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1.1. Introduction: Epidemiology and risk factors of Parkinson's disease

Parkinson's disease (PD) is the most common movement disorder with strikingly rapid increase in both prevalence and incidence (*Dorsey R et al, 2018*). The incidence of PD was estimated to be 21 cases per 100 000 individuals per year as observed in the Minnesota population-based study in 2013 (*Savica R et al., 2013*). Current world-wide prevalence of PD is estimated to nearly 7 million individuals and is expected to double over the next 20 years. The reason behind this phenomenon has not yet been fully understood, but it is mainly considered to be a combined effect of increasing industrial and agricultural pollution along with ageing population (*Dorsey R et al, 2018; Murata H et al, 2022*). Physiological ageing has been determined as a major risk factor for developing PD with typical age of onset (AAO) after the age of 60 years, although cases of early-onset PD are widely reported with high proportion of genetic causes linked to PD (*Poewe W et al, 2017*). Monogenic causes of PD account for 5-10% of cases (*Klein C et al, 2012; Blauwendraat C et al, 2020*). This heritability increases up to 30% when considering pathogenic variants with incomplete penetrance and the complex polygenic architecture (*Ohnmacht J et al 2020*). However, the underlying causes remain obscure in the majority of PD cases. Interestingly, male sex shows an increased risk for PD with male-to-female ratio 2:1 across multiple study populations (*Baldereschi M et al, 2000; Georgiev D et al, 2017*) with exception in Japanese population reporting higher prevalence of females with PD vs males (*Kimura H et al, 2002*). Such a preponderance of male sex for PD may be attributed to sex-specific hormonal differences, effect of genetic background, sex-linked differences in terms of occupation and environmental exposure (*Savica R et al, 2013; Cilia R et al; 2014; Shulman LM et al, 2007; Miller IN et al; 2010*). Among non-genetic risk factors for PD, the most established are exposure to pesticides (*Nandipati S et al, 2016; Breckenridge CB et al; 2016*) and traumatic brain injury (*Chen H et al, 2018*). Conversely, the protective factors against PD have been extensively studied with convincing body of epidemiological evidence for smoking (*Chen H et al, 2010; Thacker EL et al, 2007; Ritz B et al, 2014*), caffeine consumption (*Ascherio A et al, 2004; Liu R et al, 2012; Benedetti MD et al, 2000*) and regular physical exercise (*Chen H et al, 2005; Xu Q et al, 2010*).

1.2. PATHOPHYSIOLOGY AND ALPHA-SYNUCLEIN SPREADING IN PARKINSON'S DISEASE

The core clinical manifestation of motor PD is a slowly progressive dopamine-responsive parkinsonism with bradykinesia, rigidity and/or rest tremor due to the neurodegeneration of dopaminergic cells in brain structure *substantia nigra pars compacta* (SNpc) leading to impairment of neural circuits in basal ganglia. PD is histopathologically classified as alpha-synucleinopathy, a group of diseases further including Dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). Definitive diagnosis of PD and DLB is based on autopsy proven neuronal loss of pigmented neurons in SNpc along with pathognomic alpha-synuclein positive cytoplasmic inclusions in neuronal cells (Lewy bodies) and in neuronal axons and dendrites (Lewy body dendrites) (*Spillantini M et al, 1997; Halliday G et al, 2010*). Although the physiological functions of alpha-synuclein are not yet fully understood, this protein has been identified to be involved in complex cellular dynamics including synaptic vesicle trafficking, mitochondrial homeostasis (*Vekrellis K et al; 2011*). The form of neurotoxicity is reported to be due impaired proteostasis leading to misfolding and oligomerization of alpha-synuclein that causes cellular dysfunction and neuronal death.

Over the years, the neurodegenerative process of PD has been shown to be more complex involving not only dopamine transmitter systems, but also noradrenergic, serotonergic and cholinergic systems with wide-ranging implication on motor and non-motor symptoms (*Titova N et al, 2016; Kish SJ et al, 2008*). Dysfunction of noradrenergic system in PD was linked with autonomic, motor and sensory impairment mainly driven via neurodegeneration in locus coeruleus (*Paredes et al; 2020*), whereas depression and apathy were linked to dysfunction of serotonergic system (*Pagano G et al, 2017*). In contrast, cognitive decline and postural instability is partially accounted to the deficit in cholinergic system *(Bohnen NI et al, 2022).*

Moreover, the gradual spread of misfolded alpha-synuclein behaviour has been proposed to resemble a *prion-like* dissemination across the nervous system (*Goedert M et al, 2015*). However, whether a common origin in terms of timing

and location of the misfolded aggregates exists remain unclear. The fact that misfolded alpha-synuclein appears in the olfactory bulb, in peripheral enteric nervous and in dorsal nucleus of the vagal nerve up to 20 years before full manifestation of PD (*Shannon KM et al, 2012; Stokholm MG et al, 2016; Hilton D et al, 2014*), let to the hypothesis of *dual-hit theory*. This hypothesis presumes two distinctive triggers leading to the centripetal spread of misfolded alpha-synuclein (*Hawkes CH et al, 2007*). Subsequentially, the hypothesis was partially transformed into a concept of *body-first* vs *brain-first* subtype of PD aiming to account for the variable progression rate in PD (*Nuzum ND et al, 2022*). *Body-first* subtype represents the cases where the neurodegenerative process seems to start in the peripheral nervous system affecting dominantly the cardiac sympathetic nerves and autonomic enteric system followed by neurodegeneration in central nervous system. This subtype follows a *bottom-up* direction of pathological spread and sequentially affects brainstem, midbrain, forebrain, limbic system and cortex. Accordingly, the *body-first* subtype was linked with REM-sleep behaviour disorder (RBD) and severe autonomic dysfunction (*Borghammer P et al, 2019*). Conversely, the *brain-first* subtype is represented by a *top-down* distribution of the affected areas with generally motor-dominant asymmetric PD affecting the autonomic system in lower degree and at a later stage of the disease.

1.3. PRODROMAL AND CLINICAL PHASE OF PARKINSON' DISEASE

As the neurodegenerative process in PD has been shown to be gradual and starting decades before the appearance of the typical motor impairment, affected individuals present various combination of symptoms even before the clinical diagnosis of PD. Hence the complex clinical trajectory of PD is traditionally divided into two stages: (i) prodromal and (ii) clinical phase of the disease. Alternatively, a third disease phase is added in the scheme coined to be a *preclinical phase*; a phase preceding the first prodromal symptoms corresponding to individuals having known risk factor for PD such as a PD-causing mutation or high exposure to pesticides or heavy metals in absence of any identifiable symptoms related to PD (*Mahlknecht P et al, 2015*).

Prodromal phase is characterized by appearance of non-motor symptoms associated with PD such as (among others) hyposmia, depression, anxiety, orthostatic hypotension, constipation or RBD, whereas the cardinal motor symptoms bradykinesia, rigidity and/or rest tremor are not fully established. Finally, the clinical phase of the disease manifests with motor symptoms fulfilling the official diagnostic criteria with various combination of motor and non-motor symptoms including (in addition to above mentioned) insomnia, excessive daily sleepiness, pain, erectile dysfunction, anhedonia or urinary incontinence. In the later stage of PD, motor complications arise comprising motor fluctuations, dyskinesia, dystonia or freezing of gait as well as non-motor symptoms such as hallucinations, mild cognitive impairment or dementia (*Kalia LV et al, 2015*). However, every patient affected by PD follows a unique disease trajectory yielding a need for personalized approach, thus necessitating a stratification for regular ambulatory follow-up as well as for inclusion into targeted clinical trials.

1.4. PHENOTYPIC VARIABILITY IN PARKINSON'S - GENOTYPE PHENOTYPE INTERACTION

Even though the diagnostic criteria for PD focus principally on the motor symptoms (*Litvan I et al, 2003*), the non-motor symptoms linked to PD represent a crucial part of the clinical phenotype and have undeniably an effect on the quality of life in PD patients. Moreover, the phenotypic variability has been perplexing both clinicians as well as researchers since the first systematic description of PD in the seminal *Essay on Shaking Palsy* by James Parkinson in 1817. The accumulating knowledge on phenotypic as well as pathophysiological complexity of PD has led to an open ontological debate whether PD should be considered as one disease, or rather a class of separate disorders. On the one hand, all PD patients converge in an extrapyramidal syndrome with a common denominator (slowly progressive L-DOPA responsive parkinsonism), on the other hand they heavily diverge in the combination and intensity of motor and non-motor impairment along with different disease progression rate. Due to such a variability of clinical presentation, misdiagnosis of PD (i.e., autopsy diagnosis vs clinical diagnosis) was estimated up to 20% even when assessed by a movement disorder experts (*Hughes AJ et al, 1992; Larsen JP et al 1994; Tolosa E et al; 2006*).

The genetic forms of PD have significantly contributed to the understanding of molecular pathways underlaying the neurodegeneration in PD. The PD-linked mutations have been identified in the genes mainly involved in regulating mitochondrial function and its homeostasis (PINK1, PARKIN, DJ-1), lysosomal function (LRRK2, GBA) or encoding/ handling of the alpha-synuclein (SNCA, VPS35) (*Hernandez DG et al, 2016*). While some of PD-linked mutations are very rare (e.g., PINK1, PARKIN, DJ1) with highest frequency of PARKIN mutation in early-onset PD, mutations in LRRK2 and GBA are relatively common with average of 2% and 7-10% respectively in European PD cohorts (*Schneider SA et al, 2020; Sidransky E et al, 2009*). Not only mutation in a PD-linked gene presents a distinct pathophysiological mechanism, it is also associated with a specific genotype-phenotype interaction with its typical AAO and related motor and non-motor disease profile. For instance, PARKIN mutation manifests with

early AAO (around 30 years of age) with early dystonia, no hyposmia, low risk for dementia and very slow progression rate (*Hernandez DG et al, 2016*), whereas LRRK2 resembles typical image of idiopathic PD (iPD) with AAO in 6th decade with comparable progression rate to iPD (*Tolosa E et al; 2020*). In the middle of spectrum, carriers of mutations in GBA are associated with earlier AAO (in 5th decade) presenting with early hallucinations, higher rate of cognitive decline and dementia together with higher burden of motor symptoms and progression rate (*Mata I et al, 2016; Gan-Or Z et al, 2016*). Given the well-defined genotype-phenotype interaction in PD and specific molecular pathophysiological mechanism behind the PD, the genetic stratification will pave the way to a tailored therapy in line with concept of precision medicine (*von Linstow CU et al, 2020; Schneider SA et al, 2020*).

Beside the PD-causing mutations, well established genetic risk factor for Alzheimer disease *apolipoprotein epsion4* (APOE4) has recently gained attention in PD in terms of higher risk of dementia and higher progression rate of motor symptoms in homozygotes and heterozygotes with APOE4 genotype (*Pu JL et al, 2022, Krüger et al, 1997*). However, the effect of APOE4 genotype in PD remains controversial and several studies with high sample size were not confirming such an effect in PD (*Mengel D et al, 2016; Federoff M et al, 2012*). Additionally, it has been shown that the single nucleotide polymorphisms (SNPs) in genes associated with PD can translate into a cumulative effect of small effect-sizes increasing the risk for developing PD. Based on large genome-wide association studies (GWAS) identifying SNPs significantly associated with PD, the cumulative effect of the SNPs allows to be quantified into a polygenic risk score (PRS). This factor adds to the complexity of genetic determinants of PD and potentially represents another *layer* influencing the clinical profile and severity of PD (see Figure 1). Though PRS has been recently identified to be significantly inversely associated with AAO in PD (*Escott-Price V et al, 2015*), whether a PRS has also significant effect on clinical phenotype and disease progression rate remains to be determined.

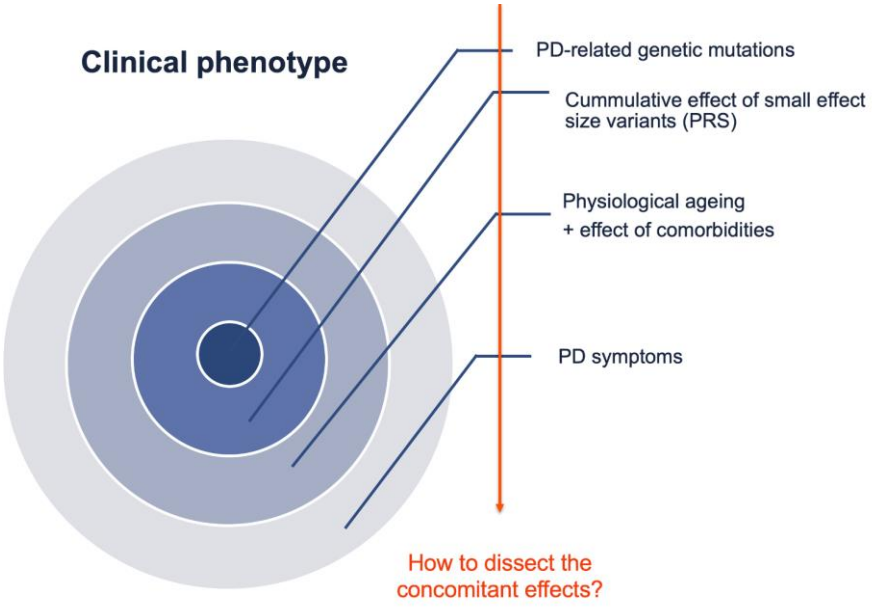

Figure 1. Illustration of the overlapping factors affecting the clinical phenotype of Parkinson's disease.

1.5. STRATIFICATION OF PARKINSON'S DISEASE

Phenotypic variability of PD has elicited several attempts to stratify patients with the aim to better understand the underlying cause of PD and its disease trajectory (Kruger et al., 2017). This approach is aligned with the concept of precision medicine based on identification and targeting of specific disease subtypes, hence allowing to tailor a future disease modifying treatment to the cause(s) of neurodegenerative process. The subtyping approaches applied so far can be categorized into three groups: (a) stratified by PD-linked mutation carrier status (b) based on single clinical variable such as AAO (early- vs late-onset PD), sex (male vs female) or presence/absence of specific symptom (e.g. RBD or freezing of gait); (c) advanced clustering and machine learning methods identifying subclasses and clusters within PD patients. Whereas approach (a) shows relatively congruent and replicable results across the studies, the approaches (b) and (c) demonstrate generally poor overlap due to varying arbitrary cut-offs and methods used for subtype identification (*Berg D et al, 2021*). In this context, age has been shown to be a key factor both in risk for developing PD as well as in its association with severity of the phenotype. Early-onset PD was identified to be associated with lower risk of dementia, slower progression rate of motor symptoms, but also early dyskinesia and dystonia (*Pagano G et al, 2016; Jellinger KA, 2003; Zhou MZ et al, 2013*). Conversely, late-onset PD was shown to progress more rapidly in terms of motor symptoms, cognitive decline and presenting higher burden of non-motor symptoms (*Ferguson LW et al; 2016; Jellinger KA et al, 2018*).

Despite high number of studies investigating the effect of AAO on the disease profile, there is very low overlap in definition of juvenile- vs early-onset vs late-onset PD, or even whether four groups should be defined (i.e., juvenile- vs early- vs middle- vs late-onset PD). Therefore, reproducibility and potential for a metanalysis of the studies focusing on the effect of ageing is relatively poor. Additionally, the cut-offs for such stratification by AAO are arbitrarily chosen and artificially influence the power of applied statistical models. As illustrated in Figure 1, the overall symptoms and burden of disability in PD patients are the result of

multiple concomitant factors overlapping by nature: physiological ageing, (ii) comorbidities and (iii) symptoms/complications of PD, merging all together in the clinical phenotype. Whether is the AAO-based phenotype determined by a specific age-dependent dynamic of PD, or it is mainly due to the concomitant ageing/comorbidities with superposed PD symptoms, remains unanswered. This open gap is ascribed to an inherit methodological problem across the studies arising due to having two variables (i.e., AAO and disease duration) that *per definitionem* determine the third (age at assessment). Therefore, using all three variables in the same regression model causes the perfect multicollinearity (*Johnson SB et al; 2002*). Such a methodological obstacle could explain why previous studies have not endeavoured to disentangle the effect of ageing vs effect of AAO in determining the phenotype in PD.

