Nonmotor Symptoms in LRRK2 G2019S Associated Parkinson’s Disease

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Abstract

Background: Idiopathic Parkinson’s disease (IPD) and LRRK2-associated PD (LRRK2-PD) might be expected to differ clinically since the neuropathological substrate of LRRK2-PD is heterogeneous. The range and severity of extra-nigral nonmotor features associated with LRRK2 mutations is also not well-defined.

Objective: To evaluate the prevalence and time of onset of nonmotor symptoms (NMS) in LRRK2-PD patients.

Methods: The presence of hyposmia and of neuropsychiatric, dysautonomic and sleep disturbances was assessed in 33 LRRK2-G2019S-PD patients by standardized questionnaires and validated scales. Thirty-three IPD patients, matched for age, gender, duration of parkinsonism and disease severity and 33 healthy subjects were also evaluated.

Results: University of Pennsylvania Smell Identification Test (UPSIT) scores in LRRK2-G2019S-PD were higher than those in IPD (23.5 ± 6.8 vs 18.4 ± 6.0; p = 0.002), and hyposmia was less frequent in G2019S carriers than in IPD (39.4% vs 75.8%; p = 0.011). UPSIT scores were significantly higher in females than in males in LRRK2-PD patients (26.9 ± 4.7 vs 19.4 ± 6.8; p < 0.01). The frequency of sleep and neuropsychiatric disturbances and of dysautonomic symptoms in LRRK2-G2019S-PD was not significantly different from that in IPD. Hyposmia, depression, constipation and excessive daytime sleepiness, were reported to occur before the onset of classical motor symptoms in more than 40% of LRRK2-PD patients in whom these symptoms were present at the time of examination.

Conclusion: Neuropsychiatric, dysautonomic and sleep disturbances occur as frequently in patients with LRRK2-G2019S-PD as in IPD but smell loss was less frequent in LRRK2-PD. Like in IPD, disturbances such as hyposmia, depression, constipation and excessive daytime sleepiness may antedate the onset of classical motor symptoms in LRRK2-G2019S-PD.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

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Introduction

Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) are the most common cause of inherited parkinsonism and account for a significant proportion of familial and sporadic Parkinson’s disease (PD) cases [1–2]. The neuropathological substrate in LRRK2-PD is in some cases quite different from idiopathic PD (IPD) and ranges from brainstem or diffuse Lewy body pathology, to nigral degeneration without distinctive histopathology, and to progressive supranuclear palsy-like pathology. Age at disease onset and parkinsonian motor features are similar between LRRK2 related PD (LRRK2-PD) and idiopathic PD (IPD) [3] but studies assessing nonmotor symptoms (NMS) in LRRK2-PD patients are limited and results at times conflicting [4–6]. Dysautonomia, sleep and mood disturbances as well as other common NMS occurring in IPD result, in part, from extra-nigral lesions in the brain and the peripheral autonomic nervous system [7]. The heterogeneous neuropathology of LRRK2-PD suggests that the range and severity of extra-nigral NMS could differ from those encountered in IPD.

The aim of the present study was to evaluate the prevalence and estimate the onset of NMS in patients with LRRK2 G2019S PD.

Methods

Subjects

A sample of 1251 PD patients from two regions of Spain, Catalonia (Hospital Clinic de Barcelona, Hospital Mutua de Terrassa, Hospital Vall d’Hebron and Hospital de Bellvitge) and Cantabria (Hospital Marqués de Valdecilla, Santander) was screened for LRRK2 G2019S and codon 1441 (R1441G/C/H) mutations as previously described [3]. PD was diagnosed according to UK Parkinson’s Disease Society criteria [8]. LRRK2 G2019S carriers were proposed to participate in this study. LRRK2-PD patients that met diagnostic criteria for dementia in PD (PDD) [9] were excluded, to avoid interference of cognitive impairment in the evaluation of NMS. A group of 33 IPD patients were recruited as controls among those from the initial sample of 1180 patients that tested negative for LRRK2 mutations. We also studied age and gender matched healthy subjects (HS) without PD, dementia, any other neurological disorder or a positive family history for PD. Patients, their relatives and the written informed consent was obtained from all study subjects. The aim of the present study was to evaluate the prevalence and estimate the onset of NMS in patients with LRRK2 G2019S PD.

