Sleep Disorders in Parkinsonian and Nonparkinsonian *LRRK2* Mutation Carriers

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Abstract

Objective

In idiopathic Parkinson disease (IPD) sleep disorders are common and may antedate the onset of parkinsonism. Based on the clinical similarities between IPD and Parkinson disease associated with *LRRK2* gene mutations (LRRK2-PD), we aimed to characterize sleep in parkinsonian and nonmanifesting *LRRK2* mutation carriers (NMC).

Methods

A comprehensive interview conducted by sleep specialists, validated sleep scales and questionnaires, and video-polysomnography followed by multiple sleep latency test (MSLT) assessed sleep in 18 LRRK2-PD (17 carrying G2019S and one R1441G mutations), 17 NMC (11 G2019S, three R1441G, three R1441C), 14 non-manifesting non-carriers (NMNC) and 19 unrelated IPD.

Results

Sleep complaints were frequent in LRRK2-PD patients; 78% reported poor sleep quality, 33% sleep onset insomnia, 56% sleep fragmentation and 39% early awakening. Sleep onset insomnia correlated with depressive symptoms and poor sleep quality. In LRRK2-PD, excessive daytime sleepiness (EDS) was a complaint in 33% patients and short sleep latencies on the MSLT, which are indicative of objective EDS, were found in 71%. Sleep attacks

occurred in three LRRK2-PD patients and a narcoleptic phenotype was not observed. REM sleep behavior disorder (RBD) was diagnosed in three LRRK2-PD. EDS and RBD were always reported to start after the onset of parkinsonism in LRRK2-PD. In NMC, EDS was rarely reported and RBD was absent. When compared to IPD, sleep onset insomnia was more significantly frequent, EDS was similar, and RBD was less significantly frequent and less severe in LRRK2-PD. In NMC, RBD was not detected and sleep complaints were much less frequent than in LRRK2-PD. No differences were observed in sleep between NMC and NMNC.

Conclusions

Sleep complaints are frequent in LRRK2-PDand show a pattern that when compared to IPD is characterized by more frequent sleep onset insomnia, similar EDS and less prominent RBD. Unlike in IPD, RBD and EDS seem to be not markers of the prodromal stage of LRRK2-PD.

Introduction

Idiopathic Parkinson disease (IPD) is a neurodegenerative condition where neuronal loss in the substantia nigra leads to motor symptoms. Patients with IPD also present a variety of non-motor symptoms such as sleep disorders, hyposmia, depression, constipation, hallucinations and dementia. Damage of brain structures outside the substantia nigra is the origin of certain nonmotor symptoms and in some cases they may antedate parkinsonism onset in what has been called the prodromal phase of PD [1].

Sleep disorders such as insomnia, excessive daytime sleepiness (EDS) and REM sleep behavior disorder (RBD) are common in IPD. RBD, and possibly EDS, may antedate the onset of parkinsonism in IPD. The origin of sleep disturbances in IPD is multifactorial and includes damage of the brain structures that modulate sleep, the coexistence of symptoms that may disrupt sleep onset and maintenance (e.g., depression, nocturnal immobility), and the effect of some medications (e.g., dopaminergic agents, antidepressants). Identification and management of sleep disorders in IPD is important since they frequently have a negative impact on quality of life [2–4].

Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) are associated with sporadic and familial PD (LRRK2-PD)[5–6]. Penetrance of LRRK2 mutations is incomplete and age-dependent, and therefore a proportion of asymptomatic individuals carrying mutations can be at the premotor stage of LRRK2-PD[7]. When manifested, clinical expression of LRRK2-PD is usually similar to IPD and consists of late onset levodopa-responsive parkinsonism, typical complications of dopaminergic therapy, and nonmotor features including hyposmia and depression[8–15].

Available data on sleep disturbances in individuals carrying *LRRK2* mutations is scarce. Based on the clinical similarities between IPD and LRRK2-PD we hypothesized that those sleep disorders occurring in IPD may also be present in LRRK2-PD. Also, we hypothesized that sleep disorders that are known to occur in premotor IPD may also be present in LRRK2 mutation carriers who have not developed parkinsonism yet. In the current study we sought to characterize the sleep disturbances occurring in parkinsonian and nonparkinsonian*LRRK2* mutation carriers through subjective and objective measures of sleep. For comparative

purposes, sleep data on *LRRK2* mutation carriers were compared with relatives who were non carriers of LRRK2 mutations and with unrelated IPD patients.

Methods

Participants and genetic assessment

The current study comprises individuals in whom the assessment of the *LRRK2* genetic status was determined at the Hospital Clinic de Barcelona, Barcelona, Spain. The study was conducted between January 2012 and September 2013 in the Hospital Clinic de Barcelona.

Sixty-eight subjects were consecutively enrolled in the study, irrespective for sleep complaints. We included the following groups for evaluations and comparisons: 1) 18PD carriers of *LRRK2* mutations (LRRK2-PD), 2) 17 first-degree relatives of LRRK2-PD patients who were carriers of LRRK2 mutations and had no parkinsonism (non-manifesting carriers, NMC) and, 3) 14 relatives of LRRK2-PD patients who were negative for LRRK2 mutations and had no parkinsonism (non-manifesting non-carriers, NMNC). During routine visits at the movement disorders clinic, we recruited a group of 19 IPD patients who were consecutively selected to match the LRRK2-PD group in age, gender, disease duration and Hoehn and Yahr stage[16].

Diagnosis of PD was made according the UK Brain Bank Criteria[17]. Exclusion criteria in LRRK2-PD and IPD groups were age at parkinsonism onset less than 30 years, Hoehn and Yahr stage ≥4[16], dementia[18], and therapy with deep brain stimulation, apomorphine continuous infusionor intestinal levodopa gel infusion. Exclusion criteria in the NMC and NMNC groups were age less than 18 years, and presence of motor symptoms, a neurological disorder or a disabling disease.

Genotyping of G2019S, R1441G and R1441C mutations in the *LRRK2* gene was performed on blood samples as previously described[9]. Patients with IPD were negative for mutations in *LRRK2* gene. LRRK2-PD patients were aware of their genetic status. Subjects in the NMC and NMNC groups were unaware of their genetic status.

In each participant all clinical and sleep assessments were done during a 2-day period. The ethical committee at our institution approved the study and all participants gave written informed consent.

Clinical assessment

Movement disorders expert neurologists (CPS and DV) conducted a medical interview that included demographic and clinical data, and current medications including calculation of total L-Dopa equivalent daily dose[19]. At the time of assessment all drugs had been stable during the previous month. Motor and non-motor features, as well as their impact on daily living activities, were evaluated with the Movement Disorders Society Unified Parkinson Disease Rating Scale (MDS-UPDRS parts I to IV)[20], Hoehn and Yahr scale[16], and Schwab and England activities of daily living scale[21]. Motor phenotype subtype was classified in postural instability and gait difficulty, tremor dominant, and indeterminate[22]. The impact of PD upon quality of life was determined with the PD questionnaire (PDQ-39)[23]and anxiety and depression by means of the Hospital Anxiety and Depression scale (HADS)[24]. Cognition was evaluated using the Montreal Cognitive Assessment (MoCA)[25]and a score of less than 21 points was suggestive of cognitive impairment (according to a cut-off score established in Spanish population)[26].

Sleep assessment

Sleep-related symptoms, video-polysomnography (V-PSG) and multiple sleep latency test (MSLT) were evaluated by sleep specialists (AFA, CG, AI and JS) who were blind to the

participant category and genetic status (LRRK2-PD, IPD, NMC or NMNC). Sleep evaluation included the following assessments:

1. Sleep-related symptomatology. This was examined with a comprehensive semi-structured sleep interview conducted by sleep experts where the presence of the bed partner or anyone who witnessed the patient's sleep was encouraged to substantiate and complete the patient sleep history. The interview covered sleep habits and complaints such as sleep onset insomnia (difficulty falling asleep within 30 minutes or more), sleep fragmentation (more than two awakenings lasting more than 15 minutes), early awakening (waking up at least two hours earlier than desired), nocturnal akinesia (difficulty in turning over in bed), excessive daytime sleepiness (EDS, inability to stay awake during the major periods of the day resulting in unintended episodes of sleep), sleep attacks (episodes of sudden onset of sleep without warning), nightmares and dream-enacting behaviors. According to the sleep interview these sleep complaints were categorized as being present or absent in each participant. In addition, overall sleep disturbances and nocturnal disability were evaluated with the Pittsburgh Sleep Quality Index (PSQI)[27] and the modified Parkinson's Disease Sleep Scale (PDSS-2)[28]. PSQI global score ≥6 points and PDSS-2 total score ≥15 were indicative of poor sleep quality[27, 29].

Subjective EDS was also assessed by means of the Epworth Sleepiness Scale (ESS), where a score ≥11 points was suggestive of EDS[30]. Sleep attacks were also identified using the Inappropriate Sleep Composite Score (ISCS).[31].

Restless legs syndrome (RLS) was diagnosed according to the International Restless Legs Syndrome Study Group criteria[32]. Individuals who fulfilled the four diagnostic criteria for RLS were then interviewed to verify its presence and exclude potential RLS mimics (e.g., stiffness, cramps, off periods). RLS severity was measured with the International RLS Study Group Rating Scale[33].