From above mentioned stratification strategies, one disease has emerged from multiple association studies as well as from studies using clustering and machine learning methods as linked to neurodegeneration in PD – RBD. Not only is idiopathic RBD the strongest predictor of conversion to alpha-synucleinopathy (up to 90% after 15 years of follow-up (*Postuma RB et al, 2019*)), but also RBD has been suggested to be associated with higher burden of autonomic dysfunction, depression and cognitive impairment (*Roguski A et al, 2020; Neikrug AB et al, 2014; Postuma RB et al; 2012*). In this context, RBD has been suggested as belonging to the *body-first subtype* of PD, a subtype with more diffuse impairment of peripheric nervous system (leading to constipation, orthostatic hypotension and sympathetic cardiac denervation) translating into a non-motor dominant PD phenotype (*Borghammer P et al, 2019*). However, the concept of *body-first vs brain first* subtype in PD has not yet been fully established and requires further investigation and validation.

1.6. CLINICO-GENETIC STRATIFICATION OF PD IN LUXEMBOURG PARKINSON'S STUDY

To understand the complex processes underlaying the development and progression of PD, Luxembourg Parkinson's Study has been established in 2015 allowing for longitudinal deep phenotyping of individuals with neurodegenerative parkinsonism in parallel with healthy controls (HC). It is one of the largest longitudinal monocentric observational studies focused on PD in the world having more than 1600 participants (*Heinzel S et al; 2017*) with unique study design including (i) patients at all disease stages, (ii) regardless of cognitive status (unimpaired cognition, mild cognitive impairment or dementia), (iii) without *a priori* tertiary referral bias, (iv) recruiting and following up the PD patients along with atypical parkinsonism and finally (v) genotyping of all individuals in the study. The cohort design, recruitment procedure and assessment batteries applied in the study were published previously in detail (*Hipp et al, 2018*).

In the presented work, we endeavoured to address the multifaceted phenotypic profiles of PD using large baseline dataset of genotyped PD and HC from Luxembourg Parkinson's Study. The key focus of this work to give an insight into the complex interaction of multiple determinants acting in PD as illustrated in Figure 1 with listed research questions addressed in the peer-reviewed articles 1 and 2 as follows:

1. How to disentangle the concomitant effect of parallel ageing and the effect of AAO on PD phenotype? Is the ageing itself accountable for the differences in PD phenotype when investigating the effect of AAO on clinical profile?
2. Is the polygenic background of PD associated with younger AAO in PD? Does higher PRS have a significant effect on clinical phenotype in PD?
3. Does pRBD stratify the patients with PD to a specific phenotype in line with the proposed *body-first* subtype of PD?
4. What is the interplay between pRBD, APOE4 genotype and clinical profile in PD? Do both factors have a concomitant/cumulative effect on cognitive decline in PD?

5. How far the sex affects the clinical phenotype in PD? Is pRBD significantly associated with male sex in PD as reported in cohorts with RBD proven by polysomnography?

2.1. RESULTS: PEER-REVIEWED ARTICLE N.1

Pavelka L, Rauschenberger A, Landoulsi Z, Pachchek S, May P, Glaab E, Krüger R; NCER-PD Consortium. Age at onset as stratifier in idiopathic Parkinson's disease - effect of ageing and polygenic risk score on clinical phenotypes. NPJ Parkinsons Dis. 2022 Aug 9;8(1):102. doi: 10.1038/s41531-022-00342-7. Erratum in: NPJ Parkinsons Dis. 2022 Sep 2;8(1):112.

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Age at onset as stratifier in idiopathic Parkinson's disease – effect of ageing and polygenic risk score on clinical phenotypes

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Several phenotypic differences observed in Parkinson's disease (PD) patients have been linked to age at onset (AAO). We endeavoured to find out whether these differences are due to the ageing process itself by using a combined dataset of idiopathic PD ($n = 430$) and healthy controls (HC; $n = 556$) excluding carriers of known PD-linked genetic mutations in both groups. We found several significant effects of AAO on motor and non-motor symptoms in PD, but when comparing the effects of age on these symptoms with HC (using age at assessment, AAA), only positive associations of AAA with burden of motor symptoms and cognitive impairment were significantly different between PD vs HC. Furthermore, we explored a potential effect of polygenic risk score (PRS) on clinical phenotype and identified a significant inverse correlation of AAO and PRS in PD. No significant association between PRS and severity of clinical symptoms was found. We conclude that the observed non-motor phenotypic differences in PD based on AAO are largely driven by the ageing process itself and not by a specific profile of neurodegeneration linked to AAO in the idiopathic PD patients.

npj Parkinson's Disease (2022)8:102 ; https://doi.org/10.1038/s41531-022-00342-7

INTRODUCTION

Although considered as one disease entity, Parkinson's disease (PD) displays substantial clinical heterogeneity with various phenotypes that translate into different combinations of both motor and non-motor symptoms. To address this heterogeneity, the age at onset (AAO) has been suggested as a key indicator associated with the clinical profile and progression of PD[1–3]. Previous studies with cross-sectional design have identified later AAO to be related with a stronger motor as well as non-motor impairment suggesting that late AAO is associated with higher progression rate of motor symptoms and cognitive decline. Conversely, early onset PD has been reported to show a specific disease profile with higher rate of motor complications such as early dyskinesia and dystonia[4–6]. Furthermore, both prospective[7] and retrospective studies with autopsy-proven PD[8] have shown similar findings, but given the heterogeneity of the study designs and various cut-offs used for categorising AAO, the reproducibility of the findings is limited. Despite reporting multiple AAO-related phenotypic differences, no study so far has endeavoured to integrate the effect of the physiological ageing process. Therefore, the associations between AAO and severity of PD phenotypes require further analysis.

Apart from AAO, the concept of polygenic risk scores (PRS) in sporadic forms of PD has recently been established to assess the complex genetic architecture of PD beyond known rare familial forms of PD with Mendelian inheritance of mutations in disease-causing genes[9]. Even though PRS were reported to be significantly negatively correlated with AAO[10], potential effects of PRS on the disease severity and the phenotypic profile have not yet been explored in detail.

Previous studies focusing on the role of AAO in PD were limited by (i) not addressing the concomitant effect of the physiological ageing process on the clinical phenotype by modelling age-related effects in a healthy control group, (ii) including relatively small numbers of PD patients from highly specific subgroups (e.g. drug naïve), (iii) using different AAO cut-offs across the studies and (iv) lacking a detailed genetic profiling of the study sample to exclude individuals with monogenic forms and variants presenting a genetic risk factor for developing PD. Therefore, our study addresses these issues by combining a mono-centric idiopathic PD dataset and healthy control group (HC) with detailed genetic data with the aim (i) to investigate the effect of AAO on clinical phenotype in idiopathic PD, (ii) to separate the PD-related ageing effect from the natural ageing effect and finally (iii) to explore the effect of the genetic background reflected by PRS on the disease severity in idiopathic PD.

RESULTS

Effect of AAO on clinical outcomes in PD

Several traits in PD phenotypic profiles were found in association with AAO. An overview of clinical outcomes, sociodemographic characteristics and comorbidities among participants of the Luxembourg Parkinson's Study is shown in Tables 1 and 2. As expected, the PD group comprised more males than females (67% vs. 33%) with mean AAO of 61.8 ± 12.0 years and mean disease duration since diagnosis of 5.5 ± 5.5 years. The mean age at assessment (AAA) was 67.3 ± 11.0 years. To investigate the effects of AAO on the clinical outcomes, a multiple regression analysis adjusting for disease duration was performed with results shown in Fig. 1. The overall motor disease severity as reflected by modified H&Y, MDS-UPDRS III, frequency of falls and gait disorder were all significantly positively associated with AAO. With regard

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Table 1. Overview of sociodemographic characteristics of study dataset including comorbidities and polygenic risk score with *p* values from Mann–Whitney *U* test for numerical variables and Fisher's exact test for binary variables.

	HC $n = 556$			PD = 430			
Demographic, PRS and comorbidities	Mean or YES in %	SD or NO/YES	n.a.	Mean or YES in %	SD or NO/YES	n.a.	*p* value
Gender (male)*	56%	243/313	0	67%	142/288	0	7.8e−04″
Age at onset (years)	–	–	556	61.84	11.99	0	–
Age at assessment (years)	59.61	11.78	0	67.30	11.04	0	6.8e−23″
Disease duration since diagnosis (years)	–	–	556	5.49	5.54	0	–
Years of education	14.27	3.88	5	13.09	4.10	0	5.4e−06″
Family history of parkinsonism*	26%	408/146	2	25%	324/106	0	5.6e−01
Family history of dementia*	32%	373/178	5	24%	325/103	2	5.4e−03′
Polygenic risk score for PD	−0.21	0.91	6	0.16	0.94	6	7.1e−09″
De novo*	–	0/0	556	8%	395/35	0	–
Treatment with DBS*	0%	556/0	0	5%	410/20	0	4.8e−08″
History or presence of RLS*	6%	520/36	0	9%	392/38	0	1.8e−01
Diabetes (type not specified)*	6%	523/33	0	10%	385/45	0	1.2e−02′
Arterial hypertension*	33%	375/181	0	44%	239/191	0	1.6e−04″
Cardiovascular disease*	9%	504/52	0	21%	340/90	0	3.8e−07″
Hypercholesterolemia*	38%	347/209	0	42%	248/182	0	1.5e−01
History of stroke*	3%	539/17	0	5%	410/20	0	2.4e−01

Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5% level respectively. The binary variables are annotated by asterisk. *n.a.* corresponds to total number of missing values per variable, *PD* Parkinson's disease, *PRS* Polygenic risk score, *HC* Healthy controls, *DBS* Deep brain stimulation, *SD* Standard deviation.

to the motor complications of PD, no significant association of AAO was found with total hours of dyskinesia/day, dystonia/day, nor OFF time/day, however, a significant negative association of AAO with the MDS-UPDRS IV total score was identified. Additionally, SCOPA-AUT total score and Starkstein Apathy scale had significant positive associations with AAO indicating that patients with higher AAO experience more non-motor symptoms including urinary incontinence. Cognition as reflected by the MoCA score was significantly negatively associated with AAO showing higher impairment in patients with an older AAO. Similarly, AAO was significantly negatively associated with olfactory dysfunction. All other putative associations were not significantly associated with AAO as shown in Fig. 1.

Analysing the difference in ageing effect in PD vs HC

When investigating the effects of AAA and AAO on the clinical phenotypes of PD, all associations were found to be comparable in both models (cf. Table 3). The reason is the strong correlation between AAA and AAO (statistically significant Kendall's tau $\rho = 0.73$, see Supplementary Fig. 1). To investigate an effect of physiological ageing on the PD phenotypes, we also included the HC group into the regression models. When investigating the ageing-associated effects in PD, we determined a significant positive association in PD between AAA and H&Y, MDS-UPDRS III, frequency of falls and urine incontinence, SCOPA-AUT, Starkstein Apathy Scale as well as significant negative association between AAA and MoCA and Sniffin' Stick test (cf. Table 3). Similarly in the HC group, we found a significant positive association between AAA and MDS-UPDRS III, SCOPA-AUT, Starkstein Apathy Scale, frequency of urine incontinence and gait disorder as well as significant negative association between AAA and MoCA and Sniffin' Stick test as demonstrated in Table 4. Surprisingly, after comparing the ageing effect between PD vs HC (i.e. comparing effect of AAA on the clinical variables; see Table 5, column AAA:status), the only significant differences between PD and HC were found for H&Y, MDS-UPDRS III, MDS-UPDRS IV and MoCA indicating that the concomitant ageing process might be the main determinant of the non-motor PD phenotypic differences when studying the isolated effect of age in PD.

Correlation between AAO and PRS and its effect on severity of the PD phenotype

Using a polygenic risk score defined by the imputed genotypic data from the Luxembourg Parkinson's Study and the summary statistics of 90 single nucleotide polymorphisms (SNP) that were previously identified to be genome-wide significantly associated with PD risk, we identified a significant negative correlation between PRS and AAO as shown in Fig. 2. However, neither Kendall's tau correlation test for continuous variables nor Mann–Whitney *U* test for binary variables estimating the effect of PRS on clinical outcomes nor multiple regression models including PRS adjusted for AAA and disease duration showed effects of PRS on the severity of the clinical phenotype as demonstrated in Tables 6 and 7 respectively.

DISCUSSION

The presented cross-sectional analysis of PD patients and HC at the baseline clinical visit uses data from one of the largest ongoing observational studies, focusing on PD with demographic and clinical parameters corresponding closely to other recently published large PD datasets[11–13]. In our study, we have identified several significant associations of different PD-associated motor and non-motor symptoms with AAO using a comprehensive set of clinical assessments. This is in line with previous cross-sectional, retrospective and prospective studies suggesting that later onset PD is associated with a more rapid progression rate of motor symptoms[4,11,14,15]. Conversely, comparing to the Cardiff community-based PD longitudinal cohort[16] and the longitudinal study at the Movement Disorders Clinic Saskatchewan[4], both demonstrating higher frequency of dyskinesia, motor fluctuations and dystonia in the younger onset groups vs. older onset groups,

Table 2. Overview of dataset with clinical variables in healthy control group (HC) and Parkinson's disease patients (PD) with *p* values from Mann–Whitney *U* test for numerical variables and Fisher's exact test for binary variables.