Nonmotor Symptoms in LRRK2 Parkinson’s Disease

Parkinsonism evaluation

Information about motor symptoms (rest tremor, bradykinesia, rigidity, postural instability and persistent asymmetry), as well as development of levodopa-induced motor complications, freezing of gait and repeated falls during the disease course, was collected by means of a structured clinical interview. All patients were assessed through the Unified Parkinson’s Disease Rating Scale (UPDRS), Schwab & England scale and H-Y stage in On condition. Medications at the time of the evaluation were also recorded and levodopa equivalent daily dose (LED) calculated [10].

Nonmotor symptoms evaluation

The presence of the following NMS was assessed: hyposmia; neuropsychiatric disorders: depression, anxiety, hallucinations and psychosis; dysautonomic symptoms: constipation and urinary dysfunction; and sleep disturbances: REM Sleep Behaviour Disorder (RBD), excessive daytime sleepiness (EDS) and insomnia. A structured clinical interview was conducted to determine the presence of each NMS at the time of the evaluation. When a NMS was present, we asked the patient to estimate when it had been developed in relation to the OMS (e.g. before, concurrently or after). Each NMS was also evaluated by using several self-administered tests. Information on the current use of medications, such as laxatives, hypnotics or antidepresants to treat some of these NMS, was also collected.

Smell loss was assessed by asking to the subjects whether they experienced a loss or change in their ability to smell. The 40-items University of Pennsylvania Smell Identification Test (UPSIT; Smell Identification Test™ Sensorsics, Spanish version) [11] was administered. Factors that could impair odor identification, such as active smoking habit, past-history of significant traumatic head injury or rhinologic disorders, were also considered. UPSIT scores obtained previously from 148 HS aged 30 to 85 year-old were used for comparison with LRRK2-PD and IPD. Since odor identification has been shown to decline with age and is better in females than in males [11–12], these 148 HS were stratified by age ($<$50 years, n = 36; 50–69 years, n = 76; and $\geq$70, n = 36) and gender (males, n = 73; females, n = 75). Mean and standard deviation (SD) UPSIT score for each age-gender HS subgroup was calculated. Hyposmia was considered to be present if the UPSIT score was lower than the mean–2SD corresponding to the age-gender matched HS subgroup.

Presence of depression and anxiety at the time of this study were diagnosed according to DSM-IV criteria [13]. In addition, a previous history of depression or anxiety was also recorded. The Hospital Anxiety and Depression scale (HADS) [14] was used to quantify the symptoms. To assess the presence of hallucinations and psychosis the NINS-NIMH work group criteria for psychosis in PD [15] and the Parkinsonian Psychosis Rating Scale (PPRS) were used [16].

Constipation was diagnosed according to the Rome criteria [17]. Bowel movements frequency was evaluated. The Bristol Scale Stool was used to assess whole gut transit time [18]. Urinary dysfunction was assessed by asking for long-lasting complaints of urinary urgency, frequency, incontinence or incomplete emptying. The SCOPA-AUT was administered [19].

RBD was considered to be present when a history of problematic sleep behaviors that were potentially harmful, disrupted sleep continuity or was annoying to self or bed partner was present [20]. EDS occurred when the subject could fall asleep at least twice a day and the total sleeping time during the daytime was more than 1 hour [21] and insomnia when there were long-lasting complaints of difficulty in initiating or maintaining sleep. The Pittsburgh sleep quality index (PSQI), the Epworth sleepiness scale (ESS) and the Parkinson’s disease sleep scale (PDSS) were administered [22–24].

Statistical analyses. Categorical variables and the frequency of each NMS in the different groups were assessed by using the Chi-square test or the Fisher’s exact test when appropriated. For continuous variables, the Kruskal-Wallis analysis and the Mann-Whitney U test were used to compare the means of groups for
Results

General demographic data and parkinsonian motor symptoms

Among 66 PD patients identified as LRRK2 G2019S carriers, only 33 were included in the study (22 from Barcelona and 11 from Santander). One LRRK2-PD patient was excluded because of severe dementia. Of the remaining 32 non-participants, 10 declined to participate, 11 had died when the study was initiated and 11 had been lost for follow up. Thirty-three IPD patients and 35 HS (22 from Barcelona and 11 from Santander in each group) were included. Mean age was not statistically different between LRRK2-PD, IPD and HS (Table 1). Family history for PD was positive in 20 (60.6%) of LRRK2-PD patients. There were no significant differences in the features of motor symptoms, or in dopamine replacement treatment (Table 1).