REM sleep behavior disorder (RBD) was diagnosed according to the International Classification of sleep disorders 3rd revision criteria (ICSD-III)[34]. In brief, RBD was diagnosed in patients with a chronic history of dream-enacting behaviors (reported by the patient or the bed partner) in whom video-polysomnography (V-PSG) demonstrated excessive electromyographic (EMG) activity in REM sleep (see below for V-PSG definition of "excessive EMG activity") linked to abnormal vigorous movements. RBD was also diagnosed in patients who were unaware of displaying dream-enacting behaviors but V-PSG demonstrated excessive EMG activity in REM sleep associated with vigorous behaviors that are typical of RBD (e.g., prominent body jerking, kicking, screaming)[34]. Besides, the RBD screening questionnaire (RBDSQ) was administered to assess its usefulness to detect true RBD in *LRRK2* mutation carriers[35]. A RBDSQ score ≥5 in nonparkinsonian subjects and a RBDSQ score ≥7 in parkinsonian subjects has been considered suggestive of RBD[36].

2. Video-polysomnography (V-PSG). This was performed in all participants with a digital polygraph (Deltamed, software version 2007, Paris, France) and consisted of electroencephalogram (F3, F4, C3, C4, O1, and O2, referred to the contralateral ear), right and left electro-oculograms, surface EMG of the mentalis muscle, surface EMG of the right and left flexor digitorum superficialis in the upper limbs, surface EMG of the right and left anterior tibialis in the lower limbs, electrocardiogram, nasal and oral air-flow assessment, nasal pressure cannula, thoracic and abdominal movement assessment, and measurement of oxyhemoglobin saturation. Sleep stages were scored according to standard criteria with the allowance for REM sleep without atonia[37]. Apneas, hypopneas, arousals and periodic leg movements in sleep (PLMS) were scored according to standard criteria[37]. The apnea/hypopnea index (AHI) was defined as the mean number of apneas and hypopneas per hour of sleep, the arousal index was defined as the mean number of arousals per hour of sleep, and the PLMS index (PLMSI) was defined as the number of PLMS per hour of sleep[37].

For the measurement of EMG activity in REM sleep we followed the SINBAR method[38] where a trained neurologist (AFA) visually quantified "any" (tonic, phasic or a combination of both) EMG activity in the mentalis muscle plus phasic EMG activity in the right and left flexor digitorum superficialis muscles. Following this method, $\geq$32% of 3-sec mini-epochs containing EMG activity in REM sleep was considered "excessive EMG activity" and indicative of RBD [38].

3. Multiple SleepLatency Test (MSLT). This test evaluated the presence of objective EDS. The MSLT was performed according to standard criteria and consisted of five naps at 2-hour intervals beginning two hours after the end of the V-PSG[39]. A mean sleep latency onset of $\leq$8 min was considered indicative of objective EDS[34]. A mean sleep latency of $\leq$8 min plus more than one sleep onset period (SOREMP) defined a narcoleptic phenotype[3].

Statistical analysis

Data are reported in mean, standard deviation, number and percentage. Quantitative variables were analyzed using Student's t-test for comparisons of two independent groups. Qualitative variables were analyzed using χ2-test and the Fisher exact test, when appropriate. No imputation was made for missing data. Subjects missing values in a particular field were not included in the analysis for that particular outcome. Secondary analysis controlling for confounders like sleep-related drug intake (e.g., benzodiazepines, dopamine agents) was performed by means of ANOVA or logistic regression in case of differences between groups. Pearson's correlation coefficients were used to examine in the LRRK2-PD group the association between variables and sleep onset insomnia and a mean sleep latency onset $\leq$8 on the MSLT. Two tailed P values of less than 0.05 were considered to be significant. Statistical analyses were performed with SPSS version 20.0 (IBM).

Results

The study comprised 18 LRRK2-PD subjects from 17 families (17 patients carrying the G2019S mutation and one the R1414G mutation), 17 NMC from 13 families (11 G2019S, three R1414G and three R1414C), 14 NMNC from nine families (11 relatives of LRRK2-PD patients carrying the G1920S mutation, and three individuals from the same family of a R1441G LRRK2-PD patient), and 19 unrelated IPD patients. All seven subjects with the R1441G mutation (one LRRK2-PD, three NMC and three NMNC) were from the same family. The three NMC subjects carrying the R1441C mutation were siblings. All participants included in this study were Caucasian Spanish except one G2019S LRRK2-PD subject of North-African origin. All participants lived in Northeast Spain (63 in Catalonia and five in Menorca). Demographic, parkinsonian and sleep related clinical data, V-PSG data and comparisons between groups are presented in Tables 1, 2 and 3.

Sleep in LRRK2-PD subjects

There were nine women and nine men with a mean age of 61.0 ± 11.2 years. Fourteen (78%) subjects had PSQI $\geq$6 and eight (44%) a PDSS-2 $\geq$15, which are indicative of subjective poor sleep quality. Six (33%) patients complained of sleep onset insomnia, ten (56%) of sleep fragmentation, nine (50%) of nocturnal akinesia, 11 (61%) of nocturia, and seven (39%) of early awakening. Sleep onset insomnia correlated with HADS total score (r = 0.512, p = 0.003), HADS depression score (r = 0.707, p = 0.001), PSQI score (r = 0.740, p<0.001) and PDSS-2 score (r = 0.688, p = 0.002).

Six (33%) patients complained of EDS andnone of them reported that this symptom antedated the onset of motor symptoms. Of these six patients, four had an ESS score $\geq$11. MSLT

Table 1. Demographic and parkinsonian clinical data and comparisons between groups.

	LRRK2-PD (n = 18)	IPD(n = 19)	NMC (n = 17)	NMNC (n = 14)	p value (LRRK2-PD vs. IPD)	p value(LRRK2-PD vs. NMC)	p value(NMC vs. NMNC)
Age (years)	61.0 ± 11.2	63.1 ± 11.2	44.4 ± 12.3	50.8 ± 16.0	0.996	<0.001	0.557
Female gender n (%)	9 (50.0)	8 (42.1)	9 (52.3)	7 (50.0)	0.758	0.849	0.858
Body mass Index (Kg/m^2)	26.4 ± 3.7	26.4 ± 2.8	25.8 ± 2.7	24.4 ± 3.8	0.966	0.677	0.299
Age at PD diagnosis (years)	53.2 ± 10.2	55.9 ± 11.2	NA	NA	0.440	NA	NA
PD duration (years)	7.8 ± 5.8	7.3 ± 3.7	NA	NA	0.746	NA	NA
L-dopa intake n (%)	11 (61.1)	11 (57.9)	0 (0.0)	0 (0.0)	0.860	NA	NA
Dopaminergic agonist intake n (%)	16 (88.8)	11 (57.9)	0 (0.0)	0 (0.0)	0.021	NA	NA
L-Dopa daily equivalent dose (mg)	871.4 ± 669.2	550.2 ± 478.9	0 ± 0	0 ± 0	0.109	NA	NA
Antidepressants intake n (%)	4 (22.2)	4 (21.0)	2 (11.7)	2 (14.3)	0.867	0.510	0.941
Benzodiazepine intake n (%)	10 (55.5)	2 (10.5)	1 (5.8)	3 (21.4)	0.003	0.003	0.249
UPDRS-I score (n)	10.2 ± 7.4	12.3 ± 7.6	3.1 ± 4.2	3.0 ± 4.0	0.398	0.002	0.931
UPDRS- II score (n)	8.9 ± 5.2	11.6 ± 7.9	0.2 ± 0.4	0.3 ± 0.5	0.239	<0.001	0.605
UPDRS-III score (n)	14.4 ± 7.7	21.8 ± 10.8	1.1 ± 1.9	0.2 ± 0.4	0.022	<0.001	0.085
UPDRS-IV score (n)	2.3 ± 3.5	1.4 ± 2.3	0 ± 0	0 ± 0	0.329	0.015	NA
PIGD subtype n (%)	10 (55.5)	10 (52.6)	NA	NA	0.756	NA	NA
Indeterminate subtype n (%)	4 (22.2)	1 (5.3)	NA	NA			
Tremor dominant subtype n (%)	4 (22.2)	4 (21.0)	NA	NA			
Hoehn and Yahr stage (n)	2.0 ± 0.7	1.9 ± 0.6	0 ± 0	0 ± 0	0.931	0.036	0.600
Schwab and England score (n)	86.1 ± 10.4	87.0 ± 10.8	100 ± 0	100 ± 0	0.797	<0.001	NA
PDQ-39 score (n)	35.9 ± 15.2	36.2 ± 23.3	5.5 ± 6.4	7.4 ± 9.4	0.960	<0.001	0.544
HADS total score (n)	12.2 ± 6.1	10.5 ± 4.7	8.9 ± 5.8	8.3 ± 6.7	0.346	0.116	0.804
HADS-anxiety score (n)	6.8 ± 3.8	6.4 ± 2.3	5.9 ± 3.5	5.6 ± 4.3	0.658	0.491	0.806
HADS-depression score (n)	5.4 ± 3.2	4.3 ± 2.9	2.9 ± 2.5	2.8 ± 3.1	0.277	0.023	0.890
MoCA score (n)	24.2 ± 4.7	24.9 ± 2.4	26.3 ± 2.4	27.4 ± 1.9	0.714	0.108	0.192

Data are presented as mean, standard deviation, number and percentage.

HADS: Hospital Anxiety and Depression scale; **IPD**: Idiopathic Parkinson Disease; **LRRK2-PD**: LRRK2 Parkinson Disease; **MoCA**: Montreal Cognitive Assessment; **NA**: Not applicable; **NMC**: Non-manifesting carriers; **NMNC**: Non-manifesting non-carriers; **PDQ-39**: 39-item Parkinson Disease Questionnaire; **PIGD**: postural instability gait difficulty; **PD**: Parkinson disease; **UPDRS**: Unified Parkinson Disease Rating Scale.