	HC $n = 556$			PD = 430			
Clinical symptoms and scales	Mean or YES in %	SD or NO/YES	n.a.	Mean or YES in %	SD or NO/YES	n.a.	*p* value
H&Y	0.00	0.00	2	2.24	0.81	2	1.5e−196″
MDS-UPDRS III	3.45	4.76	6	34.70	17.02	9	3.1e−150″
MDS-UPDRS II	1.21	2.37	6	11.69	8.32	8	6.2e−126″
LEDD (g/day)	0.0035	0.037	0	0.53	0.42	0	7.5e−160″
Gait disorder*	2%	546/10	0	57%	185/245	0	6.9e−97″
Repetitive falls*	1%	552/4	0	18%	351/79	0	1.1e−25″
MDS-UPDRS IV	0.00	0.00	4	1.88	3.52	5	1.4e−43″
Dyskinesia/day (hours)	0.00	0.00	0	0.69	2.73	1	1.2e−21″
OFF time/day (hours)	0.00	0.00	0	0.53	1.44	2	3.3e−34″
Dystonia/day (hours)	0.00	0.00	0	0.048	0.22	2	6.8e−12″
Dyskinesia*	0%	556/0	0	13%	375/55	0	1.9e−21″
Motor fluctuations*	0%	556/0	0	17%	357/73	0	1.1e−28″
Freezing of gait*	0%	556/0	0	23%	331/99	0	1.6e−39″
MoCA	27.03	2.55	3	24.28	4.41	8	4.4e−28″
Sniffin' stick test	12.86	2.39	2	8.03	3.41	13	1.6e−94″
PDQ-39	10.31	13.20	16	39.69	26.31	39	1.3e−80″
SCOPA-AUT	7.34	5.81	16	14.82	8.02	21	6.9e−53″
MDS-UPDRS I	4.58	4.42	9	10.47	7.04	10	9.0e−51″
BDI-I	5.29	5.03	15	9.97	7.11	23	2.3e−30″
Starkstein Apathy Scale	9.41	4.71	16	13.93	5.70	26	8.2e−35″
PDSS	122.81	19.61	13	105.17	23.85	28	2.3e−34″
Probable RBD*	8%	496/42	18	24%	305/95	30	1.2e−11″
Excessive daily sleepiness*	3%	541/15	0	30%	299/131	0	2.4e−36″
Insomnia*	8%	514/42	0	24%	327/103	0	9.1e−13″
Hallucinations*	0%	554/2	0	17%	357/73	0	1.2e−25″
Impulse Control Disorder*	0%	555/1	0	9%	392/38	0	1.8e−13″
Orthostatic hypotension*	6%	525/31	0	27%	312/118	0	8.2e−22″
Dysphagia*	1%	552/4	0	25%	323/107	0	5.6e−37″
Constipation*	5%	528/28	0	42%	250/180	0	3.6e−47″
Urinary Incontinence*	5%	530/26	0	32%	293/137	0	3.6e−31″

Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5 % level respectively.
Clinical symptoms and scales are described in Supplementary Material. The binary variables are annotated by asterisk.
n.a. (not acquired) corresponds to total number of individuals with missing value, *SD* Standard deviation.

we could not identify such associations with AAO. Only an overall burden of motor complications reflected by MDS-UPDRS IV score was significantly negatively associated with AAO in our study. The significant positive association of olfactory dysfunction and significant negative association of cognitive performance with AAO observed in our study correlate with previous findings[17,18] and in terms of cognitive impairment it might point to a decreased ability of senescent brain to cope with the pathological neurodegenerative process known as cognitive resilience[19]. Additionally, another large multi-centric study using the Quebec Parkinson Network (QPN) dataset of over 1000 PD individuals showed comparable results with a positive association between late-onset PD and higher motor burden reflected by H&Y, higher cognitive decline and higher frequency of falls, but differed on significantly higher frequency of constipation and hallucinations late-onset PD (defined as AAO > 50 years) compared to early onset PD[11]. However, most scales applied in QPN differ from our study and different categorical approaches were used in QPN both for AAO and disease duration, influencing the comparability of results. To summarise our results, the earlier AAO, patients experience a lower level of motor impairment, lower cognitive impairment and less global autonomic dysfunction, apathy and olfactory deficit, but present with more motor complications even after adjusting for disease duration as a main determinant of disease severity.

These phenotypic differences observed in PD based on different AAO were previously not clearly separated from the physiological ageing process and challenged the concept that phenotypic differences are related specifically to the age at which the disease first manifests. This intriguing aspect evolves from the inherent close correlation between the main co-variates (AAA, AAO and disease duration) and thus raises a major methodological concern in most of the cross-sectional studies when aiming at determining the effect of all three co-variates on the clinical outcomes in a single model as discussed by Johnson et al. 2002[20]. Therefore, we tried to disentangle the effect of ageing on the clinical phenotype in the cross-sectional setting by determining the ageing effect in individuals with and without PD. Surprisingly, the effect of ageing (AAA) on clinical outcomes in PD vs HC differed significantly only in motor disease severity (H&Y, MDS-UPDRS III), motor complications (MDS-UPDRS IV) and cognitive performance. These results suggest

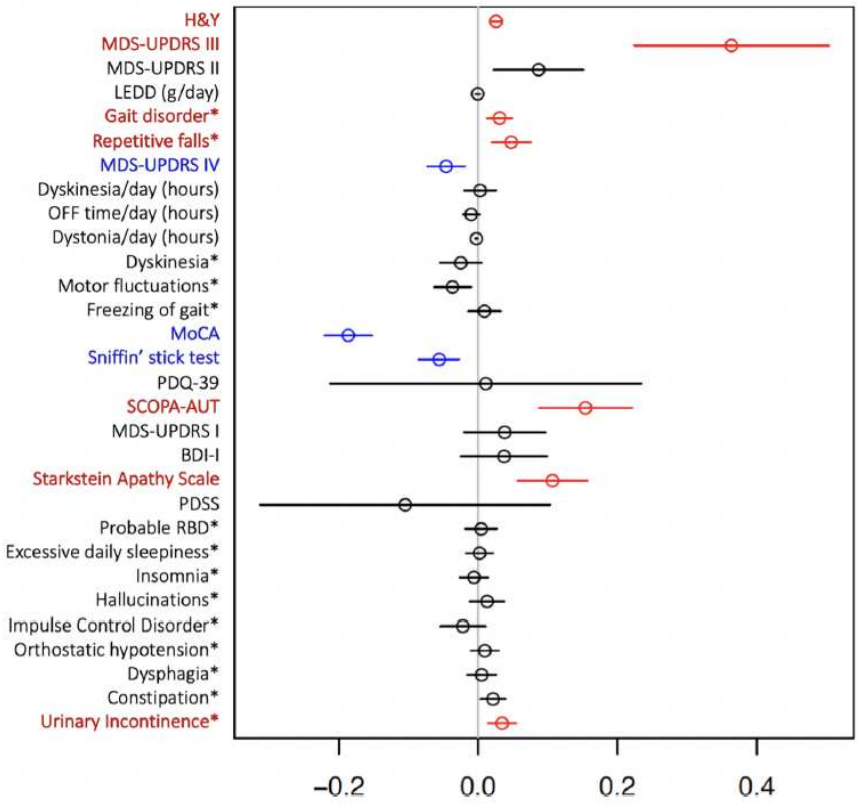

Fig. 1 Forrest plot with estimated coefficients and corresponding confidence intervals (±1.96 × standard error) for AAO, from linear/logistic regression of numerical/binary outcome on disease duration and AAO. The colour blue indicates significant negative effects of AAO on the clinical outcome, and the colour red indicates significant positive effects at the Bonferroni-adjusted 5% level. The binary variables are annotated by asterisk. Clinical symptoms and scales are described in Supplementary Material.

that the majority of the observed significant non-motor phenotypic differences in PD should be attributed rather to the physiological ageing process itself than age-specific dynamics of PD.

When considering the effect and role of AAO and age in classification of the respective PD phenotypes, potential underlying genetic determinants need to be considered. It is well known that rare disease-causing mutations in monogenic PD (e.g. in PARKIN, PINK1, SNCA or GBA[21–23]) have an effect on both AAO and PD phenotype. However, until now only few studies have explored the cumulative effects of common genetic variants with small effect sizes (as defined by PRS) on the clinical phenotype[24]. Here our results are in line with several recent studies observing no significant association between PRS and cognitive decline, severity of motor symptoms[25] or ICD[26] in contrast to other longitudinal prospective study[27]. It is worth noting that our statistical models included individuals without any known PD causing monogenic mutation or genetic risk variant (i.e. PD-associated variants in the GBA gene). Nevertheless, the significance of the PRS effect on clinical outcomes did not change in the models including PD-associated mutation or genetic variant carriers. Together with the significant negative correlation between AAO and PRS (cf. Fig. 2), our findings suggest that PRS may increase the risk to develop PD but might not have an effect on the severity of the disease phenotype. This observation is in favour of the hypothesis that initiation of the disease on one hand and the disease progression rate on the other might be driven by distinct factors.

Besides the mentioned strengths of our study design, several limitations need to be considered. First, the cross-sectional design does not allow for the identification of causal relations between AAO and clinical phenotypes. Second, we cannot consider the Luxembourg Parkinson's Study as community-based by design, although some clinical indicators (such as mean AAO and male-to-female ratio) correspond closely to several community-based studies[28–31]. Third, we observe a relatively high frequency of positive family history of parkinsonism in the HC group (26% vs. 25% in PD) as well as high frequency of a family history of dementia in HC (32% vs 24%). We assume that there are two principal reasons why we observe increased frequencies of neurodegenerative diseases in HC group: (i) HC with personal experience with parkinsonism and/or dementia in their family are more aware to support research and (ii) family members of study participants are more inclined to participate in the study. To address these points and eliminate a potential bias, we excluded 1st, 2nd and 3rd degree relatives from our statistical models.

In summary, our study sought to overcome limitations identified in previous studies on the role of AAO in PD by (i) including substantially higher number of PD patients and HCs in the model accounting for the independent effect of ageing, (ii) our study being based on monocentric data collection and including PD patients of all disease stages regardless of the cognitive status, (iii) investigating an idiopathic dataset of PD and PD-related mutation free HC, (iv) refuting the categorisation bias by a priori arbitrary AAO grouping, and finally (v) exploring the effect of PRS on severity of the PD phenotype in a large genotyped sample.

METHODS

Study population

All subjects were recruited from March 2015 until 10th December 2020 in the frame of the nation-wide monocentric observational longitudinal Luxembourg Parkinson's Study. The diagnosis of PD was based on

Table 3. Multiple regression of clinical outcomes on age at onset (AAO), age at assessment (AAA) and disease duration for Parkinson's disease group.

Clinical symptoms and scales	Intercept	Disease duration	AAA	Intercept	Disease duration	AAO	Intercept	AAA	AAO
H&Y	0.23	**0.05″**	**0.03″**	0.23	**0.07″**	**0.03″**	0.23	**0.07″**	**−0.05″**
MDS-UPDRS III	5.95	**0.76″**	**0.36″**	6.04	**1.13″**	**0.36″**	5.98	**1.13″**	**−0.76″**
MDS-UPDRS II	2.38	**0.63″**	0.09′	2.39	**0.72″**	0.09′	2.41	**0.72″**	**−0.63″**
LEDD (g/day)	0.37	**0.04″**	0.00	0.37	**0.03″**	0.00	0.38	**0.03″**	**−0.04″**
Gait disorder*	−2.23	**0.08″**	0.03′	−2.23	**0.12″**	0.03′	−2.22	**0.12″**	**−0.08″**
Repetitive falls*	−5.79	**0.14″**	**0.05″**	−5.74	**0.19″**	**0.05″**	−5.80	**0.19″**	**−0.15″**
MDS-UPDRS IV	3.43	**0.28″**	−0.05′	3.46	**0.24″**	−0.05′	3.43	**0.24″**	**−0.28″**
Dyskinesia/day (hours)	−0.16	**0.12″**	0.00	−0.16	**0.12″**	0.00	−0.15	**0.12″**	**−0.12″**
OFF time/day (hours)	0.87	**0.06″**	−0.01	0.87	**0.05″**	−0.01	0.87	**0.05″**	**−0.06″**
Dystonia/day (hours)	0.15	**0.01″**	0.00′	0.16	**0.01″**	0.00′	0.15	**0.01″**	**−0.01″**
Dyskinesia*	−1.57	**0.18″**	−0.02	−1.55	**0.15″**	−0.02	−1.56	**0.15″**	**−0.18″**
Motor fluctuations*	−0.35	**0.18″**	−0.04′	−0.39	**0.14″**	−0.04′	−0.33	**0.14″**	**−0.18″**
Freezing of gait*	−2.82	**0.16″**	0.01	−2.87	**0.17″**	0.01	−2.79	**0.17″**	**−0.16″**
MoCA	37.04	−0.05	**−0.19″**	37.10	**−0.24″**	**−0.19″**	37.03	**−0.23″**	**0.05**
Sniffin' stick test	12.40	**−0.11″**	**−0.06″**	12.41	**−0.17″**	**−0.06″**	12.40	**−0.17″**	**0.11″**
PDQ-39	29.72	**1.78″**	0.01	29.46	**1.79″**	0.01	29.87	**1.77″**	**−1.76″**
SCOPA-AUT	2.20	**0.43″**	**0.15″**	2.16	**0.58″**	**0.15″**	2.23	**0.58″**	**−0.42″**
MDS-UPDRS I	6.05	**0.35″**	0.04	5.96	**0.39″**	0.04	6.08	**0.38″**	**−0.35″**
BDI-I	6.14	**0.24″**	0.04	6.19	**0.28″**	0.04	6.15	**0.28″**	**−0.24″**
Starkstein Apathy Scale	6.75	−0.01	**0.11″**	6.81	0.10	**0.11″**	6.74	0.10	0.00
PDSS	117.20	**−0.98″**	−0.10	117.45	**−1.08″**	−0.10	117.11	**−1.06″**	**0.96″**
Probable RBD*	−2.13	**0.11″**	0.00	−2.14	**0.12″**	0.00	−2.12	**0.11″**	**−0.11″**
Excessive daily sleepiness*	−1.31	**0.07″**	0.00	−1.37	**0.07″**	0.00	−1.29	0.07′	**−0.06″**
Insomnia*	−1.00	0.04′	−0.01	−1.01	0.04	−0.01	−1.00	0.04	**−0.04′**
Hallucinations*	−3.08	**0.09″**	0.01	−3.06	**0.10″**	0.01	−3.08	**0.11″**	**−0.09″**
Impulse Control Disorder*	−1.62	**0.11″**	−0.02	−1.64	0.09′	−0.02	−1.60	0.09′	**−0.11″**
Orthostatic hypotension*	−1.88	0.05′	0.01	−1.92	0.06′	0.01	−1.87	0.06′	−0.05′
Dysphagia*	−1.77	0.06′	0.00	−1.80	0.06′	0.01	−1.76	**0.06′**	−0.06′
Constipation*	−2.14	**0.07″**	0.02′	−2.19	**0.10″**	0.02′	−2.13	**0.09″**	**−0.07″**
Urinary Incontinence*	−3.40	0.04′	**0.04″**	−3.36	**0.08″**	**0.03″**	−3.41	**0.08″**	−0.05′

Regression coefficients for different outcomes (rows) from three equivalent models with each two out of three features (columns). Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5% level respectively. The bold indicates significant effect where minus value indicates negative significant effect and positive value positive significant effect respectively. The binary variables are annotated by asterisk. Clinical symptoms and scales are described in Supplementary Material.

UKPDSBB diagnostic criteria[32]. The initial visit dataset of 430 PD patients and 556 HC genetically screened by both NeuroChip and PacBio were analysed after exclusion of 6 PD and 39 HC individuals for 1st, 2nd and 3rd degree relationships and after exclusion of 53 PD carriers and 27 HC carriers of pathogenic PD-associated variants. The overall study design, inclusion and exclusion workflow are illustrated in Fig. 3.

All participants taking part in Luxembourg Parkinson's Study agreed and signed a written informed consent. The study has been approved by the National Ethics Board (CNER Ref: 201407/13). The patients with PD were included regardless of the disease duration, cognitive status, age or disease stage. The HC were partially recruited from the pool of independent observational studies in Luxembourg (ORISCAV-LUX study; EHES-LUX) or were recruited from Luxembourg or the surrounding area of Greater Region based on individual interest not meeting any of the exclusion criteria (presence of a neurodegenerative disorder, active cancer; age under 18 and pregnant women)[33].

Clinical assessment and data. A description of the design of the Luxembourg Parkinson's Study was previously published[33]. Sociodemographic characteristics and clinical outcomes validated for PD were chosen from the basic clinical assessment battery and listed in Tables 1 and 2. Validated self-administered questionnaires and scales for PD were used. All patients have been evaluated in medication ON state and where applicable, in deep brain stimulation ON state. AAO is defined as age at diagnosis of PD. The clinical symptoms as scales are defined in detail in the Supplementary material.

Missing data statement. The absolute number of missing data per variable are shown in Tables 1 and 2. Given the low proportions of missing values in the outcome variables and 0% of missing values in the co-variates (AAA, AAO and disease duration), we used a pairwise deletion for all statistical models.