Nonmotor symptoms

Olfaction. Awareness of smell loss occurred in a similar frequency in both LRRK2-PD and IPD patients (54.5% vs 63.6%; p = 0.45; Table 2) and more frequently than in HS. Mean UPSIT scores, though, were significantly different among the three groups. LRRK2-PD patients had a mean UPSIT score significantly higher than IPD patients (23.5 ± 6.8 vs 18.4 ± 6.0; p = 0.002) and lower than HS (29.5 ± 4.3; p = 0.001) (Figure 1 A). Hyposmia was present in 13 (39.4%) of G2019S carriers, significantly lower than in IPD (25 (75.0%); p = 0.01) (Table 2). No significant differences were found in the frequency of active smoking, rhinologic pathology or history of head trauma between groups.

Mean UPSIT score in LRRK2-PD cases was significantly higher in females than in males (28.9 ± 4.7 vs 19.4 ± 6.8, p < 0.01; Figure 1 B). Hyposmia was present in only 3 (16.7%) LRRK2-PD females, in contrast to 10 (66.7%) LRRK2-PD males. Such significant difference in UPSIT scores by gender was not observed in IPD patients (18.6 ± 5.8 males vs 18.2 ± 6.3 females; p = 0.66) or HS (29.1 ± 4.9 males vs 30.7 ± 3.4 females; p = 0.10). LRRK2-PD females were significantly older than LRRK2-PD males (67.8 ± 8.9 vs 61.1 ± 13.3; p = 0.04), but disease duration or severity, and frequency of active smoking, rhinologic pathology or history of head trauma, were similar in both genders. Mean UPSIT score in LRRK2-PD females was significantly higher than in IPD females (p < 0.01) and significantly lower than in HS females (p = 0.01). In contrast, mean UPSIT score in LRRK2-PD males was similar to

Table 1. General demographic data and parkinsonian motor symptoms in patients with LRRK2 G2019S associated Parkinson’s disease, idiopathic Parkinson’s disease and healthy subjects.

|                      | LRRK2 PD (n = 33) | IPD (n = 33) | HS (n = 33) | P       |
|----------------------|------------------|-------------|------------|---------|
| **Age (years)**      | 64.8 ± 11.4      | 65.1 ± 10.0 | 64.8 ± 10.2| 0.99    |
| **Sex (male, %)      | 15 (45.4%)       | 15 (45.4%)  | 15 (45.4%) | 1.0     |
| **Disease duration (years)** | 9.2 ± 5.7 (2–28) | 9.0 ± 6.1 (1–30)| 0.79    |
| **Motor symptoms during disease course; n (%)** |                      |             |            |         |
| Rest tremor          | 29 (87.9%)       | 27 (81.8%)  | 30 (90.9%) | 0.49    |
| Action tremor        | 20 (60.6%)       | 23 (69.7%)  | 27 (81.8%) | 0.44    |
| Bradykinesia         | 33 (100%)        | 33 (100%)   | 33 (100%) | 1.0     |
| Postural instability | 16 (48.5%)       | 11 (33.3%)  | 15 (45.4%)| 0.21    |
| Repeated falls        | 9 (27.3%)        | 7 (21.2%)   | 10 (30.3%)| 0.57    |
| Freezing of gait     | 18 (54.6%)       | 14 (42.4%)  | 22 (66.7%)| 0.32    |
| Persistent Asymmetry | 31 (93.9%)       | 31 (93.9%)  | 33 (100%)| 1.0     |
| Fluctuations         | 18 (54.5%)       | 17 (51.5%)  | 20 (60.6%)| 0.80    |
| Dyskinesias          | 19 (57.6%)       | 16 (48.5%)  | 24 (72.7%)| 0.46    |
| **UPDRS part II**    | 10.3 ± 7.5 (0–26)| 8.3 ± 4.9 (0–24)| 0.48    |
| **UPDRS part III**   | 24.0 ± 14.0 (0–62)| 19.9 ± 12.2 (2–62)| 0.16    |
| **UPDRS part IVA+VIB**| 3.21 ± 3.7 (0–13)| 2.0 ± 2.7 (0–10)| 0.18    |
| **Hoehn & Yahr stage**| 2.1 ± 1.0 (1–4)  | 1.8 ± 0.8 (1–4)  | 0.24    |
| **Schwab & England**| 82.1 ± 14.7 (50–100)| 85.8 ± 10.3 (60–100)| 0.44    |
| Treatment with levodopa; n (%) | 31 (93.9%) | 30 (90.9%) | 1.0    |
| Treatment with dopamine agonists; n (%) | 27 (81.8%) | 25 (75.8%) | 0.55    |
| **Levodopa equivalent daily dose (in mg)** | 793.7 ± 482.1 (105–2474) | 823.0 ± 516.4 (0–1770) | 0.77    |

