A comparative study of *LRRK2*, *PINK1* and genetically undefined familial Parkinson's disease

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ABSTRACT

Genetic classification of Parkinson's disease (PD) subtypes may become the preferred diagnostic tool for neurologists. Herein we compare clinical features from a large cohort of patients with familial PD of unknown aetiology or attributable to distinct genetic forms. Comprehensive neurological examinations were performed in 231 familial PD patients from Tunisia. Analysis was previously performed to screen for mutations in *leucine rich repeat kinase 2* (*LRRK2*), *PTEN induced kinase 1* (*PINK1*) and *parkin* (*PRKN*). Clinical features were compared between patients with genetically undefined PD (n=107) and those with *LRRK2* (n=73) and *PINK1* (n=42) mutations using regression analyses adjusted for gender, age of onset and disease duration. *PRKN* cases (n=9) were too few for meaningful statistical analysis. In comparison with genetically undefined patients, *LRRK2* mutation carriers had more severe motor symptoms (median Unified Parkinson's Disease Rating Scale scores ~1.6 times higher, $p<0.001$), a higher rate of dyskinesia (OR 4.21, $p=0.002$) and use of dopamine agonists (OR 3.64, $p<0.001$), and less postural tremor (OR 0.21, $p<0.001$). *PINK1* mutation carriers presented an increased rate of drug induced dyskinesia (OR 3.81, $p=0.007$) and a lower rate of postural tremor (OR 0.16, $p<0.001$) than genetically undefined patients. As expected, *PINK1* patients had younger ages and ages at disease onset, and a longer disease duration compared with *LRRK2* mutation carriers and genetically undefined patients. Clinical differences between *LRRK2*, *PINK1* and genetically undefined familial PD appear more pronounced than previously appreciated, and may prove useful in clinical practice. As future therapies are targeted to specific protein abnormalities, identifying the genetic causes and associated clinical and pathological features will determine diagnosis, preventative medicine and drug intervention strategies.

INTRODUCTION

Parkinson's disease (PD) is a complex neurodegenerative disorder in which an increasing proportion of familial disease has been ascribed to genetic mutations.[1] Parkinsonism resulting from *α-synuclein* (*SNCA*; MIM 163890) and *leucine rich repeat kinase 2* (*LRRK2*; MIM 609007) mutations has dispelled the belief that sporadic PD and familial disease are distinct entities.[2–4] However, clinical differences are apparent between known forms of genetically defined parkinsonism.

The observed frequency of specific *LRRK2* mutations has made this gene the major, dominantly inherited, genetic factor implicated in late onset familial and sporadic PD identified to date.[5–9] Although genetic mutations in patients with sporadic PD are generally rare, the first common functional risk variants have been identified in Asian populations (Lrrk2 p.G2385R and p.R1628P) with low OR of disease (~2) but a high frequency in control subjects (~5%).[3 4] Perhaps even more remarkable are the findings from the Lrrk2 p.G2019S mutation. Initially found in 1–2% of sporadic PD and 5–6% of familial parkinsonism in Europe and North America, these values dramatically increase up to 37% (sporadic PD) and 41% (familial parkinsonism) among North African Arabs.[9–13]

Our recent study in a Tunisian sample of PD patients found frequencies of 30% for Lrrk2 p.G2019S.[12] We also identified a high proportion of patients with homozygous mutations in *PTEN induced kinase 1* (*PINK1*; MIM 608309) (~18%)[14] and a small number with *Parkin* (*PRKN*; MIM 602544) homozygous mutations. The high prevalence of pathogenic mutation carriers provides a unique opportunity to compare clinical phenotypes both in early and late onset forms of the disease. Thus we sought to compare the clinical characteristics between patients with familial PD not genetically defined (mutation negative patients), *LRRK2* patients and *PINK1* patients from a single population. This type of clinical–genetic analysis may help refine the nosological classification of PD and its subtypes, thereby benefiting both neurologists and patients in a diagnostic setting.