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was performed in 17 LRRK2-PD subjects and 12(71%)(six women and six men, 11 carrying the G2019S mutation and one the R1441G mutation) showed a mean sleep latency of ≤8 minutes on the MSLT, which is indicative of objective EDS. Only four of these 12 patients with abnormal sleep latency on the MSLT complained of EDS. There was no correlation between the mean sleep latency on the MSLT with any demographic, clinical and V-PSG variable such as age, PD duration and severity, therapy with dopaminergics, antidepressants and

Table 2. Sleep related clinical data and comparisons between groups.

	LRRK2-PD (n = 18)	IPD (n = 19)	NMC (n = 17)	NMNC (n = 14)	p value(LRRK2-PD vs. IPD)	p value(LRRK2-PD vs. NMC)	p value(NMC vs. NMNC)
PSQI score (n)	8.3 ± 3.5	5.7 ± 3.6	3.6 ± 3.3	4.4 ± 4.8	0.033	<0.001	0.590
PSQI score ≥6 n (%)	14 (77.7)	8 (42.1)	1 (5.8)	3 (21.4)	0.025	<0.001	0.330
PDSS-2 score (n)	14.4 ± 7.7	10.2 ± 6.9	6.3 ± 6.2	3.4 ± 6.2	0.102	0.003	0.226
PDSS-2 score ≥15 n (%)	8 (44.4)	6 (31.6)	2 (11.7)	1 (7.1)	0.486	0.060	1.00
Sleep onset insomnia n (%)	6 (33.3)	0 (0.0)	2 (11.7)	3 (21.4)	0.005	0.242	0.564
Sleep fragmentation n (%)	10 (55.5)	5 (26.3)	4 (23.5)	2 (14.3)	0.054	0.158	0.411
Early awakening n (%)	7 (38.8)	7 (36.8)	1 (5.8)	2 (14.3)	0.804	0.046	0.501
Nocturia n (%)	11 (61.1)	12 (63.1)	10 (58.8)	6 (42.9)	0.944	1.000	0.198
Nocturnal tremor n (%)	2 (11.1)	3 (15.8)	0 (0.0)	0 (0.0)	0.723	0.489	NA
Nocturnal rigidity n (%)	5 (27.7)	3 (15.8)	0 (0.0)	0 (0.0)	0.335	0.049	NA
Nocturnal akinesia n (%)	9 (50.0)	9 (47.4)	0 (0.0)	0 (0.0)	0.758	0.001	NA
Early morning dystonia n (%)	4 (22.2)	1 (5.3)	0 (0.0)	0 (0.0)	0.117	0.108	NA
Restless legs syndrome (%)	3 (16.6)	3 (15.8)	2 (11.7)	2 (14.3)	0.911	1.000	0.941
IRLSGRS score (n)	4.0 ± 8.0	1.0 ± 4.0	2.0 ± 5.0	2.0 ± 6.0	0.505	0.723	0.382
Excessive daytime sleepiness complaint n (%)	6 (33.3)	9 (47.3)	3 (17.6)	0 (0)	0.463	0.442	0.077
ESS score (n)	7.8 ± 5.0	8.3 ± 2.7	7.3 ± 4.5	5.5 ± 2.2	0.756	0.766	0.171
ESS score ≥11 n (%)	4 (22.2)	5 (26.3)	4 (23.5)	0 (0.0)	1.00	1.000	0.100
Sleep attacks n (%)	3 (16.6)	1 (5.3)	0 (0.0)	0 (0.0)	0.242	0.233	NA
RBDSQ score (n)	3.1 ± 2.2	3.7 ± 2.6	2.7 ± 2.7	1.1 ± 1.9	0.395	0.648	0.072

Data are presented as mean, standard deviation, number and percentage.

ESS: Epworth Sleepiness Scale; **IPD:** Idiopathic Parkinson Disease; **IRLSGRS:** International Restless Legs Syndrome Study Group Scale; **LRRK2-PD:** LRRK2 Parkinson Disease; **NA:** Not applicable; **NMC:** Non-manifesting carriers; **NMNC:** Non-manifesting non-carriers; **PSQI:** Pittsburg Sleep Quality Index; **PD:** Parkinson disease; **PDSS-2:** Parkinson's Disease Sleep Scale 2; **RBD:** REM sleep behavior disorder; **RBDSQ:** RBD screening questionnaire.

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benzodiazepines, pain, HADS score, PSQI score, PDSS-2 score, sleep complaints, body mass index, nocturnal total sleep time, sleep efficiency, PLMI and AHI.

Three (17%) patients, two women and one man, reported sleep attacks occurring several years after the onset of parkinsonism. One patient reported a temporal association between the introduction of pramipexol and the onset of sleep attacks. His sleep efficiency on the V-PSG was 93%, no apneas were detected, and the mean sleep latency on the MSLT was 1 min. Another patient with sleep attacks was taking L-Dopa, pramipexol and alprazolam, the sleep efficiency in the V-PSG was 70%, AHI was 19 and sleep latency on the MSLT was 4 min. The remaining patient experiencing sleep attacks was receiving L-Dopa, pramipexol and escitalopram, sleep efficiency in the V-PSG was 91%, AHI was 25, and sleep latency on the MSLT was 5 min.

None of the LRRK2-PD patients had a narcoleptic phenotype. None had two SOREMPs on the MSLT. A single SOREMP was detected in one subject who was sleep and REM sleep deprived (nocturnal PSG showed a sleep efficiency of 30%and REM sleep was not recorded).

Three (17%) unrelatedG2019SLRRK2-PD had RLS (Table 4). RLS severity was mild in one patient, moderate in one and severe in one. One patient reported that RLS symptomatology started before and two after parkinsonism onset.

In three patients REM sleep was not recorded during V-PSG (none of these three subjects reported a history of dream-enacting behaviors suggestive of RBD). Three (17%) unrelated LRRK2-PD G2019S carriers had RBD (Table 5). All three were men and had thepostural

Table 3. Polysomnography and Multiple Sleep Latency Test data and comparisons between groups.

	LRRK2-PD (n = 18)	IPD(n = 19)	NMC(n = 17)	NMNC (n = 14)	p value (LRRK2-PD vs. IPD)	p value(LRRK2-PD vs. NMC)	p value(NMC vs. NMNC)
Age (years)	61.0 ± 11.2	63.1 ± 11.2	44.4 ± 12.3	50.8 ± 16.0	0.996	<0.001	0.557
Total sleep time (min)	362.8 ± 95.8	396.2 ± 81.3	411.4 ± 65.7	409.2 ± 49.5	0.174	0.107	0.471
Sleep efficiency (%)	75.0 ± 19.2	82.2 ± 16.0	83.9 ± 13.2	83.5 ± 11.0	0.187	0.177	0.498
Wake time after sleep onset (min)	75.6 ± 66.7	70.2 ± 63.5	47.4 ± 37.0	54.1 ± 40.8	0.539	0.154	0.777
N1 (%)	15.2 ± 6.9	11.2 ± 6.1	18.1 ± 20.3	12.7 ± 4.0	0.047	0.583	0.896
N2 (%)	67.3 ± 9.2	62.2 ± 12.4	52.9 ± 13.4	59.3 ± 8.1	0.136	0.001	0.190
N3 (%)	6.9 ± 6.9	10.0 ± 8.3	14.1 ± 7.1	10.8 ± 5.5	0.195	0.006	0.206
REM sleep (%)	10.6 ± 7.4	16.5 ± 9.9	18.5 ± 6.1	17.2 ± 6.5	0.059	0.003	0.541
Arousal Index (n)	18.5 ± 13.4	19.2 ± 12.5	15.0 ± 11.1	18.1 ± 8.6	0.953	0.428	0.222
AHI (n)	9.0 ± 11.6	15.3 ± 15.4	10.0 ± 14.7	14.7 ± 17.8	0.054	0.826	0.485
AHI ≥15 n (%)	5 (27.7)	8 (42.1)	4 (23.5)	5 (35.7)	0.428	1.000	0.700
PLMSI (n)	7.2 ± 13.3	8.0 ± 13.4	3.3 ± 5.3	4.9 ± 7.6	0.274	0.284	0.835
PLMSI ≥15 n (%)	4 (22.2)	4 (21.0)	1 (5.8)	3 (21.4)	1.000	0.346	0.330
REM sleep behavior disorder (%)	3 (16.6)	10 (52.6)	0 (0.0)	0 (0.0)	0.037	0.224	1.000
REM sleep 3- secmini-epochs (n)	825.5 ± 584.7	909.9 ± 583.2	1264.3 ± 534	1079.4 ± 363	0.663	0.033	0.289
SINBAR EMG activity (%)	12.2 ± 14.9	42.2 ± 25.6	8.7 ± 4.8	9.7 ± 7.9	<0.001	0.482	0.757
Mentalis EMG activity (%)	10.1 ± 12.1	29.1 ± 24.1	5.5 ± 3.5	5.4 ± 3.8	0.009	0.169	0.958
Mentalisplus AT EMG activity (%)	15.4 ± 15.6	41.4 ± 23.7	14.5 ± 8.9	8.5 ± 4.9	0.007	0.352	1.000
MSLT sleeplatency (min)	6.5 ± 4.6	8.0 ± 4.9	9.3 ± 5.3	7.2 ± 4.3	0.334	0.119	0.371
MSLT sleep latency <5min n (%)	7 (41.2)*	8 (42.1)	4 (23.5)	4 (28.6)	0.955	0.472	0.700
MSLT sleep latency < 8min n (%)	12 (70.6)*	8 (42.1)	6 (35.3)	10 (71.4)	0.106	0.153	0.466
SOREMPs on MSLT n (%)	1 (5.8)*	1 (5.3)	0 (0.0)	1 (7.1)	1.000	1.000	0.483

Data are presented as mean, standard deviation, number and percentage.