LRRK2 G2019S PD; LRRK2 G2019S related Parkinson’s disease; IPD: Idiopathic Parkinson’s disease; HS: healthy subjects.

1 Mean ± Standard deviation (Range).

2 Kruskal-Wallis analysis;

3 Mann-Whitney U test;

4 Chi-square test;

5 Fisher’s exact test.

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Figure 1. University of Pennsylvania Smell Identification Test (UPSIT) scores. UPSIT scores in LRRK2 G2019S Parkinson's disease patients, idiopathic Parkinson's disease patients and healthy controls (Figure 1.A). UPSIT score in each group separated by sex (Figure 1.B). Circles represent individual values, while the bar refers to the mean UPSIT score in each group. IPD: idiopathic Parkinson's disease; LRRK2-PD: LRRK2 associated Parkinson's disease, HS: healthy subjects.
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Table 2. Nonmotor symptoms in patients with LRRK2 G2019S associated Parkinson’s disease, idiopathic Parkinson’s disease and healthy subjects.

|                | LRRK2 PD (n = 33) | IPD (n = 33) | Controls (n = 33) | Three groups comparison | FDR p | LRRK2 vs IPD | LRRK2 vs controls | IPD vs controls |
|----------------|-------------------|--------------|------------------|-------------------------|-------|--------------|------------------|-----------------|
| Change in smell sense | 18 (54.5%)         | 21 (63.6%)   | 5 (15.2%)        | 0.021*                  | 0.055 | 0.45*        | 0.02*            | 0.007*          |
| UPSIT Score1   | 23.5±7.8          | 18.4±6.0     | 29.5±4.3         | 0.001*                  | 0.007 | 0.002*       | 0.001*           |                 |
| Hyposmia2      | 13 (39.4%)        | 25 (75.8%)   | 13 (30%)         | 0.001*                  | 0.007 | 0.01*        | 0.003*           | 0.001*          |
| History of depression | 19 (57.6%)        | 12 (36.4%)   | 4 (12.1%)        | 0.024*                  | 0.055 | 0.08*        | 0.006*           | 0.02*           |
| Active depression | 6/19 (31.5%)      | 8/12 (66.6%) | 1/4 (25.0%)      | 0.445*                  |       | 0.703        |                  |                 |
| Antidepressive treatment | 5/19 (26.3%)    | 9/12 (75.0%) | 1/4 (25.0%)      | 0.24*                   |       | 0.45         |                  |                 |
| History of anxiety | 8 (24.2%)         | 9 (27.3%)    | 5 (15.2%)        | 0.609*                  |       | 0.794        |                  |                 |
| Current active anxiety | 2/8 (25.0%)      | 6/9 (66.7%)  | 3/5 (60.0%)      | 0.559*                  |       | 0.772        |                  |                 |
| Treatment for anxiety | 3/8 (37.5%)      | 2/9 (22.2%)  | 3/5 (60.0%)      | 0.642*                  |       | 0.802        |                  |                 |
| HADS total     | 10.4±5.5          | 10.1±6.6     | 8.0±5.6          | 0.26*                   |       | 0.459        |                  |                 |
| HADS depression | 5.2±2.7           | 5.0±3.9      | 3.0±2.9          | 0.001*                  | 0.007 | 0.54*        | 0.001*           | 0.02*           |
| HADS anxiety   | 4.9±3.6           | 5.1±3.8      | 5.0±3.5          | 0.98*                   |       | 0.985        |                  |                 |
| Hallucinations | 3 (9%)            | 5 (15%)      | NA               |                         |       | 0.40*        |                  |                 |
| PPRS score1    | 0.3±0.5           | 0.5±0.9      | 0.03±0.7         | 0.018*                  | 0.055 | 0.54*        | 0.012*           | 0.001*          |
| Constipation   | 10 (30.3%)        | 13 (39.4%)   | 9 (27.3%)        | 0.74*                   |       | 0.853        |                  |                 |