MATERIALS AND METHODS

A total of 231 prevalent familial PD patients from 90 families were recruited through the Institut National de Neurologie, Tunis, Tunisia.[14] PD was considered familial if at least one additional family member of first, second or third degree was affected. This referral centre provides specialised neurological service to the entire country of Tunisia. Appropriate local ethics committee approval was obtained prior to recruitment. Patients were informed of all aspects pertaining to their participation in the study and gave either written or proxy consent. Comprehensive standardised interviews and neurological examinations were performed by neurologists specialised in movement disorders (FH, SBS, MK). Individuals were diagnosed as affected if they

satisfied the UK PD Society Brain Bank criteria for probable PD.[15] Diagnostic scales administered included the Hoehn and Yahr staging in 'on' medication conditions and the Unified Parkinson's Disease Rating Scale (UPDRS) in 'on' and 'off' medication conditions ('off' medication was defined as no dopaminergic treatment for at least 12 h). Levodopa equivalent per day (LED) was determined as previously described.[16] Patients were screened for mutations in *LRRK2*, *PINK1* and *PRKN* using standard protocols.[12 14] Patients not harbouring a mutation in *LRRK2*, *SNCA*, *PINK1*, *PRKN* or *DJ1* were considered genetically undefined.

Of note, there were a number of genetically undefined (n=44), *LRRK2* (n=31) and *PINK1* (n=23) patients for whom information was unavailable regarding Hoehn and Yahr staging and UPDRS scores. It is unlikely that this information was unavailable for any systematic reason (eg, missing data for patients in more advanced disease stages) but rather due to incomplete data acquisition. There were no noticeable differences in demographic information between patients with and without Hoehn and Yahr staging and UPDRS score information for genetically undefined, *LRRK2* or *PINK1* patients (data not shown).

Numerical patient demographics (age, age at onset, disease duration) and clinical features (Hoehn and Yahr score, UPDRS scores, LED) were summarised with the sample median, minimum and maximum. Binary categorical clinical features (dyskinesia, dystonia, motor fluctuations, dopamine agonist use, tremor, initial symptom) were summarised with number and percentage. The primary aim was to compare clinical features between genetically undefined patients and those with *LRRK2* or *PINK1* disease, and between patients with *LRRK2* and *PINK1* disease. *PRKN* patients were not included in any formal statistical analysis owing to the small number of these patients but clinical features are provided for descriptive purposes.

To account for the lack of independence between members of the same family, clinical features were compared between genetically undefined, *LRRK2* and *PINK1* patients using linear mixed effects regression models including a random effect for family (numerical features) and generalised estimating equations[17] (binary categorical features). Single variable models were utilised with only disease group as a covariate, as well as multivariable models adjusting for age at onset, disease duration and gender. Regression coefficients and 95% CIs were estimated from linear mixed effects regression models while odd ratios (ORs) and 95% CIs were estimated from generalised estimating equations. The natural logarithm of the Hoehn and Yahr score and UPDRS scores was used in linear mixed effects regression analysis due to their skewed distributions.

Because of the relatively large number of statistical tests that were performed, some adjustment for multiple testing is required in order to control the family-wise error rate at 5%. Overall tests of difference between disease groups (genetically undefined, *LRRK2*, *PINK1*) were performed for 14 clinical features. Therefore, for this family of statistical tests, we considered $p \leq 0.0036$ statistically significant after a Bonferroni adjustment for multiple testing. For each clinical feature where there was at least marginal evidence ($p \leq 0.005$) of a difference between disease groups in multivariable analysis, we performed three pairwise comparisons (genetically undefined vs *LRRK2*, genetically undefined vs *PINK1* and *LRRK2* vs *PINK1*); for these families of pairwise comparisons we considered $p \leq 0.0167$ statistically significant after a Bonferroni adjustment for multiple testing. Statistical analyses were performed using SPLUS (V.8.0.1; Seattle, WA) and the SAS software package (SAS Institute, Chicago, Illinois, USA).

Table 1 Demographics of patients with genetically undefined, *LRRK2*, *PINK1* and *PRKN* Parkinson's disease

Variable	Genetically undefined PD (n=107)	*LRRK2* (n=73)	*PINK1* (n=42)	*PRKN* (n=9)
Age (years)	69 (27–96)	70 (38–91)	51 (25–76)	47 (32–79)
Age at onset (years)	60 (9–85)	60 (30–87)	35 (13–59)	32 (21–74)
Gender (Male) (n (%))	66 (62)	36 (49)	22 (52)	3 (33)
Disease duration (years)	6 (<1–49)	6 (1–28)	14 (<1–40)	11 (4–34)

The sample median (minimum-maximum) is given for age, age at onset and disease duration. *LRRK2, leucine rich repeat kinase 2*; *PINK1, PTEN induced kinase 1*; *PRKN*, *parkin*.