*MSLT was performed in 17 out of 18 LRRK2-PD patients since one refused to undergo this test. Percentages on MSLT measures are therefore based on these 17 subjects.

AHI: Apnea-hipopnea index; **LRRK2-PD**: LRRK2 Parkinson disease; **Mentalis**: polysomnographic montage quantifying "any" (phasic and tonic) type of EMG activity in the mentalis muscle during REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 18%)[38]; **Mentalis plus AT**: polysomnographic montage quantifying "any" (phasic and tonic) type of EMG activity in the mentalis muscle plus phasic EMG activity in the right and left anterior tibialis during REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 46.4%) [38]; **MSLT**: Multiple Sleep Latency Test; **NMC**: non-manifesting carriers; **NMNC**: non-manifesting non-carriers; **NA**: Not applicable; **PLMS**: Periodic Leg Movements in Sleep; **PLMSI**: Periodic Leg Movements in Sleep Index; **RBD**: REM Sleep Behavior Disorder; **SINBAR**: polysomnographic montage quantifying "any" (phasic or tonic) type of EMG activity in the mentalis muscle and phasic EMG activity in the right and left flexor digitorumsuperficialis muscles during REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 32%)[38]; **SOREMPs**: Sleep Onset REM Sleep Periods.

Table 4. Subjects with restless legs syndrome.

Subject	LRRK2-PD-03	LRRK2-PD-09	LRRK2-PD-14	IPD-14	IPD-15	IPD-16	NMC-05	NMC-08	NMNC-04	NMNC-09
Clinical status	LRRK2 PD	LRRK2 PD	LRRK2 PD	IPD	IPD	IPD	NMC	NMC	NMNC	NMNC
LRRK2 mutation	G2019S	G2019S	G2019S	No	No	No	G2019S	R1441C	R1441G	G2019S
Age (years)	66	77	64	73	75	51	47	61	34	58
Gender	Male	Female	Female	Female	Male	Male	Male	Female	Male	Female
Age at parkinsonism onset (years)	64	65	56	69	69	44	NA	NA	NA	NA
Antiparkinsonian drugs	No	L-dopa, pramipexole, rasagiline	L-dopa, pramipexole, rasagiline	L-dopa, pramipexole	L-dopa, entacapone	Ropinirole	No	No	No	No
LEDD (mg)	0	1310	805	775	399	140	0	0	0	0
Age at RLS onset (years)	25	70	59	69	69	20	20	56	29	56
RLS Family History	No	No	No	No	No	Yes	Yes	No	No	No
IRLSGRS score (n)	21	8	18	14	12	2	15	11	0	23
ESS score (n)	5	7	17	11	8	8	12	4	4	4
PSQI score (n)	13	10	15	11	12	6	3	5	2	18
PDSS-2 score (n)	33	15	30	13	16	4	22	10	2	21
Subjective sleep complaints	Sleep onset insomnia, sleep fragmentation	Sleep fragmentation, early awakening	Sleep onset insomnia, sleep fragmentation	Sleep fragmentation, early awakening	Sleep fragmentation, early awakening	No	Sleep fragmentation	Sleep fragmentation	No	Sleep onset insomnia, sleep fragmentation, early awakening
PLMSI (n)	26	43	0	10	15	4	19	10	17	21
Sleep efficiency (%)	71	70	91	79	35	92	88	65	91	71
Mean Sleep Latency onset on MSLT (min)	14	4	5	12	18	2	5	9	4	13

ESS: Epworth Sleepiness Scale; **IRLSGRS:** International Restless-legs syndrome Study Group Scale; **LEDD:** L-Dopa Equivalent daily dose; **LRRK2-PD:** LRRK2 Parkinson disease subjects;**MSLT:** Multiple Sleep Latency Sleep Test; **NA:** Not applicable; **NMC:** Non-manifesting carriers; **NMNC:** Non-manifesting non-carriers; **PSQI:** Pittsburg Sleep Quality Index; **PDSS-2:** Parkinson Disease Sleep Scale 2; **PLMSI:** Periodic Leg Movements in Sleep Index; **RLS:** Restless Legs Syndrome.

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Table 5. LRRK2-Parkinson Disease patients with associated REM sleep behavior disorder.

Patient	LRRK2-PD-2	LRRK2-PD-6	LRRK2-PD-10
Age (years), gender	56, male	75, male	71, male
LRRK2 mutation	G2019S	G2019S	G2019S
Age at RBD onset (years)	Unknown	74	68
Age at parkinsonism onset (years)	40	62	58
UPDRS-III score (n)	6	10	18
HoehnandYahr stage (n)	2	2.5	3
Motor subtype	PIGD	PIGD	PIGD
Falls / Freezing	Yes / No	Yes / No	Yes / Yes
Motor fluctuations	Yes	Yes	Yes
LEDD (mg)	664	1674	1940
MoCA (n)	28	25	22
Hallucinations	No	No	No
RBDSQ (n)	2	8	4
Unpleasant dream recall	No	Attacked and chased by someone, attacked by animals	No
Presence of a bed partner	No	Yes	Yes
Patient awareness of dream-enacting behaviours	No	No	No
Reported dream-enacting behaviors by the bed partner	No	Talking, screaming, biting the bedpartner	Punching, kicking, knocking off the nightstand, talking, screaming, moaning
REM sleep behaviors detected during V-PSG	Prominent limb jerking, talking	Prominent body jerking, kicking	Prominent limb jerking, screaming and moaning
SINBAR EMG activity (%)	39.8	42.1	35.9
M + AT EMG activity (%)	39.4	42.8	36.8
Mentalis EMG activity (%)	28.3	37.4	32.4

LEDD: L-Dopa Equivalent daily dose; **M+AT**: polysomnographic montage quantifying "any" (tonic or phasic) type of EMG activity in the mentalis muscle plus bilateral anterior tibialis phasic EMG activity in REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 46.4%)[38]; **Mentalis**: EMG activity quantification of "any" (tonic and phasic) type of EMG activity in the mentalis muscle during REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 18.2%.)[38]; **MoCA**: Montreal Cognitive Assessment; **PIGD**: postural instability gait difficulty motor subtype; **SINBAR**: polysomnographyc montage quantifying "any" (tonic or phasic) type of EMG activity in the mentalis muscle and phasic EMG activity in the right and left flexor digitorumsuperficialis muscles during REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 32%)[38]; **RBD**: REM sleep behaviour disorder; **RBDSQ**: REM sleep behavior disorder screening questionnaire; **UPDRS-III**: Unified Parkinson Disease Rating Scale motor exam; **V-PSG:** video-polysomnography.

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instability and gait difficulty motor subtype. In two, the onset of RBD was reported to occur 10 and 12 years after the onset of parkinsonism, respectively. These two patients were unaware of displaying dream-enacting behaviors that were reported by their wives who did not link the onset of RBD symptomatology with the introduction of any medication. The remaining RBD patient had no bed partner, and he reported no dream recall and lack of abnormal sleep behaviors. However, V-PSG was demonstrative of RBD showing excessive EMG activity in REM sleep linked to prominent body jerking and talking. One additional patient reported talking, laughing and kicking and recalled dreams of being attacked and arguing with a RBDSQ score of 8 points (which is suggestive of RBD). In this patient, however, V-PSG showed normal muscle atonia in REM sleep (SINBAR EMG activity of 10%)and therefore RBD was ruled out[38]. V-PSG showed AHI of 65. Videotape analysis disclosed gesturing and talking that only
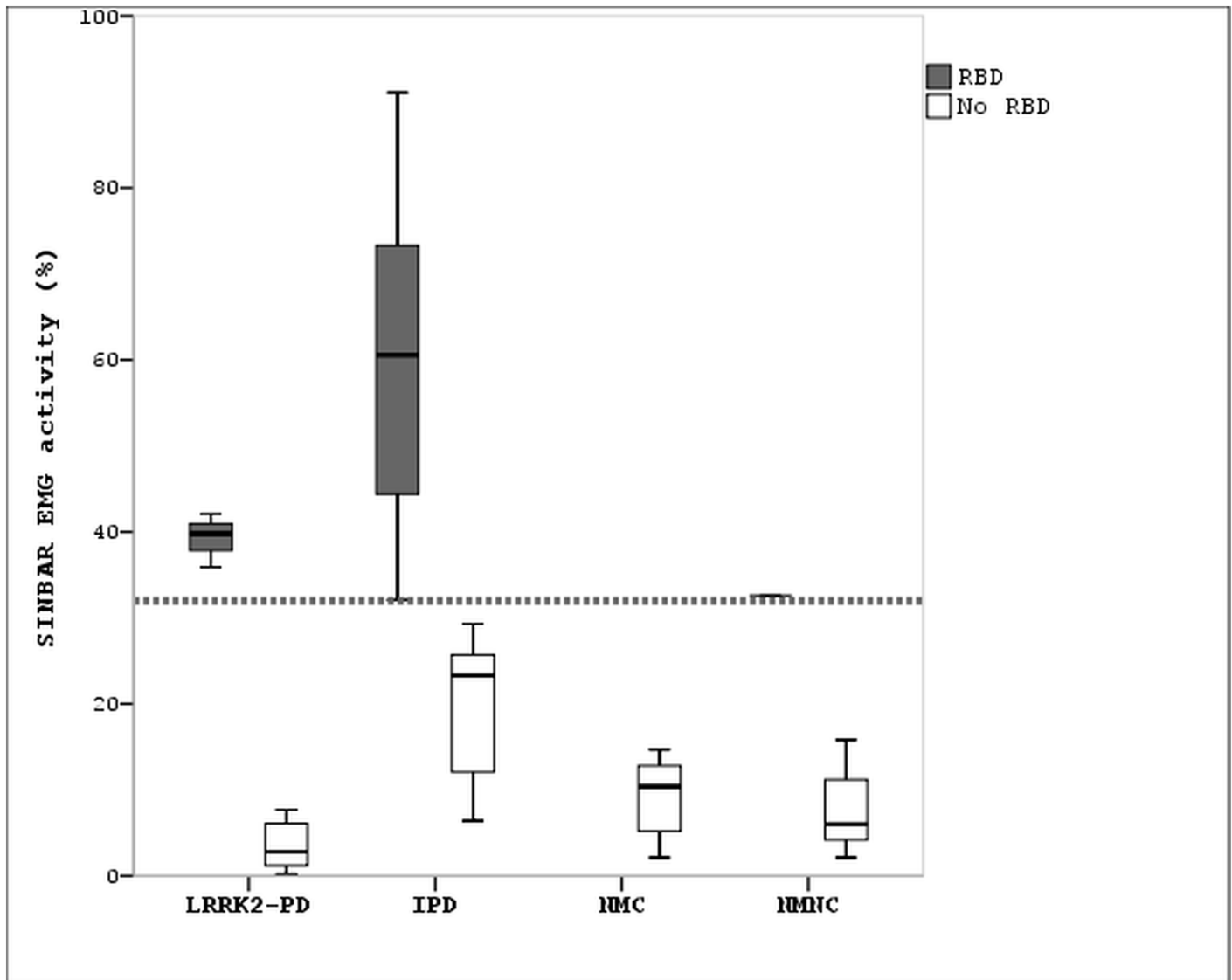
Fig 1. Electromyographic activity in REM sleep. Bars represent the percentage of electromyographic activity found during REM sleep in the different groups included in the study. Grey bars represent the percentage of electromyographic activity found in individuals who were diagnosed with RBD. White bars represent the percentage of electromyographic activity found in individuals who had no RBD. The dotted transversal line indicates that 32% of electromyographic activity is the cut-off value for the diagnosis of RBD according to the SINBAR method. Electromyographic activity ≥32% during REM sleep is indicative of RBD[38]. IPD: Idiopathic Parkinson disease; LRRK2-PD: LRRK2 Parkinson disease; NMC: non-manifesting carriers; NMNC: non-manifesting non-carriers; RBD: REM sleep behavior disorder; SINBAR: polysomnographic montage quantifying "any" (phasic or tonic) type of EMG activity in the mentalis muscle and phasic EMG activity in the right and left flexor digitorum superficialis muscles during REM sleep using 3-sec mini-epochs.