| Bowel movement (week)1 | 6.5±2.7         | 5.6±1.7      | 6.5±2.0          | 0.13*                   |       | 0.278        |                  |                 |
| Bristol scale1 | 3.5±1.1           | 2.9±0.9      | 3.8±1.1          | 0.002*                  | 0.012 | 0.03*        | 0.282*           | 0.001*          |
| Urinary dysfunction | 17 (51.5%)       | 15 (45.5%)   | 8 (24.2%)        | 0.283*                  |       | 0.472        |                  |                 |
| SCOPA-AUT1     | 16.4±8.7         | 13.5±8.5     | 10.5±5.8         | 0.02*                   | 0.055 | 0.17*        | 0.001*           | 0.14*           |
| G-I            | 4.3±3.4           | 4.1±3.5      | 2.1±2.3          | 0.024*                  | 0.055 | 0.83*        | 0.16*            | 0.02*           |
| Urinary       | 6.4±4.8           | 5.4±3.8      | 4.9±3.2          | 0.56*                   |       | 0.077        |                  |                 |
| RBD symptoms  | 7 (21.2%)         | 14 (42.4%)   | 1 (30.0%)        | 0.008*                  | 0.03  | 0.11*        | 0.054*           | 0.01*           |
| Insomnia      | 22 (66.7%)        | 19 (57.6%)   | 20 (60.6%)       | 0.932*                  |       | 0.985        |                  |                 |
| EDS           | 6 (18.2%)         | 13 (39.4%)   | 0                | 0.003*                  | 0.013 | 0.057*       | 0.02*            | 0.001*          |
| Global PSQI1   | 7.3±5.4           | 6.7±4.8      | 5.2±4.7          | 0.16*                   |       | 0.32         |                  |                 |
| Total PDSS1    | 116.4±16.8        | 116.7±22.3   | 130.1±3.4        | 0.001*                  | 0.007 | 0.72*        | 0.001*           | 0.02*           |
| ESS Score1    | 8.1±4.6           | 9.1±5.3      | 5.6±2.8          | 0.003*                  | 0.013 | 0.046*       | 0.02*            | 0.001*          |

LRRK2 G2019S PD: LRRK2 G2019S-related Parkinson’s disease; IPD: Idiopathic Parkinson’s disease; HS: healthy subjects. G-I: Gastro-intestinal; RBD: REM behavior disorder; EDS: excessive daytime sleepiness.

1Chi-square test; 2Fisher’s exact test; 3Kruskal-Wallis analysis; 4Mann-Whitney U test; 5Statistically significant: P<0.05; 6Mean ± Standard deviation (Range); 7Hyposmia was arbitrarily defined as an UPSIT score lower than the mean–2 SD UPSIT score obtained in a subset of healthy subjects of the same gender and similar age. doi:10.1371/journal.pone.0108982.t002
IPD males ($p = 0.71$), and significantly lower than in HS males ($p < 0.01$). No correlation was found between UPSTI score and disease duration or severity in LRRK2-PD or IPD patients.

**Neuropsychiatric symptoms.** Current active depression and current treatment with antidepressive drugs were more frequent in IPD than in LRRK2-PD, but these differences were not statistically significant (Table 2). There were no differences in the frequency of anxiety disorder between LRRK2-PD, IPD, and HS. Nine IPD patients were under antidepressive treatment but only 8 had active depression. Similarly, 3 LRRK2-PD patients were under anxiety treatment but only 2 had active anxiety. These patients were treated with antidepressants and benzodiazepines because of past history of depression and anxiety despite improvement of symptoms. The HADS subscore for depression was similar between LRRK2-PD and IPD, but higher than HS. The HADS subscore for anxiety was similar between groups (Table 2). Hallucinations occurred in similar frequency in LRRK2-PD and IPD. In all LRRK2 and IPD patients, hallucinations were visual in nature. Delusions or severe psychosis did not occur in any LRRK2-PD or IPD patient.