RESULTS

Patient characteristics for genetically undefined PD patients (n=107) and those with *LRRK2* (n=73), *PINK1* (n=42) or *PRKN* disease (n=9) are shown in table 1 and figure 1. All patients with a *LRRK2* mutation carried the p.G2019S substitution (20 of them in a homozygote state) whereas *PINK1* carriers were homozygous for p.Q129X, p.Q129fsX157, p.G440E or p.Q456X. Data for *PRKN* patients are presented for descriptive purposes only and therefore only comparisons between genetically undefined, *LRRK2* and *PINK1* patients are mentioned. As expected, age and age at onset were lower in *PINK1* patients compared with genetically undefined and *LRRK2* patients while disease duration was longer in *PINK1* patients compared with the two other patient groups. Age, age at onset and disease duration were similar in genetically undefined and *LRRK2* patients.

A comparison of clinical features between PD groups is shown in table 2 (numerical features) and table 3 (categorical features). Comparisons are shown without adjustment for any other variables (single variable analysis) in an exploratory analysis and when adjusting for the potentially confounding variables of age at onset, disease duration and gender in the primary multivariable analysis. Multivariable models were not additionally adjusted for age due to its high degree of correlation with age of onset (Pearson's correlation=0.83, $p<0.001$). When adjusting for age at onset, disease duration and gender, there was evidence of a difference in the numerical features of UPDRS III score 'on', UPDRS III score 'off' and UPDRS total score between patients with genetically undefined, *LRRK2* and *PINK1* disease (all

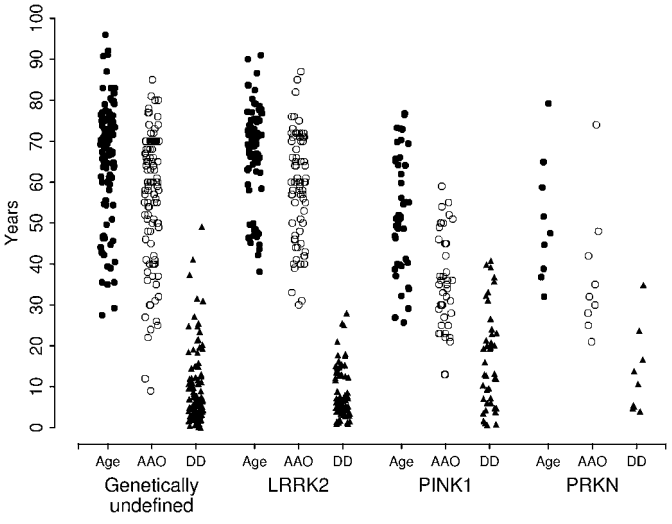

Figure 1 Patient demographic information for genetically undefined, *LRRK2*, *PINK1* and *PRKN* patients. AAO, age at onset; DD, disease duration; *LRRK2, leucine rich repeat kinase 2*; *PINK1, PTEN induced kinase 1*; *PRKN*, *parkin*.

Table 2 Comparison of numerical clinical features between Parkinson's disease subgroups

Numerical feature	Median (minimum–maximum)				Single variable analysis p Value	Adjusting for gender, AAO, and DD p Value
	Genetically undefined (n=107)	LRRK2 (n=73)	PINK1 (n=42)	PRKN (n=9)		
Hoehn and Yahr "on"	1 (0–5)	2 (0–5)	2 (1–4)	2 (2–4)	0.004	0.006
UPDRS III score "on"	6 (0–27)	10 (2–22)	8 (1–19)	7 (3–24)	0.002	0.003
UPDRS III score "off"	20 (3–80)	31 (8–80)	34 (2–67)	41 (20–78)	0.003	0.002
UPDRS total score "on"	27 (4–115)	41 (11–116)	44 (2–101)	52 (23–112)	0.004	0.002
LED	375 (0–1250)	375 (0–1125)	375 (0–1250)	500 (120–625)	0.51	0.25

p Values for numerical features result from linear mixed effects regression models including a random effect for family, comparing genetically undefined, *LRRK2* and *PINK1* patients. Data for *PRKN* patients was included for descriptive purposes only and was not included in any formal statistical analysis.
There was a significant amount of missing data for Hoehn and Yahr and UPDRS scores; information for these variables was available for 63 genetically undefined patients, 42 *LRRK2* patients, 19 *PINK1* patients and six *PRKN* patients.
AAO, age at onset; DD, disease duration; LED, levodopa equivalent per day; *LRRK2, leucine rich repeat kinase 2*; *PINK1, PTEN induced kinase 1*; *PRKN*, *parkin*; UPDRS, Unified Parkinson's Disease Rating Scale.

p≤0.003), even after adjustment for multiple testing (p≤0.0036 considered statistically significant). There was also a trend (p=0.006) towards a difference in Hoehn and Yahr score between the three groups, with genetically undefined patients (median=1) having lower scores than *LRRK2* (median=2) and *PINK1* (median=2) patients.