Genotyping and quality-control analyses. DNA samples were genotyped using the NeuroChip array (v.1.0 and v1.1; Illumina, San Diego, CA) that was specifically designed to integrate rare and common neurodegenerative disease-related variants[34]. Quality-control (QC) analysis was performed as follows: samples with call rates < 95% and whose genetically determined sex deviated from reported sex in clinical data were excluded from the analysis, and the filtered variants were checked for cryptic relatedness and excess of heterozygosity. Samples exhibiting excess heterozygosity (F statistic > 0.2) and first-degree relatedness were excluded. Once sample QC was completed, SNPs with Hardy–Weinberg equilibrium P value < 1E−6, and missingness rates >5% were excluded. All samples except for twelve from all individuals entering the analysis after exclusion of the 1st, 2nd and 3rd degree relatives and presence of PD-linked mutation and genetic risk factors passed the QC (424 PD and 550 HC). The data were then imputed using the Haplotype Reference Consortium r1.1 2016 and the Michigan Imputation Server and filtered for

Table 4. Simple regression of clinical outcomes with healthy controls.

Clinical symptoms and scales	Intercept	AAA
H&Y	0.00	0.00
MDS-UPDRS III	−3.70	**0.12″**
MDS-UPDRS II	−0.21	0.02′
LEDD (g/day)	−0.02	0.00′
Gait disorder*	−12.56	**0.13″**
Repetitive falls*	−5.14	0.00
MDS-UPDRS IV	0.00	0.00
Dyskinesia/day (hours)	0.00	0.00
OFF time/day (hours)	0.00	0.00
Dystonia/day (hours)	0.00	0.00
Dyskinesia*	−26.57	0.00
Motor fluctuations*	−26.57	0.00
Freezing of gait*	−26.57	0.00
MoCA	29.84	**−0.05″**
Sniffin' stick test	15.44	**−0.04″**
PDQ-39	10.68	−0.01
SCOPA-AUT	2.53	**0.08″**
MDS-UPDRS I	3.12	0.02
BDI-I	4.06	0.02
Starkstein Apathy Scale	5.11	**0.07″**
PDSS	130.37	−0.13
Probable RBD*	−1.58	−0.02
Excessive daily sleepiness*	−6.53	0.05
Insomnia*	−2.00	−0.01
Hallucinations*	−3.31	−0.04
Impulse Control Disorder*	−4.32	−0.04
Orthostatic hypotension*	−3.92	0.02
Dysphagia*	−7.28	0.04
Constipation*	−2.37	−0.01
Urinary Incontinence*	−8.43	**0.08″**

Regression coefficients are shown from linear regression of numerical outcome and from logistic regression of binary outcome on age at assessment (AAA). Single and double ticks indicate significance at the 5% level and the Bonferroni adjusted 5% level, the bold indicates significant effect where minus value indicates negative significant effect and positive value positive significant effect respectively. The binary variables are annotated by asterisk. Clinical symptoms and scales are described in Supplementary Material.

imputation quality (RSQ > 0.8)[35]. Genetic analysis and QC was done using PLINK v1.9. Additionally, all samples underwent targeted sequencing of the GBA locus using single-molecule sequencing on a Sequel II sequencer from Pacific BioScience[36]. Variants were called with DeepVariant 1.0[37]. PD causing rare variants were defined by the ClinVar classification 'pathogenic/likely-pathogenic'. All PD causing variants (listed in Supplementary material) identified by any method were Sanger validated and all samples with a validated PD causing variant were excluded from further analysis.

Polygenic risk score (PRS). We generated PRSs with PRSice-2 under default settings. PRSs for each individual were calculated using the imputed genotype data from Luxembourg Parkinson's Study as a target sample. The base GWAS data used to determine PRS for PD was the summary statistics of the 90 SNPs that were previously found to be genome-wide significantly associated with PD risk[38]. The criteria for linkage disequilibrium (LD) clumping of SNPs were pairwise LD r2 < 0.1 within the 250 kb window. Briefly, PRSs were calculated by summing the weighted effects of GWAS PD risk genetic variants present in the target samples, with a possible proxy of $R^2 > 0.9$, meeting p value thresholds ranging from 5e−08 to 0.5. The values of PRS were Z-normalised.

Table 5. Multiple regression model with PD and HC investigating the difference in effect of ageing in HC (AAA) and in PD (AAA:status) adjusted for disease duration.

Clinical symptoms and scales	Intercept	AAA	Status	Disease duration	AAA:Status
H&Y	0.00	0.00	0.23	**0.05″**	**0.03″**
MDS-UPDRS III	−3.70	0.12′	9.65	**0.76″**	**0.24″**
MDS-UPDRS II	−0.21	0.02	2.59	**0.63″**	0.06′
LEDD (g/day)	−0.02	0.00	0.39	**0.04″**	0.00
Gait disorder*	−12.56	0.13′	10.34	**0.08″**	−0.10′
Repetitive falls*	−5.14	0.00	−0.66	**0.14″**	0.04
MDS-UPDRS IV	0.00	0.00	3.43	**0.28″**	**−0.05″**
Dyskinesia/day (hours)	0.00	0.00	−0.16	**0.12″**	0.00
OFF time/day (hours)	0.00	0.00	0.87	**0.06″**	−0.01
Dystonia/day (hours)	0.00	0.00	0.15	**0.01″**	0.00′
Dyskinesia*	−20.57	0.00	18.99	**0.18″**	−0.02
Motor fluctuations*	−20.57	0.00	20.22	**0.18″**	−0.04
Freezing of gait*	−20.57	0.00	17.75	**0.16″**	0.01
MoCA	29.84	**−0.05″**	7.20	−0.05	**−0.14″**
Sniffin' stick test	15.44	**−0.04″**	−3.03	**−0.11″**	−0.01
PDQ-39	10.68	−0.01	19.04	**1.78″**	0.01
SCOPA-AUT	2.53	0.08′	−0.33	**0.43″**	0.07
MDS-UPDRS I	3.12	0.02	2.94	**0.35″**	0.01
BDI-I	4.06	0.02	2.09	**0.24″**	0.02
Starkstein Apathy Scale	5.11	**0.07″**	1.64	−0.01	0.04
PDSS	130.37	−0.13	−13.17	**−0.98″**	0.03
Probable RBD*	−1.58	−0.02	−0.54	**0.11″**	0.02
Excessive daily sleepiness*	−6.53	0.05	5.22	**0.07″**	−0.05
Insomnia*	−2.00	−0.01	0.99	0.04′	0.00
Hallucinations*	−3.31	−0.04	0.23	**0.09″**	0.05
Impulse Control Disorder*	−4.32	−0.04	2.70	**0.11″**	0.01
Orthostatic hypotension*	−3.92	0.02	2.04	0.05′	−0.01
Dysphagia*	−7.28	0.04	5.51	0.06′	−0.03
Constipation*	−2.37	−0.01	0.23	**0.07″**	0.03
Urinary Incontinence*	−8.43	**0.08″**	5.03	0.04′	−0.05′

Regression coefficients are shown for different outcomes (rows). Status takes the value 0 for HC and 1 for PD, the AAA:status is the interaction term of AAA and being PD (status = 1). Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5% level respectively, the bold indicates significant effect where minus value indicates negative significant effect and positive value positive significant effect respectively. The column AAA:status indicates whether the effect of AAA on clinical outcomes differs between PD and HC. The binary variables are annotated by asterisk. Clinical symptoms and scales are described in Supplementary Material.

Statistical analysis

Firstly, we performed an intergroup comparison (PD vs HC) of socio-demographic and clinical characteristics as well as polygenic risk score and comorbidities with the Mann–Whitney U test for numerical variables and

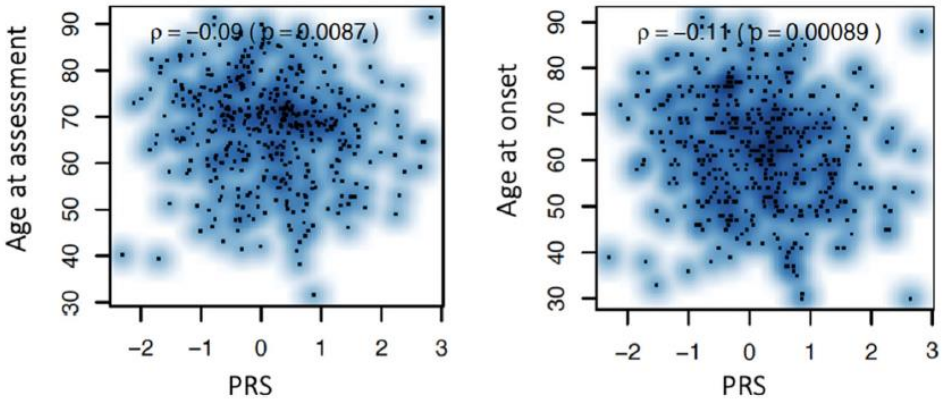

Fig. 2 Pairwise association between age at onset (AAO), age at assessment (AAA) (y-axis) and polygenic risk score (PRS) (x-axis) with Kendall correlation coefficient. Significant inverse association was determined between AAO and PRS and AAA and PRS indicating the younger the AAO of PD, the higher cummulative burden of small effect size variants (represented by PRS).

Table 6. Kendall correlation coefficient between clinical outcome (row) and polygenic risk score (PRS) for healthy controls (HC) (left) and Parkinson's disease patients (PD) (right), with annotation by bold indicating significant effect where minus value indicates negative significant effect and positive value positive significant effect respectively (Kendall correlation test).

Clinical symptoms and scales	HC	PD
H&Y	0.0272	0.0272
MDS-UPDRS III	−0.0088	−0.0341
MDS-UPDRS II	−0.0242	0.0058
LEDD (g/day)	0.0091	−0.0147
Gait disorder*	0.4070	−0.0177
Repetitive falls*	0.6209	0.1690
MDS-UPDRS IV	0.0625	0.0625
Dyskinesia/day (hours)	0.0576	0.0576
OFF time/day (hours)	0.0100	0.0100
Dystonia/day (hours)	0.0491	0.0491
Dyskinesia*	–	0.1426
Motor fluctuations*	–	0.0864
Freezing of gait*	–	0.0084
MoCA	0.0542	−0.0493′
Sniffin' stick test	0.1012	0.0576
PDQ-39	−0.0519	0.0386
SCOPA-AUT	−0.0113	0.0150
MDS-UPDRS I	−0.0556	0.0105
BDI-I	−0.0155	0.0290
Starkstein Apathy Scale	−0.0425	−0.0068
PDSS	−0.0128	−0.0332
Probable RBD*	−0.1142	−0.0703
Excessive daily sleepiness*	0.1638	−0.1261
Insomnia*	−0.0356	0.1387
Hallucinations*	−0.9806	−0.1225
Impulse Control Disorder*	−2.1177	−0.0872
Orthostatic hypotension*	0.0839	−0.1331
Dysphagia*	−0.6401	−0.2650′
Constipation*	0.2701	−0.0740
Urinary Incontinence*	0.1011	0.0434

Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5% level. The binary variables are annotated by asterisk. Clinical symptoms and scales are described in Supplementary Material.

Table 7. Multiple regression model with coefficients shown from linear regression of numerical outcome and from logistic regression of binary outcome on disease duration, age at assessment (AAA) and polygenic risk score (PRS) in PD group.

Clinical symptoms and scales	Intercept	Disease duration	AAA	PRS
H&Y	0.17	**0.05″**	**0.03″**	−0.02
MDS-UPDRS III	4.44	**0.76″**	**0.39″**	−0.57
MDS-UPDRS II	1.81	**0.63″**	0.10′	−0.47
LEDD (g/day)	0.32	**0.04″**	0.00	0.01
Gait disorder*	−2.29	**0.09″**	0.03′	−0.04
Repetitive falls*	−5.91	**0.14″**	0.05′	0.15
MDS-UPDRS IV	3.37	**0.28″**	−0.04′	0.03
Dyskinesia/day (hours)	−0.36	**0.12″**	0.01	0.04
OFF time/day (hours)	0.87	**0.06″**	−0.01	−0.04
Dystonia/day (hours)	0.15	**0.01″**	0.00′	0.01
Dyskinesia*	−1.62	**0.18″**	−0.02	−0.05
Motor fluctuations*	−0.12	**0.18″**	−0.04′	−0.12
Freezing of gait*	−2.66	**0.17″**	0.01	−0.13
MoCA	37.25	−0.07′	**−0.19″**	0.40′
Sniffin' stick test	12.64	**−0.11″**	**−0.06″**	−0.22
PDQ-39	30.96	**1.86″**	−0.01	−2.98′
SCOPA-AUT	2.74	**0.47″**	**0.15″**	−0.98′
MDS-UPDRS I	6.29	**0.36″**	0.03	−0.84′
BDI-I	6.81	**0.27″**	0.03	−0.76′
Starkstein Apathy Scale	6.99	0.00	**0.10″**	−0.18
PDSS	116.69	**−1.00″**	−0.10	0.12
Probable RBD*	−2.07	**0.12″**	0.00	−0.21
Excessive daily sleepiness*	−1.23	**0.07″**	0.00	−0.20
Insomnia*	−1.04	0.04′	−0.01	0.11
Hallucinations*	−2.95	**0.10″**	0.01	−0.23
Impulse Control Disorder*	−1.37	**0.11″**	−0.03	−0.25
Orthostatic hypotension*	−1.64	0.06′	0.01	−0.20
Dysphagia*	−1.77	**0.07″**	0.00	−0.37′
Constipation*	−2.07	**0.08″**	0.02′	−0.13
Urinary Incontinence*	−3.67	0.05′	**0.04″**	0.06

Single and double ticks indicate significance at the 5% level and the Bonferroni adjusted 5% level, the bold indicates significant effect where minus value indicates negative significant effect and positive value positive significant effect respectively. The binary variables are annotated by asterisk. Clinical symptoms and scales are described in Supplementary Material.

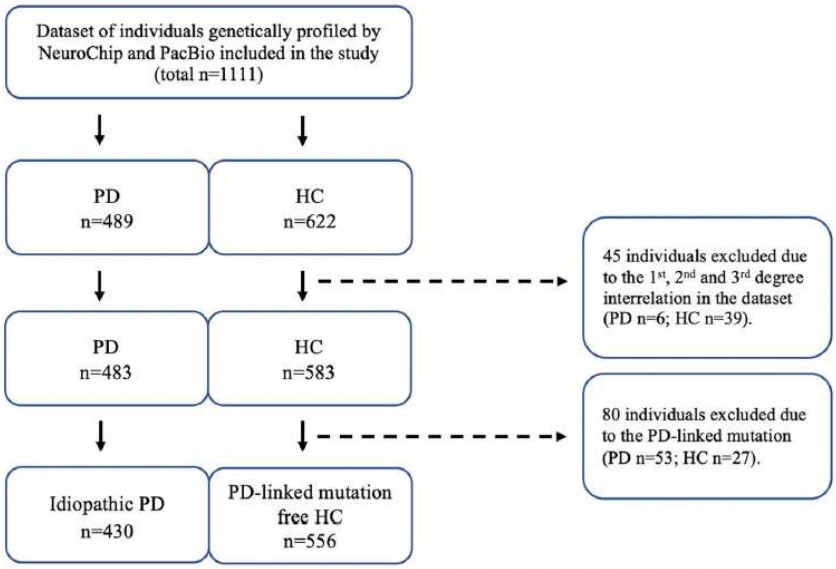

Fig. 3 Description of the study design and study dataset. PD individuals with Parkinson's disease, HC healthy control.

Fisher's exact test for binary variables (Tables 1 and 2). Secondly, we used multiple regression models (linear and logistic) to identify effects of AAO (as a numerical variable) on numerical or binary clinical outcomes accounting for disease duration (Fig. 1). Subsequently, we performed a multiple regression model for both HC and PD (Table 5) to examine whether the effect of ageing (AAA) on clinical outcomes differs between HC and PD adjusted for disease duration. For this, we included the main effects of the continuous variable AAA and the binary variable status (HC: status = 0, PD: status = 1), their interaction effect (HC: status*AAA = 0, PD: status*AAA > 0), and the main effect of the continuous variable disease duration (HC: duration = 0, PD: duration > 0). To investigate the role of PRS in PD, a pairwise association analysis with Kendall's tau correlation test between PRS and AAO and AAA was performed (Fig. 2). Furthermore, we performed a Kendall correlation test between PRS and clinical outcome for PD and HC respectively (Table 6). As a last step, we employed a multiple regression model including PRS adjusting for AAA and disease duration, to investigate the effect of PRS on the clinical phenotype in PD (Table 7). At all instances, the significance at the 5% level and the Bonferroni-adjusted 5% level was set.