**Dysautonomic symptoms.** No significant differences were found in the presence of constipation between LRRK2-PD, IPD and HS groups (Table 2). Mean Bristol scale stool score was significantly higher in LRRK2-PD than in IPD (Table 2). Mean SCOPA-AUT subscore for gastro-intestinal dysfunction was similar in LRRK2-PD and IPD. IPD patients, but not LRRK2-PD patients, showed mean SCOPA-AUT subscore for gastro-intestinal dysfunction significantly higher compared to HS. The presence of urinary dysfunction was not statistically different between three groups. No differences in mean SCOPA-AUT subscore for urinary dysfunction was identified among the three groups (Table 2).

**Sleep disturbances.** A trend to an increased frequency of RBD symptoms in IPD compared to LRRK2-PD was found. Frequency of insomnia and use of hypnotics were similar among the three groups. IPD patients had a borderline increased frequency of EDS compared to LRRK2-PD (Table 2). Mean Global PSQI, total PDSS and EDSS scores were not significantly different between LRRK2-PD and IPD. LRRK2-PD and IPD patients had a mean Global PSQI score that was not significantly different from HS. In contrast, LRRK2-PD and IPD patients showed a mean total PDSS significantly lower than HS. Mean ESS score in LRRK2 and IPD were significantly higher compared to HS (Table 2).

**Estimated onset of NMS in LRRK2-PD**

LRRK2-PD patients frequently reported that several NMS occurred before OMS (table 3). Smell loss, depression, constipation and EDS were reported to develop before OMS in more than 40% of the LRRK2-PD patients in whom these symptoms were present at the time of examination. Smell loss and depression were reported to occur at variable time intervals before OMS but constipation and EDS frequency were estimated to occur more than 10 years before OMS in most LRRK2-PD (Table 3). In LRRK2-PD subjects, RBD was reported to occur usually coincidentally or after OMS. In IPD patients smell loss, depression and constipation were also reported to appear before OMS, but EDS and RBD were reported to appear coincidentally or after OMS in most cases. Anxiety symptoms were reported coincidentally or developing after OMS in most LRRK2-PD cases but more frequently before OMS in IPD.

**Discussion**

Our study shows that NMS occur frequently in LRRK2 G2019S PD patients, in a frequency similar to a group of IPD subjects of similar disease duration, severity of motor symptoms, and dopaminergic treatment. The only NMS that significantly differed between LRRK2-PD and IPD was smell loss. While reported awareness of smell loss occurred as frequently in LRRK2-PD as in IPD, UPSTI scores were significantly higher in LRRK2-PD. Hyposmia was present in only 39% of our LRRK2-PD patients, in contrast to 75% of IPD patients. Also in other studies the prevalence of abnormal olfaction in LRRK2-PD has been found to range from 36 to 49%, significantly inferior to IPD (75–81%) [5].

Reasons for the differences in smell between LRRK2-PD and IPD encountered in this and other studies remain unclear. Heterogeneous pathology or less severe involvement of olfactory structures in LRRK2-PD has been proposed to explain such differences. In Parkin gene associated PD, absence of smell loss is common [25] and neuropathological changes are usually limited to the substantia nigra without LB pathology [26]. One study involving four LRRK2-PD brains, reported α-synuclein accumulation in the olfactory bulb, olfactory tract, and primary olfactory cortex providing a pathophysiological substrate for olfactory deficit[6]. Still only 20% of LRRK2 G2019S cases are thought to present different pathology to that seen in IPD [27], and therefore neuropathological heterogeneity may not adequately explain the preservation of the olfactory sense in most LRRK2 patients.

In contrast to previous studies, we found that smell was particularly preserved in G2019S LRRK2-PD females. Such difference could suggest a gender effect in the expression of LRRK2-PD. Gender-related susceptibility factors likely play a role in PD, a disorder more common in males [28] and female predominance has been suggested to occur in G2019S LRRK2-PD [29]. One study has also reported an earlier age at disease onset in women than in men with the G2019S mutation [4].