When considering categorical features, there was evidence of a difference in the rate of dyskinesia, motor fluctuations, dopamine agonist use, any tremor, postural tremor and resting tremor in the single variable analysis (all p≤0.006). Multivariable analysis was not possible for drug induced dystonia, tremor, resting tremor and dystonia not related to treatment, due to the small number of patients who did or did not experience these features. In multivariable analysis, only drug induced dyskinesia, dopamine agonist use and postural tremor showed evidence of a difference between genetically undefined, *LRRK2* and *PINK1* patients after adjustment for multiple testing (all p≤0.004).

Pairwise comparisons are shown in table 4, and were performed only in the presence of at least marginal evidence (p≤0.005) of an overall difference between groups in the multivariable analysis. The differences in UPDRS scores between the three groups all appeared to be due to a difference between *LRRK2* and genetically undefined patients where median UPDRS scores were all approximately 1.6 times higher in the *LRRK2* group (all p<0.001) (figure 2). Although not statistically significant (all p≤0.083), there were trends towards higher UPDRS scores in *LRRK2* patients compared with *PINK1* patients. There was no evidence of a difference in UPDRS scores between genetically undefined and *PINK1* patients (p>0.56). In comparison with genetically undefined patients, there was strong evidence of a higher rate of dyskinesia in *LRRK2* patients (OR 4.21, 95% CI 1.71 to 10.35, p=0.002) and in *PINK1* patients (OR 3.81, 95% CI 1.44 to 10.07, p=0.007). Dopamine agonist use was also more common in *LRRK2* patients compared with genetically undefined patients (OR 3.64, 95% CI 1.83 to 7.23, p<0.001) and, although not statistically significant, in *PINK1* patients compared with genetically undefined patients (OR 3.16, 95% CI 1.06 to 9.47, p=0.040). There was strong evidence of a lower rate of postural tremor in both *LRRK2* patients and *PINK1* patients compared with genetically undefined patients (ORs 0.21 and 0.16, respectively, both p<0.001), and no evidence of a difference in the rate of dyskinesia, dopamine agonist use or postural tremor between *LRRK2* and *PINK1* patients.

DISCUSSION

Herein we have compared the clinical features of patients with familial PD of unknown aetiology and those carrying *LRRK2* or *PINK1* pathogenic mutations in a Tunisian population with a high prevalence of Lrrk2 p.G2019S (32%) and *PINK1* (18%) mutation carriers. Analysis of the detailed clinical data collected shows that those Tunisian patients harbouring Lrrk2 p.G2019S have a more severe motor phenotype than mutation negative patients, despite similar ages, ages at onset and disease duration.

Table 3 Comparison of categorical clinical features between Parkinson's disease subgroups

Categorical feature	Fraction (%)				Single variable analysis p Value	Adjusting for gender, AAO, and DD p Value
	Genetically undefined (n=107)	LRRK2 (n=73)	PINK1 (n=42)	PRKN (n=9)		
Initial symptom (non-tremor)	14/106 (13%)	12/72 (17%)	16/41 (39%)	2/9 (22%)	0.012	0.39
Drug induced dyskinesia	11/107 (10%)	18/73 (25%)	21/42 (50%)	3/9 (33%)	<0.001	0.004
Drug induced dystonia	10/107 (10%)	6/72 (8%)	8/41 (20%)	1/9 (11%)	0.13	N/A ψ
Drug induced motor fluctuations	10/106 (9%)	15/73 (21%)	14/42 (33%)	4/9 (44%)	<0.001	0.042
Dopamine agonist use	57/104 (55%)	57/71 (80%)	36/41 (88%)	6/8 (75%)	<0.001	<0.001
Tremor	101/107 (94%)	69/73 (95%)	33/42 (79%)	8/9 (89%)	0.006	N/A ψ
Tremor–postural	70/101 (69%)	20/69 (29%)	11/33 (33%)	5/8 (63%)	<0.001	<0.001
Tremor–resting	80/101 (79%)	68/69 (99%)	32/32 (100%)	8/8 (100%)	0.006†	N/A ψ
Dystonia not related to treatment	8/107 (7%)	8/73 (11%)	6/42 (14%)	3/9 (33%)	0.44	N/A ψ