DATA AVAILABILITY

The dataset for this manuscript is not publicly available as it is linked to the Luxembourg Parkinson's Study and its internal regulations. Any requests for accessing the dataset can be directed to *request.ncer-pd@uni.lu*.

CODE AVAILABILITY

The code for the statistical models is available at: https://doi.org/10.17881/hr67-ba06.

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AUTHOR CONTRIBUTIONS

L.P.: Conceived, organised, and executed the research project; co-executed the statistical analysis and interpretation of results; wrote the manuscript; substantially participated in data collection, data exportation and data curation. R.K.: Conceived, organised, and co-executed the research project; participated in interpretation of results; critically revised the manuscript. A.R.: Executed the statistical analysis and interpretation, critically revised the manuscript, substantially participated in data curation. E.G.: Co-executed the research project; participated in interpretation of results; critically revised the manuscript. P.M.: Executed the genetic analysis; participated in interpretation of results; critically revised the manuscript. S.P.: Contributed genetic data; co-executed the research project; critically revised the manuscript. Z.L.: Executed the genetic analysis; participated in interpretation of results; critically revised the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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2.2. RESULTS: PEER-REVIEWED ARTICLE N.2

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Research Report

Body-First Subtype of Parkinson's Disease with Probable REM-Sleep Behavior Disorder Is Associated with Non-Motor Dominant Phenotype

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Abstract.
Background: The hypothesis of *body-first* vs. *brain-first* subtype of PD has been proposed with REM-Sleep behavior disorder (RBD) defining the former. The body-first PD presumes an involvement of the brainstem in the pathogenic process with higher burden of autonomic dysfunction.
Objective: To identify distinctive clinical subtypes of idiopathic Parkinson's disease (iPD) in line with the formerly proposed concept of *body-first* vs. *brain-first* subtypes in PD, we analyzed the presence of probable RBD (pRBD), sex, and the *APOE* ε4 carrier status as potential sub-group stratifiers.
Methods: A total of 400 iPD patients were included in the cross-sectional analysis from the baseline dataset with a completed RBD Screening Questionnaire (RBDSQ) for classifying as pRBD by using the cut-off RBDSQ ≥ 6. Multiple regression models were applied to explore (i) the effect of pRBD on clinical outcomes adjusted for disease duration and age, (ii) the effect of sex on pRBD, and (iii) the association of *APOE* ε4 and pRBD.
Results: iPD-pRBD was significantly associated with autonomic dysfunction (SCOPA-AUT), level of depressive symptoms (BDI-I), MDS-UPDRS I, hallucinations, and constipation, whereas significantly negatively associated with quality of life (PDQ-39) and sleep (PDSS). No significant association between sex and pRBD or *APOE* ε4 and pRBD in iPD was found nor did we determine a significant effect of *APOE* ε4 on the PD phenotype.

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Conclusion: We identified an RBD-specific PD endophenotype, characterized by predominant autonomic dysfunction, hallucinations, and depression, corroborating the concept of a distinctive *body-first* subtype of PD. We did not observe a significant association between *APOE* ε4 and pRBD suggesting both factors having an independent effect on cognitive decline in iPD.

Keywords: Idiopathic Parkinson's disease, probable REM-Sleep behavior disorder, RBDSQ, non-motor symptoms, *APOE*, stratification

INTRODUCTION

The phenotypic heterogeneity of Parkinson's disease (PD) has been a challenge for both clinicians and researchers for decades. Several efforts were made to identify an underlying pattern explaining this heterogeneity by subtyping PD patients. They can be grouped into two distinct methods. The first approach uses a single clinical or genetic metric determining the clinical phenotype, such as age at onset, sex, motor phenotype, or being a carrier of the PD-causing rare genetic mutations. The second approach has been using hypothesis-free data-driven models identifying phenotypic clusters in PD based on clinical symptoms, but this approach failed reproducibility checks, possibly due to a limited methodological overlap between the studies and a wide variety of clinical metrics entering the models [1]. Interestingly, both approaches systematically reported REM-sleep behavior disorder (RBD) as a relevant clinical variable. Not only is RBD currently known as the most robust prodromal marker of future pheno-conversion to the alpha-synucleinopathies (i.e., PD, dementia with Lewy bodies or multiple system atrophy) [2], but it was suggested that RBD is associated with more rapid progression of motor symptoms, a higher burden of non-motor symptoms and lower quality of life [3–5].

RBD received increasing attention in the last years, with several cross-sectional and longitudinal studies investigating the association between RBD and the clinical phenotype of PD. On the one hand, we observe an overall consensus regarding a non-motor dominant profile of PD with higher autonomic dysfunction and more rapid cognitive decline. On the other hand, prior studies have reported contradictory findings on the effect of comorbid RBD on motor progression in PD [5–8]. Moreover, genetic risk factors and PD-causing rare mutations with a substantial effect on the clinical phenotype were rarely systematically addressed in the context of concomitant RBD and PD and their effect on the severity of the clinical phenotype. Recently, the *APOE* epsilon4 (*APOE* ε4) genotype has been linked to faster cognitive decline and motor progression in PD [9], although studies on the role of *APOE* ε4 and clinical progression of PD remain controversial [10, 11]. Whether an additive or multiplicative potentiation effect of RBD and *APOE* ε4 on cognitive decline in PD exists has not been adequately addressed so far. Currently, no association of the *APOE* ε4 carriers status with idiopathic RBD has been observed [12, 13], but a potential role of the *APOE* ε4 genotype as a modifier of the clinical phenotype of PD with RBD has not yet been explored.

RBD has been suggested to represent a key element in distinguishing body-first from brain-first subtype of PD, a concept recently proposed to explain the phenotypic differences and variability of dynamics in PD and supported by several clinical and imaging studies [14, 15]. It has been proposed that the body-first subtype of PD starts in the peripheral nervous system with spreading of neurodegeneration via brainstem thus associated with RBD, higher burden of autonomic dysfunction and higher rate of cognitive decline [16].

In order to test the hypothesis of body-first subtype of PD with comorbid pRBD, we used a large baseline visit dataset from the Luxembourg Parkinson's Study, a monocentric longitudinal observational study with a previously described recruitment design [17]. In our study, we primarily aimed to determine the effect of pRBD on clinical outcomes in idiopathic PD (iPD) by excluding known PD-linked rare mutations or genetic risk variant carriers. Next, we investigated potential confounding effects of sex and the *APOE* ε4 carrier status as potential stratifiers of iPD.

MATERIALS AND METHODS

Study population

The data used in this study were acquired from participants recruited in the frame of the nationwide monocentric observational longitudinal Luxembourg Parkinson's Study [17]. The diagnosis of PD relied on the UK Parkinson's Disease Society Brain Bank

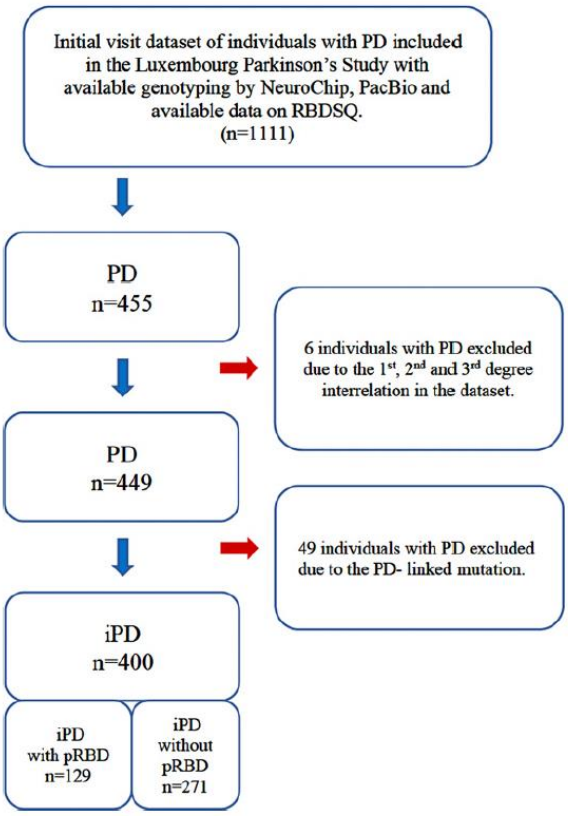

Fig. 1. Description of the study design and study dataset. PD, individuals with Parkinson's disease; iPD, idiopathic Parkinson's disease; pRBD, probable REM-sleep behavior disorder; RBDSQ, REM sleep behavior disorder screening questionnaire.

(UKPDSBB) diagnostic criteria [18]. All participants were genotyped for disease-causing mutations and PD-associated risk variants using both NeuroChip® and PacBio sequencing. Available data on RBDSQ were analyzed after excluding six PD patients for 1st, 2nd, and 3rd degree relationships and after excluding 49 PD patients carrying PD-associated mutations. The overall study design, inclusion, and exclusion workflow are illustrated in Fig. 1. Though the diagnostic gold standard of RBD remains polysomnography (PSG) [19], the accessibility of the sleep laboratory and performing PSG on a large scale is problematic due to the sleep laboratory capacities and costs. We therefore applied a classification of probable RBD (pRBD) by REM-sleep behavior disorder screening questionnaire (RBDSQ) as used in several previous studies [20–24]. The group assignment of pRBD in iPD individuals uses the criterion RBDSQ ≥ 6 to optimize the specificity and sensitivity for pRBD in line with the Oxford Discovery Study [24].

All participants taking part in the Luxembourg Parkinson's Study agreed and signed a written informed consent. The study has been approved by the National Research Ethics Committee (CNER Ref: 201407/13).

Clinical assessment and data

The design and recruitment of the Luxembourg Parkinson's Study were previously published in detail [17]. Sociodemographic characteristics and clinical outcomes validated for PD were chosen from the basic clinical assessment battery and are listed in Tables 1 and 2. All patients have been evaluated in medication ON state and, where applicable, in deep brain stimulation ON state. The clinical symptoms as scales are defined in detail in the Supplementary Material.

Missing data statement

The absolute number of missing data per variable is described in Tables 1 and 2. Given the low proportions of missing values in the dataset, we used a pairwise deletion for all statistical models.

Genotyping and quality-control analyses

The methods for genotyping in our dataset have been described previously [25]. PD causing rare variants were defined by the ClinVar classification as "pathogenic/likely pathogenic". All PD-causing variants (listed in the Supplementary Material) identified by any method were Sanger validated, and all samples with a validated PD-causing variant were excluded from further analysis with a list of excluded variants described in the Supplementary Material.

APOE genotyping

APOE genotypes were called for all individuals from two SNPs investigated by NeuroChip array (rs429358, rs7412) that distinguish the $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$ alleles classifying the respective *APOE* carriers. The NeuroChip provides high accuracy of 98.1% for genotyping of *APOE* $\varepsilon 4$ [26], and the approach was aligned with other large studies [27].

Statistical analysis

Mann-Whitney's *U* test was used for numerical variables and Fisher's exact test for binary variables in

Table 1
Descriptive and comparative statistics of demographic data and frequency of *APOE* ε4 genotype in PD individuals with (right) and without (left) probable REM-sleep behavior disorder (pRBD). For intergroup comparisons, *p*-values are shown from Mann-Whitney *U* test for numerical variables and Fisher's exact test for binary variables. Binary variables are annotated by asterisk. Results are shown as mean and standard deviation (SD) for numerical variables, number of zeros ('NO') and ones ('YES') for binary variables and percentage of YES, and number of missing values (NA). Single and double ticks indicate significance at the 5% level, and the Bonferroni-adjusted 5% level. Age at onset was calculated based on the year of the PD diagnosis. PD, Parkinson's disease

	PD non-pRBD ($n=271$)			PD pRBD ($n=129$)			p
	Mean or YES in %	SD or NO/YES	NA	Mean or YES in %	SD or NO/YES	NA	
Disease duration since diagnosis (y)	4.20	4.55	0	7.86	6.36	0	8.2e-11″
Age at assessment (y)	66.19	11.29	0	68.31	9.85	0	1.2e-01
Age at onset (y)	62.01	11.64	0	60.48	11.98	0	2.5e-01
Sex (male)*	65%	96/175	0	74%	34/95	0	8.6e-02
APOE (ε2/ ε4; ε3/ ε4;ε4/ε4)*	21%	213/58	0	26%	95/34	0	3.1e-01
Years of education	13.29	4.12	0	12.99	3.90	0	6.7e-01
Total languages spoken	2.86	1.06	0	2.89	1.04	0	8.0e-01

Table 2
Descriptive and comparative statistics of clinical outcomes for iPD group with and without probable REM-sleep behavior disorder (pRBD). Results are shown as mean and standard deviation (SD) for numerical variables, number of zeros ('NO') and ones ('YES') for binary variables and percentage of YES, and number of missing values (NA). For intergroup comparisons, *p*-values are shown from Mann-Whitney *U* test for numerical variables and Fisher's exact test for binary variables. Binary variables are annotated by asterisk. Single and double ticks indicate significance at the 5% level, and the Bonferroni-adjusted 5% level. All clinical outcomes are defined and described in the Supplementary Material

	PD non-pRBD ($n=271$)			PD pRBD ($n=129$)			p
	Mean or YES in %	SD or NO/YES	NA	Mean or YES in %	SD or NO/YES	NA	
H&Y	2.12	0.78	2	2.37	0.75	0	1.2e-04″
MDS-UPDRS III	32.00	16.11	5	38.02	16.76	2	4.5e-04″
MDS-UPDRS II	9.79	7.45	3	14.50	8.64	3	1.0e-07″
LEDD (g/day)	0.45	0.38	0	0.68	0.41	0	2.8e-08″
Gait disorder*	48%	141/130	0	71%	37/92	0	1.0e-05″
Repetitive falls*	11%	240/31	0	29%	91/38	0	1.7e-05″
MDS-UPDRS IV	1.37	3.01	2	2.75	3.98	3	5.2e-05″
Dyskinesia/day (hours)	0.47	2.29	0	1.21	3.57	1	9.3e-05″
OFF time/day (hours)	0.40	1.41	0	0.72	1.38	2	3.2e-04″
Dystonia/day (hours)	0.027	0.15	1	0.088	0.31	1	7.3e-03′
Dyskinesia*	9%	246/25	0	20%	103/26	0	3.5e-03′
Motor fluctuations*	11%	241/30	0	27%	94/35	0	8.1e-05″
Freezing of gait*	16%	227/44	0	34%	85/44	0	9.4e-05″
MoCA	24.85	3.93	5	24.02	4.45	2	6.9e-02
Sniffin' stick test	8.52	3.34	7	7.50	3.27	3	1.0e-02′
PDQ-39	33.65	23.88	12	52.23	27.05	6	7.2e-11″
SCOPA-AUT	12.59	6.97	2	19.59	8.11	0	6.7e-15″
MDS-UPDRS I	8.54	5.78	6	13.62	7.36	4	5.1e-12″
BDI-I	8.79	6.65	7	12.62	7.33	3	6.2e-08″
Starkstein Apathy Scale	13.46	5.31	4	14.67	6.24	3	1.2e-01
PDSS	111.40	21.55	4	92.64	23.05	3	2.3e-13″
Probable RBD*	0%	271/0	0	100%	0/129	0	1.4e-108″
Excessive daily sleepiness*	23%	208/63	0	41%	76/53	0	3.8e-04″
Insomnia*	24%	205/66	0	21%	102/27	0	5.3e-01
Hallucinations*	9%	247/24	0	29%	91/38	0	4.8e-07″
Impulse Control Disorder*	6%	255/16	0	16%	108/21	0	1.4e-03′
Orthostatic hypotension*	23%	210/61	0	36%	82/47	0	3.9e-03′
Dysphagia*	20%	218/53	0	33%	87/42	0	5.6e-03′
Constipation*	31%	187/84	0	63%	48/81	0	2.8e-09″
Urinary Incontinence*	27%	197/74	0	39%	79/50	0	2.8e-02′

intergroup comparison analyses (iPD pRBD vs. iPD non-pRBD; male sex iPD vs. female sex iPD). Multiple linear and logistic regression models were applied to investigate the effect of pRBD on clinical outcomes in iPD, adjusted for age at assessment (AAA) and disease duration. To investigate the potential effect of the *APOE* genotype on clinical outcomes, we pooled the heterozygotes ($\varepsilon2/\varepsilon4$; $\varepsilon3/\varepsilon4$) and homozygotes ($\varepsilon4/\varepsilon4$), allowing us to quantify a potential association between *APOE* $\varepsilon4$ genotype and pRBD in iPD. Furthermore, we applied regression of clinical symptoms in PD on *APOE* $\varepsilon4$, AAA and disease duration. For all analyses, we assessed significance at the 5% level and the Bonferroni-adjusted 5% level.