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occurred during arousals at the end of obstructive apneic events during REM sleep and to a lesser extent during no-REM sleep. We concluded that this patient had severe obstructive sleep apnea mimicking RBD symptomatology ("pseudo-RBD")[40].

The LRRK2-PD patient carrying theR1441G mutation complained of early awakening but he had no subjective EDS, no sleep attacks, no RLS, no RBD, and V-PSG showed sleep efficiency of 95%, absence of apneas and PLMS, and sleep onset latency on the MSLT of 4 min without SOREMPs.

Table 6. Patients with idiopathic Parkinson Disease associated with REM sleep behavior disorder.

Patient	IPD-01	IPD-02	IPD-04	IPD-06	IPD-08	IPD-010	IPD-14	IPD-18	IPD-19	IPD-20
Age (years), gender	57, female	69, male	75, male	60, male	62, male	63, female	73, female	72, male	78, female	56, female
Age at RBD onset (years)	32	59	73	54	48	56	65	Unknown	75	56
Age at parkinsonism onset (years)	42	64	74	49	56	56	68	62	65	52
UPDRS-III (n)	20	28	23	9	24	30	26	44	16	24
Hoehn and Yahr stage (n)	2	2	1	2	2.5	2	3	2.5	2	1.5
Motor subtype	Indeterminate	TD	TD	PIGD	PIGD	PIGD	PIGD	PIGD	PIGD	TD
Falls / Freezing	No / No	No / No	No / No	Yes / No	No / No	No / No	No / Yes	No / Yes	Yes / No	No / No
Motor fluctuations	Yes	No	No	Yes	Yes	Yes	No	No	Yes	No
LEDD (mg)	410	120	100	773	900	700	775	960	1597	0
MoCA (n)	ND	ND	22	26	24	25	ND	ND	ND	ND
Hallucinations	No	No	No	Yes	No	No	Yes	No	Yes	No
RBDSQ (n)	3	2	5	4	7	3	9	2	8	2
Unpleasant dream recall	Falling from a cliff	Arguing with someone, attacked by someone, falling from a cliff	No	No	Attacked by someone, falling from a cliff	Falling from a cliff, being robbed	No	No	Arguing with someone, chased by someone, children being kidnapped	No
Presence of a bedpartner	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Patient awareness of dream-enacting behaviours	No	Yes	No	No	No	No	No	No	Yes	No
Reported dream-enacting behaviorsby the bed partner	Gesturing, screaming, talking, laughing	Gesturing, punching, knocking off the nightstand, screaming	Gesturing, punching, talking	Gesturing, punching kicking, screaming, talking, laughing	Gesturing, screaming, talking, laughing	Talking, kicking	Gesturing, punching, kicking, knocking off the nightstand, talking, screaming, laughing, crying	No	Gesturing, punching, kicking, knocking off the nightstand, creaming, talking, crying, laughing, falling out of bed causing lacerations	Screaming
REM sleep behaviors detected during V-PSG	Prominent limb and body jerking, moaning	Prominent limb and body jerking, gesturing, punching	Limb and body jerking, talking moaning	Prominent body jerking, gesturing	Prominent limb jerking	Prominent body jerking, gesturing	Talking, moaning, swearing, prominent, body jerking, punching	Prominent limb and body jerking	Prominent limb jerking, murmuring	Prominent limb jerking, kicking and punching
SINBAR EMG activity (%)	41.7	91.1	58.6	63.8	32.1	44.4	85.3	62.5	73.3	49.6
M + AT EMG activity (%)	38.9	84.4	53.8	66.3	33.1	42.3	76.8	63.3	68.1	54.0

(Continued)

Table 6. (*Continued*)

Patient	IPD-01	IPD-02	IPD-04	IPD-06	IPD-08	IPD-010	IPD-14	IPD-18	IPD-19	IPD-20
Mentalis EMG activity (%)	21.7	83.4	37.7	43.5	6.9	38.3	72.0	35.4	58.7	43.7

LEDD: L-Dopa Equivalent daily dose; **M+AT:** polysomnographic montage quantifying "any" (tonic or phasic) type of EMG activity in the mentalis muscle plus bilateral anterior tibialis phasic EMG activity in REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 46.4%)[38]; **Mentalis:** EMG activity quantification of "any" (tonic and phasic) type of EMG activity in the mentalis muscle during REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 18.2%[38]; **MoCA:** Montreal Cognitive Assessment; **ND:** not done; **PIGD:** postural instability gait difficulty motor subtype; **SINBAR:** polysomnographyc montage quantifying "any" (tonic or phasic) type of EMG activity in the mentalis muscle and phasic EMG activity in the right and left flexor digitorumsuperficialis muscles during REM sleep, using 3-sec mini-epochs (the cut-off for patients with RBD is 32%[38]; **RBD:** REM sleep behaviour disorder; **RBDSQ:** REM sleep behavior disorder screening questionnaire; **TD:** tremor dominant motor subtype; **UPDRS-III:** Unified Parkinson Disease Rating Scale motor exam; **V-PSG:** video-polysomnography.

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Sleep in LRRK2-PD vs. IPD subjects

Compared to IPD, LRRK2-PD patients reported more frequently sleep onset insomnia (p = 0.005), had greater (worse) PSQI score (p = 0.033) and PSQI ≥6 (p = 0.025), more benzodiazepines usage (p = 0.003) and lower UPDRS-III score (p = 0.022). In a secondary analysis controlled for benzodiazepine and dopamine agonist intake, UPDRS-III score (p = 0.012), PSQI ≥6 (p = 0.012), and PSQI and score (p = 0.056) were still significant but not sleep onset insomnia (p = 0.998).

There were no differences between LRRK2-PD and IPD groups in percentage of patients complaining of EDS, ESS score, mean sleep latency on the MSLT, and percentage of patients with mean sleep latencies shorter than five and eight min on the MSLT.

Overall, the LRRK2-PD group had less EMG activity in REM sleep than the IPD group (Fig 1). RBD was diagnosed in three (17%) patients with LRRK2-PD and in ten (53%) with IPD (p = 0.034) (Tables 5 and 6). Among the patients with RBD, those with LRKK2-PD had less EMG activity in REM sleep than those with IPD (Fig 1). Seven IPD patients with true RBD had a RBDSQ score less than seven (which is suggestive of not having RBD according to this instrument). One IPD patient reporting dream-enacting behaviours and nightmares had a RBDSQ score of eight points (suggestive of RBD), but V-PSG demonstrated that he had not RBD but severe obstructive sleep apnea (AHI = 69)mimicking this parasomnia ("pseudo RBD")[40].

Sleep in NMC of *LRRK2* mutations

Subjects were nine women and eight men with a mean age of 44.4 ± 12.3 years. Sleep complaints in this group were not common. Only one subject had a PSQI ≥6 and two a PDSS-2 ≥15. Two complained of sleep onset insomnia, four of sleep fragmentation, one of early awakening, two had RLS, and only one used benzodiazepines.

