-PD), and those without pathogenic variants of known PD genes (PD-NM).

Patients and Methods

Patient selection

Patients were enrolled between 1990 and 2018, through the French PDG network and North African and Turkish collaborations. PD was diagnosed according to the clinical diagnostic criteria of the UK Parkinson Disease Society Brain Bank (PDSBB).[4] Cases with mutations responsible for recessive atypical parkinsonism or those carrying the common *LRRK2* Gly2019Ser variant were not included. We selected a cohort of 1587 probands from 497 AR PD families (at least two affected siblings and isolated cases born to consanguineous parents), and 1090 isolated cases, all screened for *PRKN*.

Standardized neurological examinations were performed by movement disorder experts, and 28 variables were used to obtain comparable data.

Most probands were Caucasian (*n*=1324, 83.4%; 927 French, 134 Turkish), Arab-Berber (*n*=213, 13.4%) or of other ethnicities (*n*=50, 3.2%). We included 1587 PD probands and 52 mutation-carrying relatives in the genotype/phenotype correlation analysis. Informed consent and approval from institutional review boards were obtained for sample collection.

Procedures

Probands were screened by denaturing high-performance liquid chromatography (dHPLC) and/or direct sequencing,[5-8] next-generation sequencing (NGS) with a targeted gene panel or whole-exome sequencing[9,10].

PRKN was screened in 1587 probands, *PINK1* and *DJ-1* in 1223. Sanger sequencing was performed to confirm variants and cosegregation analyses, where possible. Exon rearrangements were detected by semi-quantitative PCR for *PRKN*[5] or with the MRC Holland Salsa MLPA P051/P052 Parkinson kits. Patients with an AAO ≤40 years and lymphoblastoid cells available for RT-PCR analysis (*n*=15)[5] or unaffected relatives for cosegregation analysis (*n*=30) were investigated for possible *PRKN* rearrangements undetectable by MLPA.

Statistical analysis

The PD-NM and *PRKN*-PD groups were compared with Welch's *t*-tests for continuous variables and Fisher's exact tests for categorical variables.

We used generalized linear models (GLMs) to compare clinical features between *PRKN*-PD with bi-allelic or double heterozygous mutations and PD-NM, adjusting for sex, age-at-onset

(AAO), disease duration and dopaminergic medication. We used GLMs with identity links and normal distributions for continuous clinical features, and GLMs with logit links and Bernoulli distributions for binary clinical features. Interactions between AAO and disease duration were also included. Disease duration and dopaminergic medication were not included in models for clinical features at onset. Effects were assessed in Fisher type II tests, and effect size was estimated with Cohen's f2. Benjamini-Hochberg correction for multiple testing was performed. GLMs were generated for the 15/28 clinical features for at least 20% of the patients in each group with available data.

Results

Demographic and clinical data

In our cohort, men (*n*=951, 60%) and EO cases (mean AAO 40.2 [SD 12.1] years) were overrepresented, particularly among isolated cases ($p<0.0001$) (Table 1).

Distribution and nature of recessive PD-associated gene mutations

Bi-allelic or double heterozygous mutations of known AR PD-causing genes were present in 224 of the 1587 probands (14.1%): 27.6% (137/497) of familial and 8% (87/1090) of isolated cases. The most frequently mutated genes were *PRKN* (199/1587, 12.5%), then *PINK1* (23/1223, 1.9%), and *DJ-1* (2/1223, 0.16%). We identified 56 patients with single heterozygous variants in the three genes in whom AAO was significantly later than cases with bi-allelic or double heterozygous mutations (36.9 [SD 10.6] *vs.* 31.3 [SD 11.1], $p=0.0009$); they were removed from genotype/phenotype correlation analyses.

The 199 cases carried either homozygous (*n*=92), or compound heterozygous (*n*=59), confirmed by segregation analysis of all available unaffected relatives or double heterozygous (*n*=48) *PRKN* mutations for whom phasing was still unknown. These carriers displayed 77 different variants, including 19 absent from public databases (www.mdsgene.org)[11] (Fig 1A). RT-PCR identified a single French family with *PRKN* Ex3del/Ex3dup compound heterozygous rearrangements not detectable by MLPA but elucidated by co-segregation analysis.

Most of the 23 probands with pathogenic *PINK1* variants were Arab-Berbers (*n*=12, 52.2%); 8 carried the homozygous Gln456* mutation. Most variants were homozygous (*n*=18, 78.3%). We identified 21 different pathogenic variants, including three not in databases (Fig 1B).