RESULTS

Frequency of pRBD and effect of pRBD on clinical outcomes in iPD

According to the RBDSQ classification of pRBD, we observed a relative pRBD frequency of 32.3% in the iPD group (129 iPD pRBD out of 400). The demographic characteristics of iPD pRBD ($n=129$) and iPD non-pRBD patients ($n=271$) are shown in Tables 1 and 2. We investigated the effect of pRBD on the clinical outcomes adjusted for AAA and disease duration.

As key results, we observed a significant positive association between iPD pRBD (as opposed to iPD non-pRBD) and burden of non-motor symptoms, i.e., autonomic dysfunction (SCOPA-AUT) and frequency of constipation; MDS-UPDRS I, burden of depression symptoms assessed by BDI-I, frequency of hallucinations and PDQ-39, showing lower quality of life in iPD pRBD, as demonstrated in Fig. 2. Furthermore, a significant negative association was determined between iPD pRBD and the Parkinson's Disease Sleep Scale (PDSS), indicating lower quality of sleep in the group of iPD pRBD vs. iPD non-pRBD. Other considered clinical outcomes showed no significant associations after multiple testing correction.

APOE genotype and iPD pRBD

We found no significant association between pooled heterozygote and homozygote *APOE* $\varepsilon4$ carriers and iPD with pRBD. Additionally, no significant association was observed between *APOE* $\varepsilon4$ and the clinical outcomes of iPD with pRBD vs. iPD non-pRBD adjusted for AAA and disease duration, as shown in Fig. 3.

Effect of sex on frequency of pRBD and other clinical outcomes in iPD

Clinical and demographic characteristics and outcomes of sex-stratified iPD are shown in Table 3. We did not observe a significant effect of male sex on the frequency of pRBD in iPD. Interestingly, from all the putative variables, only olfactory performance (measured by Sniffin' Stick test) was significantly negatively, and FOG significantly positively associated with male sex in PD after adjustment for AAA and disease duration (see Fig. 4).

Effect of education and number of spoken languages on cognitive performance

We analyzed a potential confounding effect of the years of education (YoE) and the total languages spoken (TLS) on cognitive performance in our dataset. As shown in the Supplementary Table 1, only YoE (not TLS) had a significant positive effect on Montreal Cognitive Assessment (MoCA) in a multiple regression model adjusted for AAA and disease duration.

DISCUSSION

The results of our study support the classification of RBD as a distinctive characteristic of the body-first subtype by identifying a significant association of iPD pRBD with the non-motor dominant disease profile, a result that matched remarkably well with the majority of previous studies [4–8]. It favors the concept of pathological process beginning in the peripheral nervous system with further centripetal spreading of alpha-synuclein in a subgroup of PD patients and hence the associated neurodegeneration causing a significantly higher autonomic dysfunction, higher depression burden as well as hallucinations through dysregulation of dopaminergic and noradrenergic system in the brainstem. Although we assessed RBD via a screening questionnaire, our results were consistent with a prior study using PSG-proven RBD, which indicated an association of a non-motor dominant phenotype in PD with PSG-proven RBD [4]. However, we observed only a trend in the negative effect of pRBD on global cognitive performance in PD, which did not correspond to several cross-

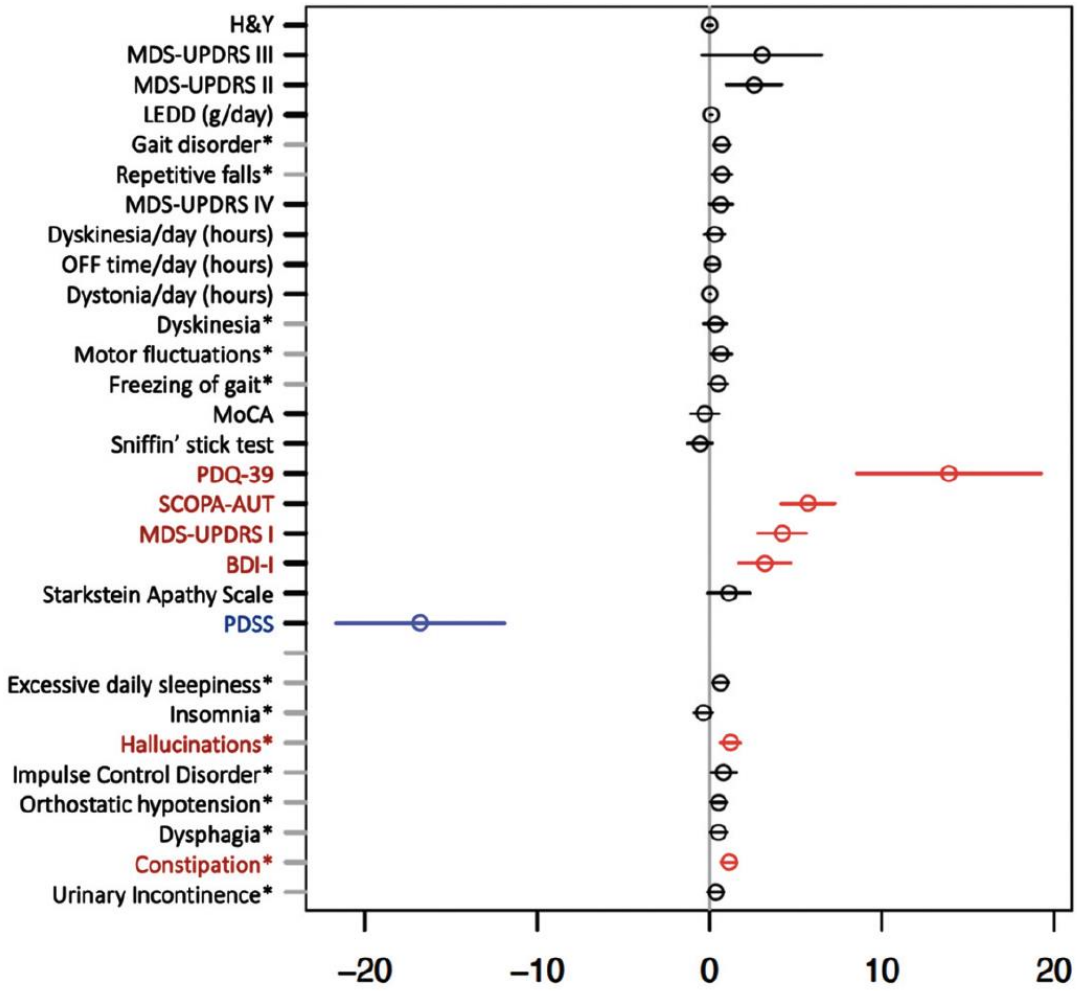

Fig. 2. Multiple regression model for investigating effect of probable REM-Sleep behavior disorder on clinical outcomes in idiopathic Parkinson's disease adjusted for age at assessment and disease duration. Forrest plot with estimated coefficients and corresponding confidence intervals (±1.96 x standard error) for pRBD, from linear/logistic regression of numerical/binary outcome on disease duration, age at assessment (AAA) and pRBD (binary outcomes are annotated by asterisk). The color blue indicates significant negative effects of pRBD on the clinical outcome, and the color red indicates significant positive effects at the Bonferroni-adjusted 5% level. Clinical symptoms and scales are described in the Supplementary Material.

sectional and longitudinal studies [8]. To assess a potential independent variable influencing cognitive performance, we identified a protective effect of YoE on cognitive decline in the overall PD group, but we did not identify a significant difference in pRBD PD vs. non-pRBD PD in terms of YoE or TLS. Therefore, we did not consider these two factors (YoE and TLS) as confounding factors for the effect of pRBD on cognitive performance assessed by MoCA in our dataset. Moreover, the *APOE* ε4 genotype, known to exacerbate beta amyloid pathology in Alzheimer's disease, has been suggested to play a role in accelerated cognitive decline in PD [27, 28]. As RBD was associated with a higher rate of cognitive decline and dementia in previous studies, we explored a potential association between pRBD and *APOE* ε4 carrier status. However, no significant association between the two was observed in our study. This would argue for an independent effect of pRBD and *APOE* ε4 status without a synergistic effect on cognitive decline in iPD. Therefore, we conclude that *APOE* ε4 genotype might not play a role as a stratifier in body-first vs. brain-first concept. It is important to stress that we excluded a potential effect of PD-linked genetic mutations and genetic risk factors for PD, which may have contributed to confounding effects on clinical phenotype in other studies, as in the case of highly prevalent mutations in the GBA gene [29].

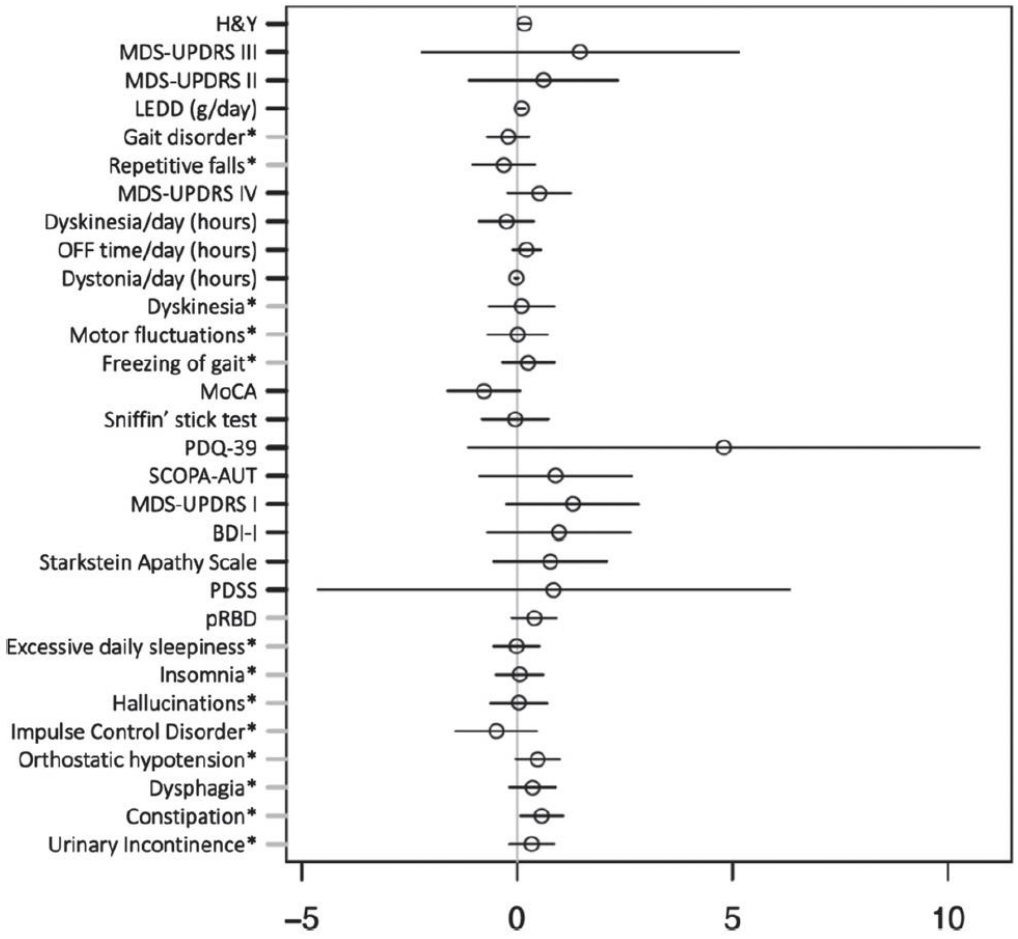

Fig. 3. Multiple regression model investigating effect of *APOE* ε4 carrier status on clinical outcomes in idiopathic Parkinson's disease adjusted for age at assessment and disease duration. Forrest plot with estimated coefficients and corresponding confidence intervals (±1.96 x standard error) for *APOE* ε4 genotype, from linear/logistic regression of numerical/binary outcome on disease duration, age at assessment (AAA), and *APOE* (binary outcomes are annotated by asterisk). The color blue indicates significant negative effects of *APOE* ε4 genotype on the clinical outcome, and the color red indicates significant positive effects at the Bonferroni-adjusted 5% level. Clinical symptoms and scales are described in the Supplementary Material.

Our investigation of potential sex-related differences in iPD phenotype did not reveal a significant association between pRBD and male sex, as suggested by several prior studies using either a similar screening questionnaire approach or PSG [30–32]. This adds to the open debate about whether there are significant differences in the prevalence of RBD in males vs. females. We would like to point out that the higher frequency of RBD in males was observed in studies using the dataset of individuals referred primarily to sleep laboratories which may cause a referral bias, given the fact that males are reported to have more violent RBD symptoms and are therefore more likely to be referred for PSG [33–36].

Next, we studied the potential confounding effects of sex on other motor and non-motor symptoms. We observed a higher frequency of males vs. females in the overall PD group (67.5% vs. 32.5%), in line with the results from recently published large cohort studies [37–39]. Interestingly, we found only olfactory dysfunction and FOG to be positively associated with males, while other putative motor and non-motor outcomes showed no significant associations with sex after multiple testing correction. These findings might indicate that sex does not play a substantial role in defining the phenotype of iPD and thus do not account for the phenotypic differences associated with pRBD.