Neuropsychiatric disorders, dysautonomic symptoms and sleep disturbances were frequent in LRRK2 G2019S carriers, similarly to that observed in IPD. Depression, anxiety and hallucinations were present in our LRRK2 patients at a frequency similar to that reported in the literature [4,30,31]. Sleep disturbances like RBD, insomnia and EDS are common in PD and have been reported to be present frequently in patients carrying the G2019S mutation. The frequency of RBD symptoms tended to be lower in LRRK2-PD cases than in IPD, although the difference was not statistically significant. The prevalence of RBD in PD reported in the literature varies from 15% to 46% [32]. A recent study has reported RBD symptoms in only 11% of the LRRK2-PD patients, compared to 42% of IPD patients [33], suggesting that RBD could be less frequently present in LRRK2-PD. Frequency of constipation and urinary dysfunction in our LRRK2 patients was similar to that reported by others [5,31,33]. In our study the frequency of constipation was similar between LRRK2 and IPD, but mean Bristol scale stool score was significantly lower in LRRK2-PD than in IPD, suggesting that intestinal motility could be less impaired in LRRK2-PD.

A substantial proportion of our LRRK2-PD patients reported that several NMS, such as hyposmia, depression, constipation, or EDS, had been present before OMS. Some NMS have been evaluated in a limited number of asymptomatic subjects carrying the LRRK2 G2019S mutation [6,31,34] and hyposmia, depression and constipation have been found to occur in some non-manifesting carriers [31,34], although some of these premotor features were found to be no more frequent in non-manifesting
Table 3. Estimated presence of nonmotor symptoms in LRRK2 G2019S Parkinson’s disease patients and idiopathic Parkinson's disease patients in relation to onset of motor symptoms.