Distribution of *PRKN* and *PINK1* mutation carriers by age-at-onset, pattern of presentation and ethnicity

The proportion of probands with *PRKN* mutations decreased with increasing AAO: 42.2% for an AAO ≤20 years, 29% (21 to 30 years), 13% (31 to 40 years), and 4.4% (41 to 60 years) (Fig 2A). This decrease was more marked in isolated than familial cases (Fig 2B). *PINK1* mutations were less frequent than *PRKN* mutations (1.9% *vs.* 12.5%) but more evenly distributed among familial cases for AAOs up to 60 years. Neither *PRKN* nor *PINK1* variants were found in patients with onset after 60 (Fig 2A-D). *PRKN* mutations were more frequent in Caucasians (179/1324, 13.5%) than in Arab-Berbers (16/213, 7.5%) (Fig 2E). Conversely, *PINK1* mutations were more common in Arab-Berbers (12/188, 6.4%) than Caucasians (9/1005, 0.9%) (Fig 2E).

Comparison of *PRKN* mutation carriers (*PRKN*-PD) with non-mutation carriers (PD-NM): genotype-phenotype correlations

We compared the clinical features between the 228/241 *PRKN*-PD and 1181/1307 PD-NM subjects without missing data (Table 2; Supplementary Table 1). The proportion of men (p_{adj}=0.016) and AAO (p_{adj}<0.0001) were greater in the PD-NM than the *PRKN*-PD group. Dopaminergic treatment was similar between groups (p_{adj}=0.69), but levodopa responsiveness was higher in *PRKN*-PD than PD-NM (p_{adj}=0.045).

After adjustment for covariables, *PRKN*-PD had a higher initial frequency of tremor (p_{adj}=0.0076), but lower frequencies of akinesia (p_{adj}=0.0003), micrographia (p_{adj}=0.010), and asymmetry (p_{adj}=0.0005) than PD-NM patients (Table 2). Dystonia at onset and cardinal symptom (bradykinesia, rest tremor and rigidity) frequencies were similar in both groups. Motor severity after adjustment for disease duration was lower in *PRKN*-PD than in PD-NM (UPDRS score, p_{adj}=0.014), and *PRKN*-PD patients developed fewer levodopa-induced motor complications (dyskinesia: p_{adj}=0.0005; motor fluctuations: p_{adj}<0.0001). Non-motor symptoms, including dysautonomia (p_{adj}<0.0001) and dementia (p_{adj}=0.014), were less frequent in *PRKN*-PD patients.

Mutational and phenotypic characteristics of *PINK1* and *DJ-1* mutation carriers

The *PINK1*-associated phenotype of the 33 carriers resembled that of *PRKN*, but with a slightly later AAO (mean 34.6 [SD 12.2] years *vs.* 31.3 [SD 10.9] years; p=0.20), a lower

frequency of tremor (16.7% *vs.* 68.5%; p=0.0005) and a higher rate of dystonia (90% *vs.* 18.2%; p<0.0001) at onset (Supplementary Table 2). Non-motor symptoms, such as dysautonomia (72.2% *vs.* 19.6%; p<0.0001) and dementia (25% *vs.* 3.8%; p=0.0007) were more frequent in patients with *PINK1* variants, who were also more likely to display levodopa-induced dyskinesia (93.3% *vs.* 54.1%; p=0.0024) and motor fluctuations (66.7% *vs.* 46.2%; p=0.06).

The two pathogenic *DJ-1* variant carriers (one Caucasian, one North African) each carried a previously unknown homozygous Glu94* and a compound heterozygous variant affecting the same highly conserved amino acid (Thr154Ile/Thr154Ala). They developed PD, with the four cardinal signs, at the ages of 29 and 28. Dystonia at onset, dyskinesia and orthostatic hypotension were noted in one patient, without cognitive signs.

Discussion

We established the spectrum and relative frequencies of mutations in a large cohort of AR PD cases, elucidating the genotype-clinical phenotype relationship. Homozygous/compound or double heterozygous pathogenic variants of *PRKN*, *PINK1*, and *DJ-1* account for 14.1% of our PD patients, with *PRKN* the most frequently mutated. This study included a large number of genotyped and extensively phenotyped patients (276 mutation carriers) compared with a group of cases not carrying mutations of known AR PD-causing genes. However, the clinical data were cross-sectional, the numbers of patients with mutations of genes other than *PRKN* were small, and our populations were biased towards EO cases. In addition, 24% (48/199) of

our PD patients with two *PRKN* mutations lacked information on their mutational phasing. However, given that we found no mutations *in cis* in a co-segregation analysis of 59 index cases, we are confident that the vast majority of patients with two mutations carry them *in trans*. Nevertheless, our findings may have major implications for patient selection for genetic testing based on AAO, pattern of disease presentation, and ethnicity. Indeed, the frequency of pathogenic variants of AR PD-associated genes i) decreased with increasing AAO, to zero for an AAO beyond 60 years, ii) in cases with a positive family history or consanguinity was more than triple that in isolated cases, and iii) was much higher for *PRKN* than *PINK1* in Caucasians, but similar for these two genes in Arab-Berbers. However, pathogenic *PRKN* variants were more frequent in our EO PD cases (<50 years, 190/1273, 14.9%) than in four other cohorts (10.1% in a Taiwanese cohort[12] and 2.8% in a Norwegian cohort[13], both with EO defined as <45 years; 5.9% in a UK series[14] and 2.8% in a larger multicenter sample[15], both with EO <50 years). A meta-analysis of more than 5800 PD patients, found *PRKN* variants in 8.6% of PD cases with an AAO <50.[15] We provide more precise data for *PRKN* and *PINK1* genes, according to AAO.