Four (23%) subjects complained of EDS and all four had an ESS score ≥11. Sleep attacks were not reported by any individual. The mean sleep latency on the MSLT was in the normal range (9.3 ± 5.3 min) and SOREMPs did not occur. The mean sleep latency on the MSLT was not correlated with any demographic, clinical, and PSG variable.

None of the NMC had RBD. One NMC with RLS reported dream-enacting behaviors (e.g., kicking, gesturing, talking) and nightmares (e.g., being attacked by animals, arguing with people), and had a RBSQ score of 9points (suggestive of RBD). However, V-PSG detected normal muscle atonia in REM sleep (SINBAR EMG activity of 10.7%) excluding RBD[38]. V-PSG

showed a PLMSI of 29 where PLMS were associated with arousals and videotape analysis showed that the leg movements also involved the trunk and upper limbs resulting in prominent body jerking associated with arousals. We concluded that in this subject the RBD-like symptomatology was likely to be secondary to PLMS ("pseudo-RBD")[41].

Sleep in LRRK2-PD vs. NMC of *LRRK2* mutations

LRRK2-PD patients were older and had higher UPDRS-I-IV scores, HADS depression subscore, early awakening, PSQI score, PSQI ≥6, PDSS-2 score and more benzodiazepine intake. In PSG, LRRK2-PD subjects had higher percentage of N2, and lower percentages of N3 and REM sleep than NMC.

Six LRRK2-PD patients and three NMC complained of EDS, and the mean ESS score was similar between both groups. The mean sleep latency was shorter in the LRRK2-PD than in NMC group but difference was not statistically significant (6.5 ± 4.6 vs. 9.3 ± 5.3 min; p = 0.119). RBD was diagnosed in three LRRK2-PD patients and in none NMC.

Sleep in NMC vs. NMNC of *LRRK2* mutations

Demographic, clinical, PSG and MSLT variables were similar between these two groups.

Discussion

To the best of our knowledge, this is the first systematic study evaluating sleep disorders with subjective measures (comprehensive sleep interview, validated sleep scales and questionnaires) and objective tools (nocturnal V-PSG followed by MSLT) in *LRRK2* mutation carriers. The study evaluated individuals who had developed motor symptoms(LRRK2-PD and IPD) and NMC who are at risk to manifest PD. Despite the limited number of subjects included, we believe that our findings are relevant considering the prevalence of LRRK2 mutation in the general population[9, 11]. Subjects were not selected on the basis of any specific sleep complaint or previous diagnosis of a sleep disorder. We found that sleep complaints were frequent in LRRK2-PD and included sleep onset insomnia, sleep fragmentation and early awakening. Short sleep onset latencies on the MSLT, indicative of objective EDS, were frequent. Sleep attacks occurred in three LRRK2-PD patients and a narcoleptic phenotype was not observed on the MSLT. RBD occurred in three LRRK2-PD patients. EDS and RBD were reported to start after onset of parkinsonism. RLS, PLMS and obstructive sleep apnea were not prominent features in LRRK2-PD. In NMC, EDS was rarely reported and RBD was absent, suggesting that these sleep disorders are not premotor markers of LRRK2-PD.

Mutations in the *LRRK2* gene are the most common cause of monogenic PD[11]. In Spain, the most frequent *LRRK2* mutations are G2019S and R1441G mutations, the latter particularly in the Basque Country (Northern Spain)[9, 13–14]. The motor phenotype of LRRK2-PD resembles IPD and is characterized by parkinsonism of late onset that responds to L-Dopa therapy and motor complications of dopaminergic therapy[11]. Nonmotor symptoms are also frequent in LRRK2-PD although they may differ when compared to IPD. Olfactory dysfunction, constipation, cardiac sympathetic denervation and neuropsychological impairment are less prevalent and less severe in LRRK2-PD than in IPD [13, 15, 42]. In contrast, depression and worse color discrimination have been reported to occur more frequently in LRRK2-PD [12, 43].

Previous studies reported that LRRK2-PD patients may complaint of sleep onset insomnia, repeated awakenings and features suggestive of RBD[11, 13, 15, 43–45]. However, these studies did not employ scales or questionnaires specifically designed to examine sleep, and V-PSG and MSLT were not used to identify objectively sleep disorders like EDS and RBD. Overall, we have

found the same sleep disorders in LRRK2-PD than those previously described in IPD (sleep onset insomnia, fragmented sleep, early awakenings, EDS, sleep attacks and RBD) but with a distinct pattern; sleep onset insomnia was more frequent, EDS was similar and RBDwas less frequent and less severe in LRRK2-PD than in IPD.

Compared to IPD, sleep onset insomnia was more frequent in LRRK2-PD and it was associated with depressive symptoms and poor sleep quality. Of note, sleep onset insomnia in LRRK2-PD was not linked to features such as age, gender,PD severity and duration, anxiety, and cognitive status. These findings may suggest that difficulty falling asleep in LRRK2-PD may be explained in part by depression. Our findings are consistent with other studies in LRRK2-PD where sleep disorders, mainly sleep onset insomnia and repeated awakenings, occurred in 65% of the cases[43]and they were related to depression[12, 43, 45].

EDS was investigated through subjective (sleep interview and ESS) and objective (MSLT) measures[46–47]. All LRRK2-PD patients who complained of experiencing EDS reported that parkinsonism preceded the onset of this symptom. MSLT in LRRK2-PD showed that an increased level of daytime sleepiness is a common feature that is frequently misperceived by patients themselves. The finding that subjects were unaware of being abnormally asleep has also been reported in 13–47% IPD patients without complaints of EDS[48–50].

In LRRK2-PD, demographic, clinical and sleep variables did not distinguish between subjects with abnormal and normal sleep latencies on the MSLT. Potential causes of EDS such as PD duration and severity, pain, depressive symptoms, the use of benzodiazepines, antidepressants or dopaminergic agents, and obstructive sleep apnea failed to reveal a relationship with reduced sleep latency on the MSLT. This suggests that the origin of EDS in LRRK2-2 PD is an intrinsic feature of the disease itself related to an abnormal sleep-wake control, and it is not the result of nocturnal sleep abnormalities, parkinsonism, or any drug.

Sleep attacks were noticed by three subjects with LRRK2-PD. As previously described in IPD, sleep attacks in our LRRK2-PD patients were linked to the introduction of a dopamine agonist, obstructive sleep apnea or an increasedlevel of daytime sleepiness on the MSLT[3, 51]. In contrast to what has been reported in IPD[48, 51], SOREMPs were not common in *LRRK2* mutation carriers. We did not identify a narcoleptic phenotype in any patient carrying *LRRK2* mutations.

Three LRRK2-PD patients had RBD confirmed by V-PSG. The pathophysiology of RBD is related to dysfunction of the brainstem nuclei that regulate REM sleep atonia, namelythe locus subcoeruleus and the magnocellularis nucleus[52]. Postmortem studies have shown that these nuclei are severely damaged in IPD associated with RBD[53]. To the best of our knowledge the state of these nuclei has not been reported in LRRK2-PD postmortem reports[54]. Our study showed that compared to RBD occurring in IPD, RBD in LRRK2-PD was less frequent and had less EMG activity during REM sleep. Thus, it can be speculated that damage of the brainstem structures that regulate REM sleep atonia is less marked in LRRK2-PD than in IPD. This might be attributed to the heterogeneous neuropathological substrate found in LRRK2-PD [54].

All three LRRK2-PD patients with RBD were men, carried the G2019S mutation, had the postural instability and gait difficulty motor subtype, no hallucinations, no cognitive impairment and lack of a previous history of impulse control disorders. RBD symptomatology developed several years after parkinsonism onset. All were unaware of displaying dream-enacting behaviors that were described by their bed partners, a finding which is also common in idiopathic RBD and RBD secondary to IPD and multiple system atrophy[53].

The diagnosis of RBD was based on clinical and V-PSG criteria showing excessive EMG activity in REM sleep associated with vigorous behaviors. Only V-PSG was able to identify true RBD patients that were unaware of displaying dream-enacting behaviors (false negatives) and

to exclude RBD in subjects with other sleep disorders (severe obstructive sleep apnea and prominent PLMS involving the trunk and upper limbs) who presented with dream-enacting behaviors and nightmares (false positives, RBD mimics). This reflects that V-PSG is necessary to diagnose RBD and that questionnaires, particularly when self-administered, may be not adequate.

The penetrance of LRRK2 mutations is incomplete and increases in an age dependent manner[11]. In *LRRK2* mutation carriers mean age of PD diagnosis is 58 years, and the estimated risk to develop PD ranges from 12% to 36% at 59 years,26% to 59% at 69 years, and 47% to 80% at 79 years[11, 55]. This indicates that asubstantial proportion of NMC with increased age could bein the prodromal stage of LRRK2-PD. The study of NMC provides a unique opportunity to characterize the premotor stage of LRRK2-PD and the search for markers of conversion. Conversion markers in prodromal LRRK2-PD may prove useful when neuroprotective strategies will become available. Available data suggest thathyposmia, depression and constipation can be present in the premotor stage of LRRK2-PD and IPD[1, 7, 10, 12]. RBD is also a marker of premotor IPD[52]. It is uncertain if EDS predicts IPD[4]. Two population-based studies in the elderly showed that subjects who reported EDS had increased risk of developing IPD[56–57]. However, most studies have demonstrated that EDS is uncommon in untreated de novo IPD[58–60]. Our study in *LRRK2* mutation carriers did not show that RBD and EDS may be features of prodromal LRRK2-PD. RBD was not detected in NMC and when present in LRRK2-PD patients the onset of parkinsonism preceded the onset of RBD for several years. The study of sleep disorders therefore does not seem to be useful to distinguishNMC who are in the premotor stage of LRRK2-PD and are likely to develop PD during lifetime. However this assumption should be taken with caution since in our study 1) the number of NMC included in our study was relatively small, 2) the mean age of NMC was only of 44 years and these subjects may still be somewhat far in time from conversion, and 3) clinical follow-up of our cohort is needed. Further studies of a larger NMC cohort with longitudinal follow-up should permit to assess if sleep disorders are markers for phenoconversion to PD.