Our study displays several specific strengths: (i) a large dataset was analyzed relative to previous studies; (ii) PD cases were genetically stratified by NeuroChip and targeted sequencing of GBA, avoid-

Table 3
Descriptive statistics for sex stratified iPD. Results are shown as mean and standard deviation (SD) for numerical variables, number of zeros ('NO') and ones ('YES') for binary variables and percentage of YES, and number of missing values (NA). The last column shows p-values from Mann-Whitney U test for numerical variables and Fisher's exact test for binary variables. Binary variables are annotated by asterisk. Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5% level. Age at onset was calculated based on the year of the PD diagnosis

	PD female ($n = 130$)			PD male ($n = 270$)			
	Mean or YES in %	SD or NO/YES	NA	Mean or YES in %	SD or NO/YES	NA	p
Disease duration since diagnosis (y)	5.44	5.53	0	5.35	5.46	0	8.1e-01
Age at assessment (y)	66.71	10.74	0	66.95	10.97	0	9.1e-01
Age at onset (y)	61.30	11.03	0	61.62	12.11	0	8.6e-01
H&Y	2.21	0.84	1	2.20	0.75	1	9.3e-01
MDS-UPDRS III	33.49	18.03	2	34.17	15.81	5	4.3e-01
MDS-UPDRS II	11.09	8.38	2	11.40	8.04	4	4.7e-01
LEDD (g/day)	0.47	0.36	0	0.55	0.42	0	1.2e-01
Gait disorder*	50%	65/65	0	58%	113/157	0	1.3e-01
Repetitive falls*	20%	104/26	0	16%	227/43	0	3.2e-01
MDS-UPDRS IV	1.90	3.61	4	1.77	3.30	1	9.3e-01
Dyskinesia/day (h)	0.87	3.22	1	0.63	2.55	0	8.6e-01
OFF time/day (h)	0.55	1.91	2	0.48	1.10	0	7.3e-01
Dystonia/day (h)	0.035	0.17	2	0.052	0.24	0	1.0e-01
Dyskinesia*	12%	115/15	0	13%	234/36	0	7.5e-01
Motor fluctuations*	11%	116/14	0	19%	219/51	0	4.3e-02′
Freezing of gait*	13%	113/17	0	26%	199/71	0	2.9e-03′
MoCA	24.92	3.84	3	24.41	4.24	4	3.4e-01
Sniffin' stick test	9.10	3.26	4	7.76	3.30	6	2.2e-04″
PDQ-39	43.28	26.38	8	37.92	26.26	10	4.0e-02′
SCOPA-AUT	14.92	8.01	2	14.83	8.08	0	1.0e+00
MDS-UPDRS I	10.22	6.32	3	10.14	6.96	7	5.5e-01
BDI-I	11.20	7.75	4	9.47	6.71	6	2.9e-02′
Starkstein Apathy Scale	13.84	5.77	6	13.86	5.60	1	9.7e-01
PDSS	102.64	25.08	4	106.68	22.94	3	1.3e-01
Probable RBD*	26%	96/34	0	35%	175/95	0	8.6e-02
Excessive daily sleepiness*	20%	104/26	0	33%	180/90	0	6.7e-03′
Insomnia*	27%	95/35	0	21%	212/58	0	2.6e-01
Hallucinations*	16%	109/21	0	15%	229/41	0	8.8e-01
Impulse Control Disorder*	7%	121/9	0	10%	242/28	0	3.6e-01
Orthostatic hypotension*	27%	95/35	0	27%	197/73	0	1.0e+00
Dysphagia*	26%	96/34	0	23%	209/61	0	4.5e-01
Constipation*	40%	78/52	0	42%	157/113	0	7.5e-01
Urinary Incontinence*	30%	91/39	0	31%	185/85	0	8.2e-01

ing a potential confounding by PD-causing mutations that are known to significantly influence the clinical phenotype; (iii) the study design included all disease stages of PD regardless of the cognitive status, and (iv) a monocentric data collection assured the consistency of the dataset.

Conversely, some limitations of our study should also be noted: We investigated the research questions using a cross-sectional analysis, and further studies on longitudinal data are still warranted. Additionally, RBD was not assessed by gold standard PSG but by a more accessible method using a screening questionnaire, potentially including in part false positive patients for RBD with another sleep pathology. Furthermore, the presence of hallucinations might be wrongly considered by the patients to classify as RBD symptoms. Nevertheless, the association of RBD in PD with hallucinations has been widely reported in the literature [40–42], thus we do not consider the significant positive association of pRBD with hallucinations in our dataset as a potential mis-classifier of pRBD vs. non-pRBD. Finally, we did not have complementary data on the time relation between pRBD and PD, i.e., describing whether pRBD preceded PD or evolved during the clinical phase of PD.

However, the overall concordance of the results on the association of pRBD in PD with a non-motor dominant phenotype indicates that applying RBDSQ may provide a useful tool for patient stratification in future studies and clinical trials. It might

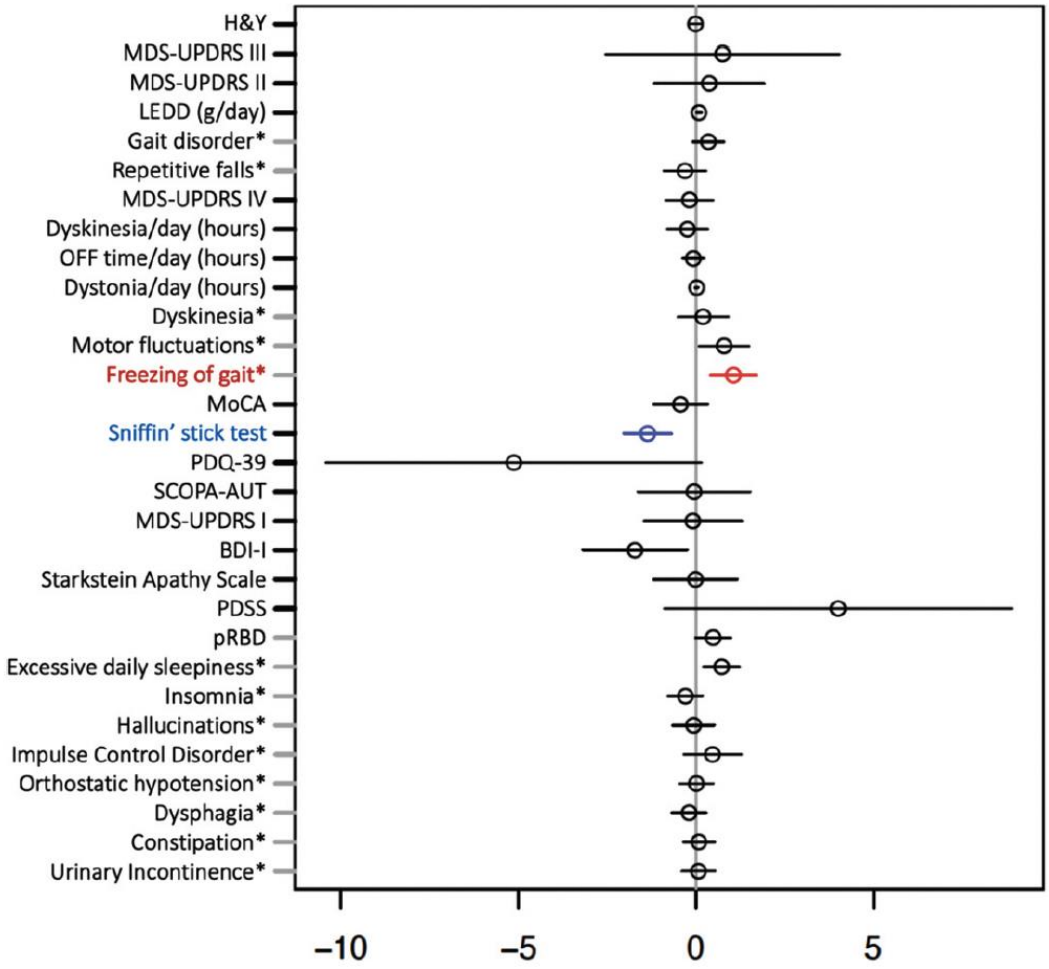

Fig. 4. Multiple regression model investigating effect of sex on clinical outcomes in idiopathic Parkinson's disease adjusted for age at assessment and disease duration. Forrest plot with estimated coefficients and corresponding confidence intervals (±1.96 x Standard error) for sex, from linear/logistic regression of numerical/binary outcome on disease duration, AAA, and sex (binary outcomes are annotated by asterisk). The color blue indicates significant negative effects of male vs. female sex on the clinical outcome, and the color red indicates significant positive effects at the Bonferroni-adjusted 5% level. Clinical symptoms and scales are described in the Supplementary Material.

prove to be a clinically relevant mean to screen for pRBD during the regular follow-up of PD patients in order to personalize and adapt the therapy and its potential secondary effects by the treating physicians. Finally, this study adds to the prior body of evidence that PD subtyping, in general, may serve the patient by providing treatment-relevant phenotype-genotype stratifications as a tool for future clinical trials.

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CONFLICT OF INTEREST

All authors have no conflict of interest to report.

DATA AND CODE AVAILABILITY STATEMENT

The dataset for this manuscript is not publicly available as it is linked to the Luxembourg Parkinson's Study and its internal regulations. Any requests for accessing the dataset can be directed to request.ncer-pd@uni.lu. The code for the statistical models is available at: https://doi.org/10.17881/sw04-1w80.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JPD-223511.

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3. DISCUSSION

The unique set-up of the Luxembourg Parkinson's Study with deep phenotyping and genotyping of all individuals with neurodegenerative parkinsonism along with HC allowed to address several key determinants of PD phenotype. Using combined baseline dataset genotyped by two modalities; i.e. (i) Neurochip (Illumina; *Blauwendraat C et al, 2017*) and (ii) targeted sequencing of GBA via PacBio technology (*Korlach J et al, 2010),* we could reliably account for potential confounders of PD phenotype by excluding all carriers of PD-linked mutations in both PD and HC group. Hence, we performed all analysis in idiopathic PD and HC not carrying mutations in any PD-related genes, granting closer look into other parallel active players in multifarious nature of iPD.

The following paragraphs will address the main research questions point-by-point as listed above summarizing the results of two published peer-reviewed articles 1 and 2 as integral part of the thesis. The tables and figures shown below with corresponding legends were taken from the two publications attached as part of the thesis (REF). All outcomes (scales, symptoms and clinical data) are defined in the Supplementary material with online-link noted in the Article n.1 and Article n.2 respectively.

1. *How to disentangle the concomitant effect of parallel ageing and the effect of AAO on PD phenotype? Is the ageing itself accountable for the differences in PD phenotype when investigating the effect of AAO on clinical profile?*

The role and effect of AAO is PD on the clinical profile has been subject of debate during the last 20 years. Many studies agree on early-onset PD being associated with reduced progression of motor symptoms, lower frequency of cognitive impairment and higher frequency of dyskinesia and dystonia. However previous studies were limited by (i) using arbitrary categorizing cut-offs for AAO and thus (ii) lowering the statistical power by artificially categorizing the continuous variables (AAO and disease duration), (iii) lacking a comprehensive genotyping

of all studied individuals and finally (iv) neglecting the potential influence of the parallel ageing process and associated comorbidities.

To overcome the above listed limitations, we have firstly analysed the effect of non-categorized AAO in idiopathic PD (iPD; n=430) on clinical phenotype using multiple regression model adjusted for disease duration. As shown in Figure 2, we found AAO to be significantly positively associated with higher motor impairment, higher burden of motor complication (MDS-UPDRS IV) and more pronounced non-motor symptoms in line with previously published studies (*Pagano G et al, 2016; Gan-Or Z et al, 2022; Diederich N et al, 2003; Wickremaratchi M et al, 2011*).

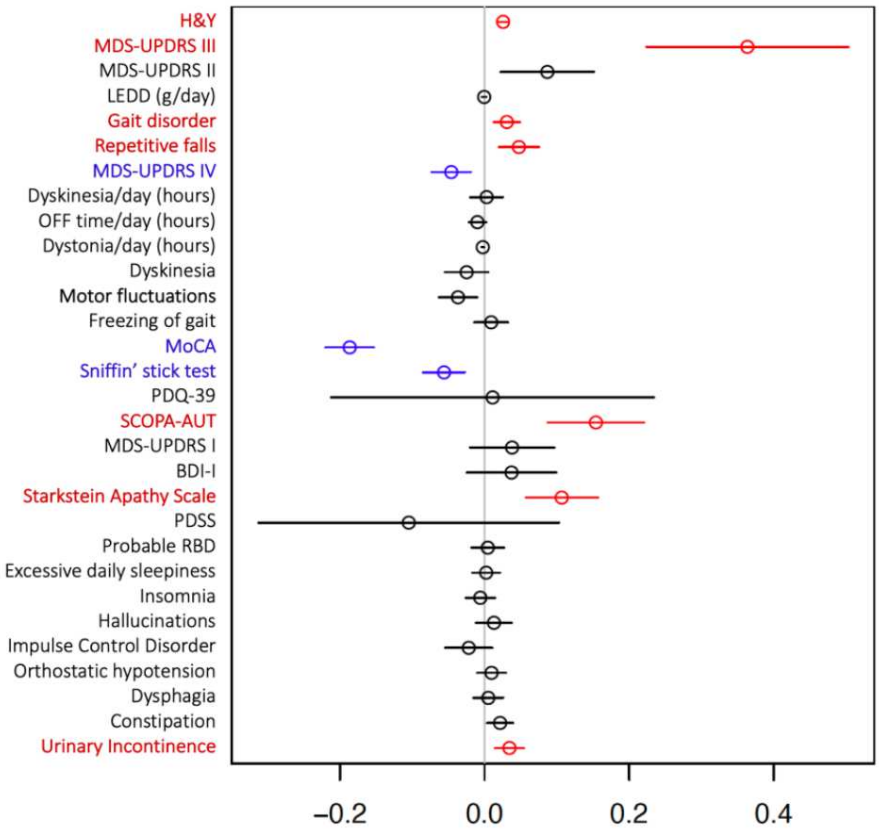

Figure 2. The colour blue indicates significant negative effects of AAO on the clinical outcome, and the colour red indicates significant positive effects at the Bonferroni-adjusted 5% level (based on the published data and Figure 1 in Pavelka L et al, 2022, Age at onset as stratifier in idiopathic Parkinson's disease - effect of ageing and polygenic risk score on clinical phenotypes).

However, these results should be interpreted with caution; whether such significant effect of AAO on clinical PD phenotype is derived from different

disease dynamics, or it is rather a result of the parallel ageing process, has not yet been sufficiently addressed so far.

Therefore, we endeavoured to disentangle the difference in effect of ageing by combining dataset of iPD and PD-linked mutation free HC (n=556) in cross-sectional analysis fitting a multiple regression model adjusting PD for disease duration as the main determinant of PD severity. To this end, the common variable age at assessment (AAA) was chosen to represent the age in both HC and patients with PD. As a result, the effect of ageing (AAA) in PD vs HC showed only significant positive association of AAA and burden of motor symptoms (MDS-UPDRS III) and negative significant association with cognitive performance (MoCA), whereas remaining non-motor symptoms showed comparable effects (see Table 1).

Table 1. Multiple regression model with PD and HC investigating the difference in effect of ageing in HC (AAA) and in PD (AAA:status) adjusted for disease duration.