†Due to a zero cell count in the *PINK1* patients, one patient in the same family who did not experience resting tremor was added to each disease group to allow for generalised estimating equation analysis.
ψNo multivariable analysis was possible due to the small number of patients who did or did not experience these features.
p Values for categorical features result from generalised estimating equations, comparing genetically undefined, *LRRK2* and *PINK1* patients. Data for *PRKN* patients was included for descriptive purposes only and was not included in any formal statistical analysis. For categorical features, fractions correspond to the number of patients for whom information was available.
No multivariable analysis was possible due to the small number of patients who did or did not experience these features.
AAO, Age at onset; DD, disease duration; *LRRK2, leucine rich repeat kinase 2*; *PINK1, PTEN induced kinase 1*; *PRKN*, *parkin*.

Table 4 Pairwise comparisons of clinical features between Parkinson's disease subgroups

	LRRK2 versus genetically undefined		PINK1 versus genetically undefined		LRRK2 versus PINK1	
Numerical feature	**Multiplicative effect (95% CI)**	**p Value**	**Multiplicative effect (95% CI)**	**p Value**	**Multiplicative effect (95% CI)**	**p Value**
UPDRS III score "on"	1.60 (1.23 to 2.08)	<0.001	1.11 (0.76 to 1.62)	0.57	1.44 (0.95 to 2.17)	0.083
UPDRS II score "off"	1.67 (1.26 to 2.21)	<0.001	1.07 (0.71 to 1.61)	0.74	1.56 (1.00 to 2.43)	0.049
UPDRS total score	1.70 (1.28 to 2.28)	<0.001	1.10 (0.72 to 1.68)	0.67	1.55 (0.98 to 2.46)	0.060
Categorical feature	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**
Drug induced dyskinesia	4.21 (1.71 to 10.35)	0.002	3.81 (1.44 to 10.07)	0.007	0.90 (0.38 to 2.13)	0.82
Dopamine agonist use	3.64 (1.83 to 7.23)	<0.001	3.16 (1.06 to 9.47)	0.040	0.87 (0.28 to 2.68)	0.81
Tremor—postural	0.21 (0.10 to 0.41)	<0.001	0.16 (0.06 to 0.41)	<0.001	0.78 (0.27 to 2.23)	0.64

p Values and multiplicative effects for numerical features result from linear mixed effects regression models adjusted for age of onset, disease duration and gender, including a random effect for family. Multiplicative effects are interpreted as the multiplicative increase on the median in comparison with the reference group. p Values and ORs for categorical features result from generalised estimating equations adjusted for age of onset disease duration, and gender.
AAO, age at onset; DD, disease duration; *LRRK2, leucine rich repeat kinase 2*; *PINK1, PTEN induced kinase 1*; *PRKN*, *parkin*; UPDRS, Unified Parkinson's Disease Rating Scale.

This may indicate a more rapid progression of motor symptoms among Lrrk2 p.G2019S carriers which could have significant prognostic implications.[18] However, an accurate comparison of disease progression rates is hampered by the cross sectional study design, and confirmation in longitudinal studies is required. Recently, in a comparative study of North African Arab PD patients, Lesage *et al* reported dyskinesia to be much more prevalent among Lrrk2 p.G2019S carriers compared with non-carrier sporadic and familial patients.[11] In good agreement with these results, our affected individuals with Lrrk2 p.G2019S had a higher prevalence of dyskinesia than patients with genetically undefined PD, despite being more likely to have received dopamine agonists.[11] Although the overall prevalence of tremor was similar, patients with *LRRK2* linked PD had more resting and less postural tremor than genetically undefined patients. Finally, we found no difference in patient demographics, LED, first symptom of disease or prevalence of dystonia.