GLM analyses revealed that AAO was lower, disease progression slower and the response to levodopa stronger in patients with *PRKN* mutations than in those without pathogenic variants, as previously reported.[12,15,16,17] However, these mutations were not associated with higher rates of dystonia at onset, a trait more strongly associated with EO (see Supplementary Table 1) than with genetic status. Patients with *PRKN* mutations also had a distinctive non-motor symptom profile, with lower frequencies of dementia and dysautonomia, consistent with previous reports.[18] After adjustment for disease duration and dopaminergic medication, these

patients had fewer treatment-induced complications, such as dyskinesia and motor fluctuations, than PD-NM patients. This very pure and slowly progressive phenotype makes patients with *PRKN* variants, very good candidates for deep-brain stimulation (DBS).[19,20]

The causal gene(s) remained unidentified for a number of families with AR PD (~72.5%), suggesting that pathogenic variants of known genes may have been missed or the involvement of unknown genes.

These findings will help to guide routine genetic testing and to establish cohorts of patients for clinical trials targeting the gene defects or their physio-pathological consequences.

Supplementary Materials

This file includes:
Supplementary Table 1
Supplementary Table 2
Supplementary Table 3

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Author Contributions

SL, J-CC, and AB contributed to the conception and design of the study. All authors contributed to the acquisition and analysis of data. SL, AL, MH, CT, ER, J-CC, AB contributed to the drafting of the text and figure preparation.

Potential Conflicts of Interest

None to report.

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Figure Legends

FIGURE 1: Schematic representation of the (A) *PRKN* and (B) *PINK1* genes and respective proteins and associated disease-linked mutations.

Exonic deletions (in red), duplications (in green), or triplications (in blue) are shown in the upper panel and point mutations (missense, frameshift, nonsense, and splice) are shown in the lower panel. Newly identified mutations are shown in bold. Numbers in brackets indicate the number of mutation carriers. *PRKN* cDNA numbering: NM_004562.2; *PINK1* cDNA numbering: NM_032409.2.

Parkin protein: UBL, ubiquitin-like; RING, really interesting new gene; IBR, in-between RING; PINK1 protein: MTS, mitochondrial targeting sequence; TM, transmembrane helix.

FIGURE 2: Distribution of *PRKN* and *PINK1* mutation carriers by age-at-onset, pattern of disease presentation and ethnicity. (A) Proportion of probands, by age-at-onset, for *PRKN* (199 carriers among 1587 probands). (B) Proportion of *PRKN* mutation carriers from 497 cases with autosomal recessive PD (in blue) *vs.* 1090 isolated cases (in orange) by age-at-onset and pattern of presentation of PD. (C) Proportion of probands, by age-at-onset for *PINK1* (23 carriers among 1223 probands). (D) Proportion of *PINK1* mutation carriers from 386 cases with autosomal recessive PD (in blue) *vs.* 837 isolated cases (in orange) by age-at-onset and pattern of presentation of PD. (E) Proportion of *PRKN* and *PINK1* mutation carriers, according to their ethnicity: Caucasians (*n*=1324, in blue) or Arab-Berbers (*n*=213, in orange) .

TABLE 1. Demographic data for our study population

	Whole PD group	*n*	AR PD, including isolated cases with consanguinity	*n*	Isolated cases	*n*	AR PD *vs.* isolated case *p* value
% men (n)	60.0 (951)	1587	57.1 (284)	497	61.2 (667)	1090	0.14
Ethnic background		1587		497		1090	<0.0001*
% Caucasian (n)	83.4 (1324)		75.5 (375)		87.1 (949)		
% Arab-Berbers (n)	13.4 (213)		22.1 (110)		9.4 (103)		
% others/mixed (n)	3.2 (50)		2.4 (12)		3.5 (38)		
% Consanguinity (n)	12.9 (202)	1564	40.9 (202)	494	0	1070	NA
Age-at-onset (SD), y	40.2 (12.1)	1543	43.8 (15.0)	485	38.6 (10.1)	1058	<0.0001*
Range	2-81		3-81		2-74		
Age at examination (SD), y	49.7 (13.2)	1575	54.2 (14.5)	495	47.7 (12.0)	1080	<0.0001*
Range	9-87		16-87		9-79		
Disease duration (SD), y	9.8 (8.6)	1530	10.9 (9.6)	482	9.2 (8.0)	1048	0.0005*
Range	0-63		0-63		0-48		

Frequencies were compared in Fisher's exact tests for qualitative traits and means were compared in t-tests for continuous variables.