| Symptom                                | LRRK2-PD | IPD  |
|-----------------------------------------|----------|------|
| Loss or change in smell sense; n       | 18       | 21   |
| Coincidentally or after OMS (n;%       | 8 (44.4%)| 8 (38.1%)|
| Before OMS (n;%                         | 8 (44.4%)| 8 (38.1%)|
| More than 10 yrs before OMS            | 2/8 (25%)| 4/8 (50%)|
| Within 10 yrs before OMS               | 4/8 (50%)| 2/8 (25%)|
| Within 2 yrs before OMS                | 2/8 (25%)| 2/8 (25%)|
| Unknown                                | 2 (11.1%)| 5 (23.8%)|
| Depression; n                          | 19       | 12   |
| Coincidentally or after OMS (n;%       | 11 (57.9%)| 7 (58.3%)|
| Before OMS (n;%                         | 8 (42.1%)| 5 (41.7%)|
| More than 10 yrs before OMS            | 2/8 (25%)| 2/5 (40%)|
| Within 10 yrs before OMS               | 3/8 (37.5%)| 2/5 (40%)|
| Within 2 yrs before OMS                | 3/8 (37.5%)| 1/5 (20%)|
| Anxiety symptoms; n                    | 8        | 9    |
| Coincidentally or after OMS (n;%       | 6 (75%)  | 1 (11.1%)|
| Before OMS (n;%                         | 2 (25%)  | 8 (88.9%)|
| More than 10 yrs before OMS            | 0        | 5/8 (62.5%)|
| Within 10 yrs before OMS               | 0        | 3/8 (37.5%)|
| Within 2 yrs before OMS                | 2/2 (100%)| 0    |
| Constipation; n                        | 14       | 15   |
| Coincidentally or after OMS (n;%       | 5 (35.7%)| 8 (53.3%)|
| Before OMS (n;%                         | 9 (64.3%)| 7 (46.7%)|
| More than 10 yrs before OMS            | 8/9 (88.9%)| 7/7 (100%)|
| Within 10 yrs before OMS               | 1/9 (11.1%)| 0    |
| Within 2 yrs before OMS                | 0        | 0    |
| Urinary dysfunction; n                 | 17       | 15   |
| Coincidentally or after OMS (n;%       | 12 (70.6%)| 12 (80%)|
| Before OMS (n;%                         | 5 (29.4%)| 3 (20%)|
| More than 10 yrs before OMS            | 0        | 0    |
| Within 10 yrs before OMS               | 1/5 (20%)| 1/3 (33.3%)|
| Within 2 yrs before OMS                | 4/5 (80%)| 2/3 (66.6%)|
| RBD symptoms; n                        | 7        | 14   |
| Coincidentally or after OMS (n;%       | 5 (71.4%)| 12 (85.7%)|
| Before OMS (n;%                         | 2 (28.6%)| 2 (14.3%)|
| More than 10 yrs before OMS            | 0        | 0    |
| Within 10 yrs before OMS               | ½ (50%)  | 0/2 (0.0%)|
| Within 2 yrs before OMS                | ½ (50%)  | 2/2 (100%)|
| Insomnia; n                            | 19       | 22   |
| Coincidentally or after OMS (n;%       | 16 (84.2%)| 14 (63.6%)|
| Before OMS (n;%                         | 3 (15.8%)| 8 (36.4%)|
| More than 10 yrs before OMS            | 2/3 (66.6%)| 6/8 (75%)|
| Within 10 yrs before OMS               | 0        | 0    |
| Within 2 yrs before OMS                | 1/3 (33.3%)| 2/8 (25%)|
| EDS; n                                 | 6        | 13   |
| Coincidentally or after OMS (n;%       | 3 (50%)  | 12 (92.3%)|
| Before OMS (n;%                         | 3 (50%)  | 1 (7.7%)|
| More than 10 yrs before OMS            | 2/3 (66.6%)| 1/1 (100%)|
| Within 10 yrs before OMS               | 1/3 (33.3%)| 0    |
| Within 2 yrs before OMS                | 0        | 0    |
carriers than in non-carriers. The presence of these symptoms in the premotor phase, as in the case of PD, suggests that the neuropathological changes occur probably in non-dopaminergic brainstem and peripheral nervous system structures before the involvement of the substantia nigra, as suggested by Braak [35]. Some limitations of our study should be mentioned. Some results are based on patient’s responses. More information could be obtained with objective tests, as occurred in smell evaluation, where no differences were identified when asked for a loss or change in smell, while the UPSIT was able to detect significant differences. Also since the mean disease duration was 9 years, we acknowledge that the patient’s responses in regards to symptoms occurring in the presymptomatic phase may not be accurate, but others studies have reported NMS such as these occurring in the premotor phase of LRRK2-PD [6,30]. Future prospective studies in asymptomatic LRRK2 G2019S carriers should clarify this issue. Finally, cognitive dysfunction which is among the most relevant and disabling NMS in PD, was not assessed in detail in our study, but only one patient was excluded for the study because of dementia. Some studies have suggested that the frequency of dementia could be lower than usually reported in IPD [5,36].

Cognitive dysfunction in LRRK2 patients deserves additional and specifically designed studies. One strength of our study, in contrast to previous ones that usually assessed one or only a few NMS, include the evaluation of several NMS in the same group of patients which has given a more global impression of the contribution of NMS to the clinical picture of LRRK2 associated PD. In addition, our study includes similar NMS assessments in a group of healthy subjects for comparison purposes.

In summary, neuropsychiatric, dysautonomic and sleep disturbances are equally frequent in non-demented LRRK2 G2019S PD and IPD. Olfactory dysfunction, however, occurs less often in LRRK2 G2019S PD, maybe reflecting less involvement of olfactory structures by the neurodegenerative process. Smell function seems to be particularly preserved in females with the G2019S mutation, suggesting a gender effect in the expression of some LRRK2-PD symptoms. Some NMS may antedate the onset of PD motor syndrome in substantial number of patients carrying LRRK2 G2019S mutation, indicating that a premotor phase similar to that occurring in IPD probably occurs in this genetic form of PD, and that common physiopathological mechanism probably underlie the onset and progression of the disease in both LRRK2 G2019S PD and IPD.

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Author Contributions

Conceived and designed the experiments: CG DV ET. Performed the experiments: CG DVJI MS IA MC JH. Analyzed the data: CG DVJI MS IG ME MB MM FV ET. Contributed reagents/materials/analysis tools: CG DVJI MS IG ME MB MM FV ET. Contributed to the writing of the manuscript: CG DV ET.

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