In contrast with our findings, a recent multicentre study found similar prevalence rates of dyskinesia in patients with any pathogenic *LRRK2* mutation (58%) compared with idiopathic patients without Lrrk2 p.G2019S but not screened for any other pathogenic mutation (54%).[9] Furthermore, Healy *et al* reported a longer time to onset of dyskinesia in patients with *LRRK2* mutations than in those without the Lrrk2 p.G2019S mutation (8.4 years vs 5.6 years, p<0.0001). Dystonia was more common in the *LRRK2* group than in the non-Lrrk2 p.G2019S group (42% vs 25%); however, no comparison was provided for severity of motor symptoms (UPDRS III).[9] Discrepant results may reflect the large size of our cohort with comprehensive data from patients of one ethnicity, seen at one centre, compared with a multicentre design.[9] In addition, all of our *LRRK2* patients harboured the same p.G2019S mutation, all pathogenic *LRRK2*, *PINK1* and *PRKN* mutations were excluded from our genetically undefined group, and our study was based exclusively on familial patients. Alternatively, population specific phenotypic differences may exist, which may also account for the relatively low rates of overall dyskinesia and motor fluctuations in our series compared with those reported in Western populations.[19]

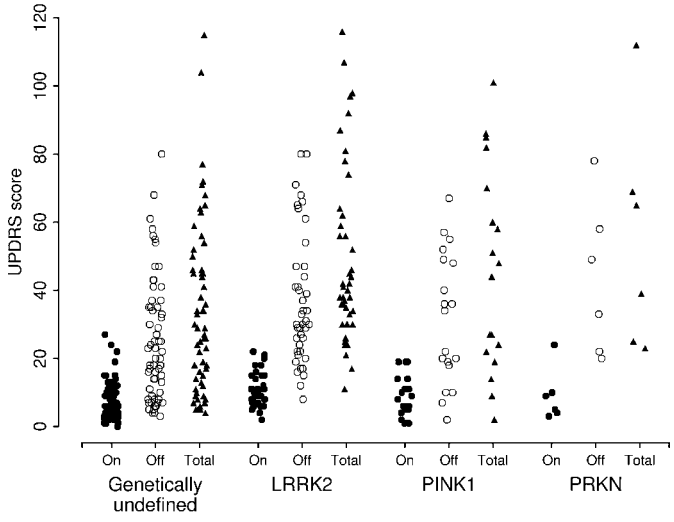

Figure 2 Unified Parkinson's Disease Rating Scale (UPDRS) scores for genetically undefined, *LRRK2*, *PINK1* and *PRKN* patients. *LRRK2, leucine rich repeat kinase 2*; *PINK1, PTEN induced kinase 1*; *PRKN*, *parkin*.

Overall, as expected, patients with *PINK1* mutations had approximately 20 years younger age at onset than patients with an *LRRK2* mutation and those with no identified genetic cause. In agreement with previous reports, patients with *PINK1* linked PD had a longer disease course and a higher prevalence of dyskinesia and motor fluctuations.[20–23] *PINK1* homozygotes had a lower overall prevalence of tremor and, when present, was more often of the resting type, a feature that has not been emphasised in other studies. Patients with *PINK1* linked disease had more dystonia than those with genetically undefined PD but the trend observed did not reach statistical significance. Also, patients with *PINK1* mutations had similar disease severity and LED. Compared with *LRRK2* mutation carriers, patients with *PINK1* mutations had younger ages and ages at disease onset, and a longer duration of disease but there were no dramatic differences in other clinical characteristics. Although the *PRKN* sample size is too small to draw conclusive results, clinically, *PRKN* patients appear to be more similar to *PINK1* patients than to those with *LRRK2* or genetically undefined PD.

Our results suggest there may be more clinical differences between *LRRK2* or *PINK1* patients and those with genetically undefined PD than previously reported. *LRRK2* mutation carriers appear to have the most severe clinical phenotype while *PINK1* carriers have a longer disease course and the lowest incidence of tremor as the initial symptom. *LRRK2* and *PINK1* carriers have an increased prevalence of resting tremor and dyskinesia compared with those not genetically defined. These differential clinical signs may help to diagnose patients, categorise PD subtypes and determine therapeutic intervention strategies with greater efficacy.

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Competing interests MJF reports (a) International Publication Number WO 2006/045392 A2; (b) International Publication Number WO 2006/068492 A1; (c) US Patent Number 7,544,786; and (d) Norwegian patent 323 175, appertaining to Lrrk2. MJF reports salary and royalty payments from the pharmaceutical industry for sponsored research on Lrrk2 biology and mouse model characterisation. As of August 2009, Mayo and MJF have received royalties from the licensing of these technologies of greater than $10 000, the US federal threshold for significant financial interest.

Ethics approval This study was conducted with the approval of the Institut National de Neurologie, Tunis, Tunisia.

Contributors All co-authors have seen and agreed with the content of the manuscript and declare no financial interests. Authors take full responsibility for the data, the analyses and interpretation, and the conduct of research; full access to all of the data; and the right to publish any and all data.

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