*$p < 0.05$
NA, Not appropriate.

TABLE 2. Demographic and clinical characteristics of patients with Parkinson's disease with pathogenic *PRKN* variants (*PRKN*-PD) and of patients without pathogenic variants (PD-NM)

	PD-NM and *PRKN*-PD unadjusted comparisons				PD-NM and *PRKN*-PD adjusted comparisons			
Characteristics	PD-NM *n*=1181	*PRKN*-PD *n*=228	*p* value	*p* value adjusted ¥	Coefficient or odds ratio (OR) (SE)	Cohen's f2	*p* value	*p* value adjusted ¥
Baseline								
Sex (% male)	727/1181 (61.6%)	118/228 (51.8%)	0.0063*	0.016*				
Age at examination (SD), y	50.5 (13.2)	45.4 (12.9)	<0.0001*	<0.0001*				
Disease duration (SD), y	8.8 (7.8)	14.1 (10.4)	<0.0001*	<0.0001*				
Age-at-onset (SD), y	41.6 (12.0)	31.2 (10.7)	<0.0001*	<0.0001*				
L-DOPA-treated	791/1181 (67%)	148/228 (64.9%)	0.54	0.69				
Levodopa responsiveness#	659/738 (89.3%)	142/149 (95.3%)	0.022*	0.045*	1.9 (0.82)	0.007	0.11	0.15
Motor symptoms and signs								
Dystonia at onset	164/990 (16.6%)	37/201 (18.4%)	0.54	0.69	0.84 (0.18)	0.001	0.42	0.46
Akinesia at onset	590/946 (61.3%)	99/206 (48.1%)	0.0002*	0.0010*	0.51 (0.09)	0.015	0.0001*	0.0003*
Tremor at onset	570/960 (59.4%)	142/205 (69.3%)	0.0091*	0.021*	1.7 (0.29)	0.007	0.0030*	0.0076*
Micrographia at onset	308/927 (33.2%)	42/204 (20.6%)	0.0003*	0.0013*	0.58 (0.11)	0.007	0.0047*	0.010*
Asymmetry	1001/1035 (96.7%)	181/198 (91.4%)	0.0016*	0.0049*	0.26 (0.09)	0.010	0.0002*	0.0005*
Bradykinesia	1033/1069 (96.6%)	201/208 (96.6%)	1.0000	1.0000	1.4 (0.63)	0.002	0.43	0.46
Rigidity	1008/1066 (94.6%)	194/204 (95.1%)	0.87	0.90	1.1 (0.42)	<0.001	0.77	0.77
Tremor	796/1056 (75.4%)	168/205 (82%)	0.031*	0.057	1.5 (0.32)	0.002	0.048*	0.072
UPDRS-Part III ON (SD)	19.6 (13.8)	15.9 (11.9)	0.0017*	0.0049*	-3.3 (1.2)	0.008	0.0081*	0.014*
Hoehn&Yahr ON (SD)	2 (0.91)	2.00 (0.93)	0.61	0.74	-0.14 (0.09)	0.003	0.13	0.16
Dyskinesia	457/667 (68.5%)	97/178 (54.5%)	0.0007*	0.0025*	0.44 (0.09)	0.020	0.0001*	0.0005*
Motor fluctuations	485/663 (73.2%)	82/177 (46.3%)	<0.0001*	<0.0001*	0.32 (0.07)	0.041	<0.0001*	<0.0001*
Non-motor symptoms and signs								
Dysautonomia	254/470 (54.0%)	36/192 (18.8%)	<0.0001*	<0.0001*	0.19 (0.05)	0.095	< 0.0001*	<0.0001*
Dementia	67/667 (10.0%)	6/153 (3.9%)	0.017*	0.037*	0.34 (0.16)	0.009	0.0087*	0.014*

Data are expressed as mean (standard deviation) for continuous variables, and as counts (percentages) for categorical variables. We used *t*-tests to compare the two groups for continuous variables and Fisher's exact tests for binary variables.

Coefficients for continuous clinical features and odds ratios (ORs) for binary clinical features and standard error (SE), Cohen's f2 and *p*-values were calculated from GLMs with mutation status, sex, age-at-onset, disease duration, L-DOPA group and age-at-onset vs. disease duration for all 15 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 links and Bernouilli distributions were used for binary variables

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

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

*$p < 0.05$