In summary, our findings show that sleep disorders in LRRK2-PD are common. Sleep onset insomnia was more frequent, EDS was similar and RBD was less frequent and severe in LRRK2-PD than in IPD. RBD and EDS seem not to occur in the premotor stage of LRRK2-PD.

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Author Contributions

Conceived and designed the experiments: CPS AI CG AFA DV FV YC RFS MF AB MC PC ODF SJ VP MS MJM JS ET. Performed the experiments: CPS AI CG AFA DV FV YC RFS MF AB MC PC ODF SJ VP MS MJM JS ET. Analyzed the data: CPS AI CG AFA DV FV YC RFS MF AB MC PC ODF SJ VP MS MJM JS ET. Contributed reagents/materials/analysis tools: CPS AI CG AFA DV FV YC RFS MF AB MC PC ODF SJ VP MS MJM JS ET. Wrote the paper: CPS AI CG AFA DV FV YC RFS MF AB MC PC ODF SJ VP MS MJM JS ET.

References

1. Tolosa E, Pont-Sunyer C. Progress in defining the premotor phase of Parkinson's disease. J Neurol Sci. 2011; 310(1–2):4–8. Epub 2011/06/18. S0022-510X(11)00288-7 [pii] doi: 10.1016/j.jns.2011.05. 027 PMID: 21679972.

2. Martinez-Martin P. The importance of non-motor disturbances to quality of life in Parkinson's disease. J Neurol Sci. 2011; 310(1–2):12–6. Epub 2011/05/31. S0022-510X(11)00237-1 [pii] doi: 10.1016/j.jns. 2011.05.006 PMID: 21621226.

3. De Cock VC, Vidailhet M, Arnulf I. Sleep disturbances in patients with parkinsonism. Nat Clin Pract Neurol. 2008; 4(5):254–66. Epub 2008/04/10. ncpneuro0775 [pii] doi: 10.1038/ncpneuro0775 PMID: 18398415.

4. Iranzo A. Sleep-wake changes in the premotor stage of Parkinson disease. J Neurol Sci. 2011; 310(1–2): 283–5. Epub 2011/09/02. S0022-510X(11)00467-9 [pii] doi: 10.1016/j.jns.2011.07.049 PMID: 21880334.

5. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron. 2004; 44(4):601–7. Epub 2004/11/ 16. S0896627304007202 [pii] doi: 10.1016/j.neuron.2004.11.005 PMID: 15541309.

6. Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron. 2004; 44(4):595–600. Epub 2004/11/16. S0896627304006890 [pii] doi: 10.1016/j.neuron.2004.10.023 PMID: 15541308.

7. Johansen KK, White LR, Farrer MJ, Aasly JO. Subclinical signs in LRRK2 mutation carriers. Parkinsonism Relat Disord. 2011; 17(7):528–32. Epub 2011/06/07. S1353-8020(11)00108-8 [pii] doi: 10.1016/j. parkreldis.2011.04.014 PMID: 21641848.

8. Brockmann K, Groger A, Di Santo A, Liepelt I, Schulte C, Klose U, et al. Clinical and brain imaging characteristics in leucine-rich repeat kinase 2-associated PD and asymptomatic mutation carriers. Mov Disord. 2011; 26(13):2335–42. Epub 2011/10/13. doi: 10.1002/mds.23991 PMID: 21989859.

9. Gaig C, Ezquerra M, Marti MJ, Munoz E, Valldeoriola F, Tolosa E. LRRK2 mutations in Spanish patients with Parkinson disease: frequency, clinical features, and incomplete penetrance. Arch Neurol. 2006; 63(3):377–82. Epub 2006/03/15. 63/3/377 [pii] doi: 10.1001/archneur.63.3.377 PMID: 16533964.

10. Gaig C, Vilas D, Infante J, Sierra M, Garcia-Gorostiaga I, Buongiorno M, et al. Nonmotor symptoms in LRRK2 G2019S associated Parkinson's disease. PLoS One. 2014; 9(10):e108982. Epub 2014/10/21. doi: 10.1371/journal.pone.0108982 PONE-D-14-12783 [pii]. PMID: 25330404; PubMed Central PMCID: PMC4201457.

11. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol. 2008; 7(7):583–90. Epub 2008/06/10. S1474-4422(08)70117-0 [pii] doi: 10.1016/S1474-4422 (08)70117-0 PMID: 18539534; PubMed Central PMCID: PMC2832754.

12. Marras C, Schuele B, Munhoz RP, Rogaeva E, Langston JW, Kasten M, et al. Phenotype in parkinsonian and nonparkinsonian LRRK2 G2019S mutation carriers. Neurology. 2011; 77(4):325–33. Epub 2011/07/15. WNL.0b013e318227042d [pii] doi: 10.1212/WNL.0b013e318227042d PMID: 21753163; PubMed Central PMCID: PMC3140802.

13. Ruiz-Martinez J, Gorostidi A, Goyenechea E, Alzualde A, Poza JJ, Rodriguez F, et al. Olfactory deficits and cardiac (123) I-MIBG in Parkinson's disease related to the LRRK2 R1441G and G2019S mutations. Mov Disord. 2011. Epub 2011/05/26. doi: 10.1002/mds.23773 PMID: 21611983.

14. Sierra M, Sanchez-Juan P, Martinez-Rodriguez MI, Gonzalez-Aramburu I, Garcia-Gorostiaga I, Quirce MR, et al. Olfaction and imaging biomarkers in premotor LRRK2 G2019S-associated Parkinson disease. Neurology. 2013; 80(7):621–6. Epub 2013/01/18. WNL.0b013e31828250d6 [pii] doi: 10.1212/ WNL.0b013e31828250d6 PMID: 23325906.

15. Alcalay RN, Mirelman A, Saunders-Pullman R, Tang MX, Mejia Santana H, Raymond D, et al. Parkinson disease phenotype in Ashkenazi Jews with and without LRRK2 G2019S mutations. Mov Disord. 2013; 28(14):1966–71. Epub 2013/11/19. doi: 10.1002/mds.25647 PMID: 24243757; PubMed Central PMCID: PMC3859844.

16. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology. 1967; 17(5):427–42. Epub 1967/05/01. PMID: 6067254.

17. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry. 1992; 55(3):181–4. Epub 1992/03/01. PMID: 1564476; PubMed Central PMCID: PMC1014720.

18. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord. 2007; 22(12):1689–707; quiz 837. Epub 2007/06/02. doi: 10.1002/mds.21507 PMID: 17542011.

19. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord. 2010; 25(15):2649–53. Epub 2010/11/12. doi: 10.1002/mds.23429 PMID: 21069833.

20. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale

presentation and clinimetric testing results. Mov Disord. 2008; 23(15):2129–70. Epub 2008/11/26. doi: 10.1002/mds.22340 PMID: 19025984.

21. Schwab JF, England AC. Projection technique for evaluating surgery in Parkinson's disease. In: Billingham FH DM, eds., editor. Third Symposium on Parkinson's disease. Edinburgh, Scotland: E. And S. Livingstone; 1969. p. 152–7.

22. Jankovic J, McDermott M, Carter J, Gauthier S, Goetz C, Golbe L, et al. Variable expression of Parkinson's disease: a base-line analysis of the DATATOP cohort. The Parkinson Study Group. Neurology. 1990; 40(10):1529–34. Epub 1990/10/01. PMID: 2215943.

23. Martinez-Martin P, Frades Payo B. Quality of life in Parkinson's disease: validation study of the PDQ-39 Spanish version. The Grupo Centro for Study of Movement Disorders. J Neurol. 1998; 245 Suppl 1: S34–8. Epub 1998/06/09. PMID: 9617722.

24. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983; 67(6): 361–70. Epub 1983/06/01. PMID: 6880820.

25. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005; 53(4):695–9. Epub 2005/04/09. JGS53221 [pii] doi: 10.1111/j.1532-5415.2005.53221.x PMID: 15817019.

26. Lozano-Gallego M H-FM, Turró Garriga O, PericotNierga I, López-Pousa S, VilaltaFranch J. Validación del Montreal CognitiveAssessment (MoCA): test de cribado para el deterioro cognitivo leve. Datospreliminares. Real Invest Demencia. 2009;(43:):4–11.

27. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989; 28(2):193–213. Epub 1989/05/01. 0165-1781(89)90047-4 [pii]. PMID: 2748771.

28. Trenkwalder C, Kohnen R, Hogl B, Metta V, Sixel-Doring F, Frauscher B, et al. Parkinson's disease sleep scale—validation of the revised version PDSS-2. Mov Disord. 2011; 26(4):644–52. Epub 2011/02/12. doi: 10.1002/mds.23476 PMID: 21312275.

29. Suzuki K, Miyamoto T, Miyamoto M, Suzuki S, Numao A, Watanabe Y, et al. Evaluation of cutoff scores for the Parkinson's disease sleep scale-2. Acta Neurol Scand. 2015; 131(6):426–30. Epub 2014/11/18. doi: 10.1111/ane.12347 PMID: 25402773.

30. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991; 14(6):540–5. Epub 1991/12/01. PMID: 1798888.

31. Hobson DE, Lang AE, Martin WR, Razmy A, Rivest J, Fleming J. Excessive daytime sleepiness and sudden-onset sleep in Parkinson disease: a survey by the Canadian Movement Disorders Group. JAMA. 2002; 287(4):455–63. Epub 2002/02/02. joc10367 [pii]. PMID: 11798367.

32. Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisi J. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med. 2003; 4(2): 101–19. Epub 2003/11/01. S1389945703000108 [pii]. PMID: 14592341.

33. Walters AS, LeBrocq C, Dhar A, Hening W, Rosen R, Allen RP, et al. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. Sleep Med. 2003; 4(2): 121–32. Epub 2003/11/01. S1389945702002587 [pii]. PMID: 14592342.

34. International classification of sleep disorders. 3rd ed. Darien I, editor2014.

35. Stiasny-Kolster K, Mayer G, Schafer S, Moller JC, Heinzel-Gutenbrunner M, Oertel WH. The REM sleep behavior disorder screening questionnaire—a new diagnostic instrument. Mov Disord. 2007; 22(16):2386–93. Epub 2007/09/27. doi: 10.1002/mds.21740 PMID: 17894337.

36. Chahine LM, Daley J, Horn S, Colcher A, Hurtig H, Cantor C, et al. Questionnaire-based diagnosis of REM sleep behavior disorder in Parkinson's disease. Mov Disord. 2013; 28(8):1146–9. Epub 2013/03/23. doi: 10.1002/mds.25438 PMID: 23519694.

37. Iber C A-IS, Chesson A, and Quan SF for the American Academy of Sleep Medicine. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. 1st ed. Medicine AAoS, editor. Westchester, Illinois:2007.

38. Frauscher B, Iranzo A, Gaig C, Gschliesser V, Guaita M, Raffelseder V, et al. Normative EMG values during REM sleep for the diagnosis of REM sleep behavior disorder. Sleep. 2012; 35(6):835–47. Epub 2012/06/02. doi: 10.5665/sleep.1886 PMID: 22654203; PubMed Central PMCID: PMC3353058.

39. Carskadon MA, Dement WC, Mitler MM, Roth T, Westbrook PR, Keenan S. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. Sleep. 1986; 9(4):519–24. Epub 1986/12/01. PMID: 3809866.

40. Iranzo A, Santamaria J. Severe obstructive sleep apnea/hypopnea mimicking REM sleep behavior disorder. Sleep. 2005; 28(2):203–6. Epub 2005/09/21. PMID: 16171244.

41. Gaig CI A.; Santamaria J.. Periodic limb movement disorder presenting as a parasomnia. J Neurol. 2013;(260 (Suppl 1)):S32.

42. Valldeoriola F, Gaig C, Muxi A, Navales I, Paredes P, Lomena F, et al. 123I-MIBG cardiac uptake and smell identification in parkinsonian patients with LRRK2 mutations. J Neurol. 2011; 258(6):1126–32. Epub 2011/01/12. doi: 10.1007/s00415-010-5896-6 PMID: 21221623; PubMed Central PMCID: PMC3101340.

43. Goldwurm S, Zini M, Di Fonzo A, De Gaspari D, Siri C, Simons EJ, et al. LRRK2 G2019S mutation and Parkinson's disease: a clinical, neuropsychological and neuropsychiatric study in a large Italian sample. Parkinsonism Relat Disord. 2006; 12(7):410–9. Epub 2006/06/06. S1353-8020(06)00083-6 [pii] doi: 10.1016/j.parkreldis.2006.04.001 PMID: 16750929.

44. Belarbi S, Hecham N, Lesage S, Kediha MI, Smail N, Benhassine T, et al. LRRK2 G2019S mutation in Parkinson's disease: a neuropsychological and neuropsychiatric study in a large Algerian cohort. Parkinsonism Relat Disord. 2010; 16(10):676–9. Epub 2010/10/12. S1353-8020(10)00226-9 [pii] doi: 10.1016/j.parkreldis.2010.09.003 PMID: 20933457.

45. Berg D, Schweitzer KJ, Leitner P, Zimprich A, Lichtner P, Belcredi P, et al. Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. Brain. 2005; 128(Pt 12): 3000–11. Epub 2005/10/28. awh666 [pii] doi: 10.1093/brain/awh666 PMID: 16251215.

46. Ataide M, Franco CM, Lins OG. Daytime sleepiness and Parkinson's disease: the contribution of the multiple sleep latency test. Sleep Disord. 2014; 2014:767181. Epub 2014/08/16. doi: 10.1155/2014/767181 PMID: 25126427; PubMed Central PMCID: PMC4121261.

47. Santamaria J. How to evaluate excessive daytime sleepiness in Parkinson's disease. Neurology. 2004; 63(8 Suppl 3):S21–3. Epub 2004/10/27. 63/8_suppl_3/S21 [pii]. PMID: 15505138.

48. Arnulf I, Konofal E, Merino-Andreu M, Houeto JL, Mesnage V, Welter ML, et al. Parkinson's disease and sleepiness: an integral part of PD. Neurology. 2002; 58(7):1019–24. Epub 2002/04/10. PMID: 11940685.

49. Cochen De Cock V, Bayard S, Jaussent I, Charif M, Grini M, Langenier MC, et al. Daytime sleepiness in Parkinson's disease: a reappraisal. PLoS One. 2014; 9(9):e107278. Epub 2014/09/10. doi: 10.1371/journal.pone.0107278 PONE-D-14-28580 [pii]. PMID: 25198548; PubMed Central PMCID: PMC4157859.

50. Merino-Andreu M, Arnulf I, Konofal E, Derenne JP, Agid Y. Unawareness of naps in Parkinson's disease and in disorders with excessive daytime sleepiness. Neurology. 2003; 60(9):1553–4. Epub 2003/05/14. PMID: 12743258.

51. Peever J, Luppi PH, Montplaisir J. Breakdown in REM sleep circuitry underlies REM sleep behavior disorder. Trends Neurosci. 2014; 37(5):279–88. Epub 2014/03/29. S0166-2236(14)00025-3 [pii] doi: 10.1016/j.tins.2014.02.009 PMID: 24673896.

52. Iranzo A, Tolosa E, Gelpi E, Molinuevo JL, Valldeoriola F, Serradell M, et al. Neurodegenerative disease status and post-mortem pathology in idiopathic rapid-eye-movement sleep behaviour disorder: an observational cohort study. Lancet Neurol. 2013; 12(5):443–53. Epub 2013/04/09. S1474-4422(13)70056-5 [pii] doi: 10.1016/S1474-4422(13)70056-5 PMID: 23562390.

53. Iranzo A, Santamaria J, Rye DB, Valldeoriola F, Marti MJ, Munoz E, et al. Characteristics of idiopathic REM sleep behavior disorder and that associated with MSA and PD. Neurology. 2005; 65(2):247–52. Epub 2005/07/27. 65/2/247 [pii] doi: 10.1212/01.wnl.0000168864.97813.e0 PMID: 16043794.

54. Kalia LV, Lang AE, Hazrati LN, Fujioka S, Wszolek ZK, Dickson DW, et al. Clinical Correlations With Lewy Body Pathology in LRRK2-Related Parkinson Disease. JAMA Neurol. 2014. Epub 2014/11/18. 1934714 [pii] doi: 10.1001/jamaneurol.2014.2704 PMID: 25401511.

55. Sierra M, Gonzalez-Aramburu I, Sanchez-Juan P, Sanchez-Quintana C, Polo JM, Berciano J, et al. High frequency and reduced penetrance of LRRK2 G2019S mutation among Parkinson's disease patients in Cantabria (Spain). Mov Disord. 2011; 26(13):2343–6. Epub 2011/09/29. doi: 10.1002/mds.23965 PMID: 21954089.

56. Gao J, Huang X, Park Y, Hollenbeck A, Blair A, Schatzkin A, et al. Daytime napping, nighttime sleeping, and Parkinson disease. Am J Epidemiol. 2011; 173(9):1032–8. Epub 2011/03/16. kwq478 [pii] doi: 10.1093/aje/kwq478 PMID: 21402730; PubMed Central PMCID: PMC3121222.

57. Abbott RD, Ross GW, White LR, Tanner CM, Masaki KH, Nelson JS, et al. Excessive daytime sleepiness and subsequent development of Parkinson disease. Neurology. 2005; 65(9):1442–6. Epub 2005/11/09. 65/9/1442 [pii] doi: 10.1212/01.wnl.0000183056.89590.0d PMID: 16275833.

58. Buskova J, Klempir J, Majerova V, Picmausova J, Sonka K, Jech R, et al. Sleep disturbances in untreated Parkinson's disease. J Neurol. 2011; 258(12):2254–9. Epub 2011/06/04. doi: 10.1007/s00415-011-6109-7 PMID: 21637949.

59. Plomhause L, Dujardin K, Duhamel A, Delliaux M, Derambure P, Defebvre L, et al. Rapid eye movement sleep behavior disorder in treatment-naive Parkinson disease patients. Sleep Med. 2013; 14(10): 1035–7. Epub 2013/07/31. S1389-9457(13)00220-7 [pii] doi: 10.1016/j.sleep.2013.04.018 PMID: 23890957.

60. Prudon B, Duncan GW, Khoo TK, Yarnall AJ, Anderson KN. Primary sleep disorder prevalence in patients with newly diagnosed Parkinson's disease. Mov Disord. 2014; 29(2):259–62. Epub 2014/01/28. doi: 10.1002/mds.25730 PMID: 24464648.