	Clinical symptoms and scales	Intercept	AAA	Status	Disease duration	AAA:status
Motor symptoms	H&Y	0.00	0.00	0.23	0.05″	0.03″
	MDS-UPDRS III	-3.70	0.12′	9.65	0.76″	0.24″
	MDS-UPDRS II	-0.21	0.02	2.59	0.63″	0.06′
	LEDD (g/day)	-0.02	0.00	0.39	0.04″	0.00
	Gait disorder	-12.56	0.13′	10.34	0.08″	-0.10′
	Repetitive falls	-5.14	0.00	-0.66	0.14″	0.04
Motor complications	MDS-UPDRS IV	0.00	0.00	3.43	0.28″	-0.05″
	Dyskinesia/day (hours)	0.00	0.00	-0.16	0.12″	0.00
	OFF time/day (hours)	0.00	0.00	0.87	0.06″	-0.01
	Dystonia/day (hours)	0.00	0.00	0.15	0.01″	0.00′
	Dyskinesia	-20.57	0.00	18.99	0.18″	-0.02
	Motor fluctuations	-20.57	0.00	20.22	0.18″	-0.04
	Freezing of gait	-20.57	0.00	17.75	0.16″	0.01
Non- motor symptoms & quality of life	MoCA	29.84	-0.05″	7.20	-0.05	-0.14″
	Sniffin' stick test	15.44	-0.04″	-3.03	-0.11″	-0.01
	PDQ-39	10.68	-0.01	19.04	1.78″	0.01
	SCOPA-AUT	2.53	0.08′	-0.33	0.43″	0.07
	MDS-UPDRS I	3.12	0.02	2.94	0.35″	0.01
	BDI-I	4.06	0.02	2.09	0.24″	0.02
	Starkstein Apathy Scale	5.11	0.07″	1.64	-0.01	0.04
	PDSS	130.37	-0.13	-13.17	-0.98″	0.03
	Probable RBD	-1.58	-0.02	-0.54	0.11″	0.02
	Excessive daily sleepiness	-6.53	0.05	5.22	0.07″	-0.05
	Insomnia	-2.00	-0.01	0.99	0.04′	0.00
	Hallucinations	-3.31	-0.04	0.23	0.09″	0.05
	Impulse Control Disorder	-4.32	-0.04	2.70	0.11″	0.01
	Orthostatic hypotension	-3.92	0.02	2.04	0.05′	-0.01
	Dysphagia	-7.28	0.04	5.51	0.06′	-0.03
	Constipation	-2.37	-0.01	0.23	0.07″	0.03
	Urinary Incontinence	-8.43	0.08″	5.03	0.04′	-0.05′

Legend: Regression coefficients are shown for different outcomes (rows). Status takes the value 0 for HC and 1 for PD, the AAA:status is the interaction effect of AAA and being PD (status=1). Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5% level respectively. The colour blue indicates significant negative effect on the clinical outcome, and the colour red indicates significant positive effect. The column AAA:status indicates whether the effect of AAA on clinical outcomes differs between PD and HC (based on the published data and Table 5 in Pavelka L et al, 2022, Age at onset as stratifier in idiopathic Parkinson's disease - effect of ageing and polygenic risk score on clinical phenotypes).

Our data showed that the observed significant effect of AAO on gait disorder, falls and the majority of non-motor symptoms in iPD could be driven by the ageing process itself (and related comorbidities) rather than a specific dynamic of neurodegeneration. This finding is novel and has not yet been reported the literature on PD so far.

2. *Is the polygenic background of PD associated with younger AAO in PD? Does higher PRS have a significant effect on clinical phenotype in PD?*

While the genotype-phenotype interaction has been clearly established for rare monogenic forms of PD (e.g. PARKIN, PINK1, DJ1, SNCA and LRRK2) and high-risk genetic variants (GBA), the cumulative effect of small-effect size polymorphism identified in PD-related genes by GWAS studies remains controversial. Such cumulative effect can be calculated into a PRS in order to account for missing heritability in PD (*Ohnmacht J et al, 2020*) and might serve as an additional genetic component playing role in the bridge between genotype and phenotype providing insight into disease mechanisms, stratifying the individuals at risk and allowing for Mendelian randomization studies (*Kullo IJ et al, 2022*). Furthermore, various PRSs can be calculated with subset of genes aiming for stratification of patient group by supposed disease mechanism, e.g. mitochondrial specific PRS presuming subtype of PD associated with predominantly mitochondrial dysfunction (*Billingsley KJ et al, 2019*).

In our study, we determined a significantly higher PRS in PD vs HC (see Table 1 in Pavelka et al, 2022, Age at onset as stratifier in idiopathic Parkinson's disease - effect of ageing and polygenic risk score on clinical phenotypes) as well as a significant inverse correlation of PRS and AAO, meaning the higher the PRS for PD, the lower AAO as shown in Figure 3.

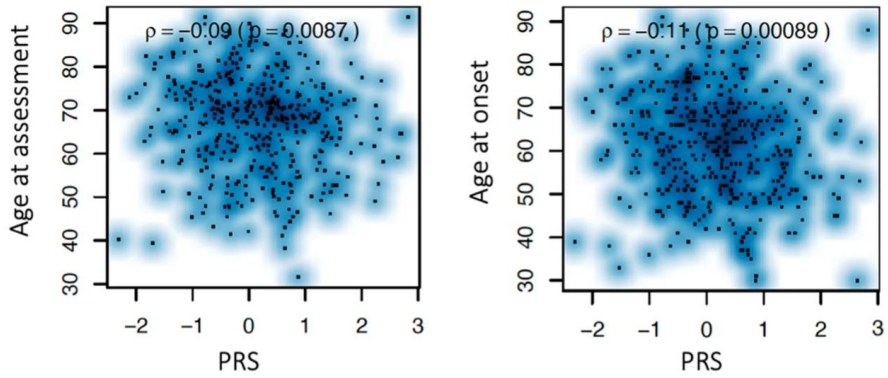

Figure 3. Pairwise association between age at onset (AAO), age at assessment (AAA) (y-axis) and polygenic risk score (PRS) (x-axis) with Kendall's tau correlation coefficient (based on the published data and Figure 2 in Pavelka L et al, 2022, Age at onset as stratifier in idiopathic Parkinson's disease - effect of ageing and polygenic risk score on clinical phenotypes).

Furthermore, we investigated the effect of PRS on the severity of clinical phenotype. Until now, studies on effect of PRS on clinical phenotype have been scarce and presenting conflicting results (*Liu G et al, 2021; Ihle J et al 2020; Paul KC et al, 2018*). Therefore, we addressed this question by fitting a multiple regression adjusting for age (AAA) and disease duration and did not identify any significant association PRS with severity of clinical phenotype in iPD (see Table 2). The discrepancy in reported studies on PRS might be due to several reasons: (i) tertiary referral bias, (ii) multicentric recruitment, (iii) set of genes included in the PRS calculation and finally (iv) using dataset that does not exclude the PD-linked mutation carriers with known genotype-phenotype interaction. For this reason, the comprehensive genotyping in our dataset and analysis in idiopathic setting presented an asset in isolating the effect of common SNPs of low effect sizes on PD phenotype. Additionally, our study used monocentric dataset that is not *a priori* biased by tertiary referral.

Table 2. Kendall correlation coefficient between clinical outcome (row) and polygenic risk score (PRS) for healthy controls (HC) and Parkinson's disease patients (PD).

	Clinical symptoms and scales	HC	PD
Motor symptoms	H&Y	0.0272	0.0272
	MDS-UPDRS III	-0.0088	-0.0341
	MDS-UPDRS II	-0.0242	0.0058
	LEDD (g/day)	0.0091	-0.0147
	Gait disorder	0.4070	-0.0177
	Repetitive falls	0.6209	0.1690
Motor complications	MDS-UPDRS IV	0.0625	0.0625
	Dyskinesia/day (hours)	0.0576	0.0576
	OFF time/day (hours)	0.0100	0.0100
	Dystonia/day (hours)	0.0491	0.0491
	Dyskinesia	-	0.1426
	Motor fluctuations	-	0.0864
	Freezing of gait	-	0.0084
Non- motor symptoms & quality of life	MoCA	0.0542	-0.0493′
	Sniffin' stick test	0.1012	0.0576
	PDQ-39	-0.0519	0.0386
	SCOPA-AUT	-0.0113	0.0150
	MDS-UPDRS I	-0.0556	0.0105
	BDI-I	-0.0155	0.0290
	Starkstein Apathy Scale	-0.0425	-0.0068
	PDSS	-0.0128	-0.0332
	Probable RBD	-0.1142	-0.0703
	Excessive daily sleepiness	0.1638	-0.1261
	Insomnia	-0.0356	0.1387
	Hallucinations	-0.9806	-0.1225
	Impulse Control Disorder	-2.1177	-0.0872
	Orthostatic hypotension	0.0839	-0.1331
	Dysphagia	-0.6401	-0.2650′
	Constipation	0.2701	-0.0740
	Urinary Incontinence	0.1011	0.0434

Legend: Significant negative correlations in shown in blue and significant positive correlations in red (Kendall correlation test). Single and double ticks indicate significance at the 5% level and the Bonferroni-adjusted 5% level (based on the published data and Table 5 in Pavelka L et al, 2022, Age at onset as stratifier in idiopathic Parkinson's disease - effect of ageing and polygenic risk score on clinical phenotypes).

Based on our results, we conclude that while polygenic background significantly contributes to the risk of PD as well as to younger AAO, it does not necessarily influence the severity and progression of PD. It is well in line with the hypothesis that pathologic processes *driving* the onset of disease could be different from the factors influencing *the progression rate* of the disease. However, we acknowledge the limitation of our study being cross-sectional and additional

validation in the longitudinal set-up will be performed in the Luxembourg Parkinson's cohort.

3. *Does pRBD stratify the patients with PD to a specific phenotype in line with the proposed body-first subtype of PD?*

RBD has been increasingly recognized as one of the most prominent prodromal signs of alpha-synucleinopathies with conversion rate to PD, DLB or rarely MSA up to 90% after 15 years of longitudinal follow-up (*Postuma RB et al, 2019*). Additionally, RBD can appear at any stage of PD giving rise to a wide range of reported frequency of RBD across the studies (30-50%; *Olson EJ et al, 2000; Schenck CH et al, 2002*). Moreover, RBD has been recently proposed to be a hallmark of *body-first subtype* of PD with more diffuse alpha-synucleinopathy leading to higher burden of autonomic dysfunction and as well as more pronounced cognitive decline (*Borghammer P et al, 2019).*

In our study design, we stratified iPD patients based on RBD screening questionnaire (RBDSQ) to iPD with and without probable RBD (pRBD). Using multiple regression model investigating effect of pRBD on clinical outcomes adjusted for AAA and disease duration (see Figure 4), we identified significant positive association of pRBD with non-motor symptoms including depression burden (BDI-I), autonomic dysfunctions (SCOPA-AUT and MDS-UPDRS I), constipation and hallucinations, as well as significant negative association with quality of life (PDQ-39) and quality of sleep (PDSS). This phenotype remarkably matched with the proposed *body-first subtype* of PD presuming a dominant centripetal spreading of pathological process from PNS leading to higher impairment of PNS and followed by lower structures of CNS (brainstem) causing constipation, high burden of autonomic dysfunction, sleep disturbances, depression and hallucinations.

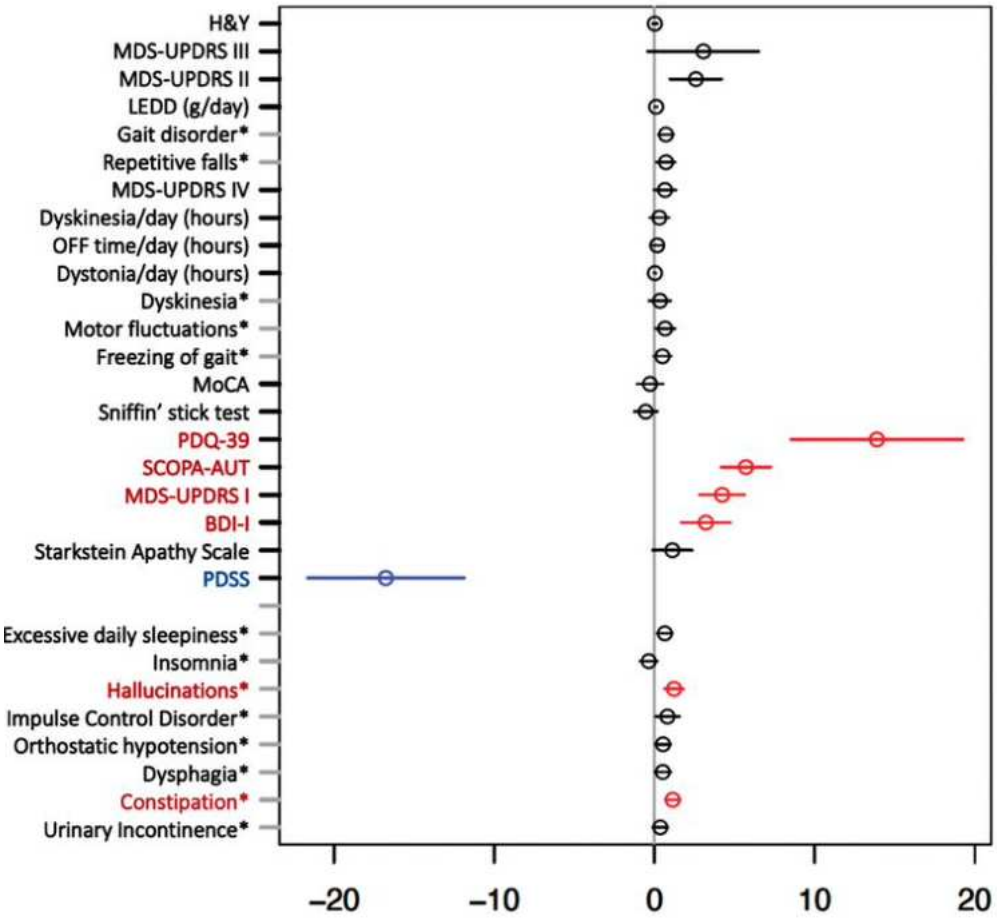

Figure 4. Multiple regression model for investigating effect of probable REM-Sleep behavior disorder on clinical outcomes in idiopathic Parkinson's disease adjusted for age at assessment and disease duration. Forrest plot with estimated coefficients and corresponding confidence intervals (±1.96 x standard error) for pRBD, from linear/logistic regression of numerical/binary outcome on disease duration, age at assessment (AAA) and pRBD (binary outcomes are annotated by asterisk). The color blue indicates significant negative effects of pRBD on the clinical outcome, and the color red indicates significant positive effects at the Bonferroni-adjusted 5% level (based on the published data and Figure 2 in Pavelka L et al, 2022, Body-First Subtype of Parkinson's Disease with Probable REM-Sleep Behavior Disorder Is Associated with Non-Motor Dominant Phenotype).

Despite the inferiority of identifying RBD by self-administered questionnaire in PD population in comparison to gold standard polysomnography (PSG), our results were in line with studies focusing on PD with PSG-proven RBD (*Neikrug AB et al; 2014*). Additionally, the study design based on screening questionnaire allowed for a higher sample size in comparison to studies with PSG proven RBD given the overall low capacities of sleep laboratories as well as its costs. Our study contributed to the body of evidence identifying a specific non-motor dominant endophenotype of PD with pRBD. Furthermore, it highlights the clinical

importance of screening for RBD informing more targeted and considered therapy of non-motor symptoms in PD with RBD.

4. *What is the interplay between pRBD, APOE4 genotype and clinical profile in PD? Do both factors have a concomitant/cumulative effect on cognitive decline in PD?*

Cognitive impairment in PD is a common non-motor symptoms with prevalence of dementia in PD (PDD) ranging between 24-50% (*Aarsland D et al, 2010, Aarsland D et al, 2005; Mayeux R et al, 1992*). Among other neurodegenerative causes of dementia, Alzheimer disease and DLB belong to the most prevalent. Overlapping with PD/PDD, DLB presents dementia before the onset of parkinsonism or at maximum of 1 year since the onset of parkinsonism (1-year rule) accompanied by visual hallucinations and cognitive fluctuations. Nevertheless, the 1 year-rule and the overlap in motor features in PD and DLB have created a grey zone blurring the diagnostic classification (*Aarsland D et al, 2017*). Additionally, complex genetic architecture behind PD, DLB and Alzheimer disease was shown to overlap in several genes including GBA and APOE4 genotype (*Chia R et al, 2021)*. While APOE4 is well established to increase risk of Alzheimer dementia via exacerbating the beta-amyloid pathology, it has been proposed that APOE4 carriers (either heterozygote or homozygote) in PD have steeper cognitive decline and higher frequency of dementia (*Tunolf JA et al, 2021; Davis AA et al, 2020*). Nevertheless, the role of APOE4 in PD remains controversial and several conflicting results have been published so far (*Mengel et al, 2016; Federoff M et al, 2012*). Among other risk factors for dementia, RBD was associated with higher cognitive impairment and dementia in PD (*Roguski A et al, 2020; Neikrug AB et al, 2014; Postuma RB et al; 2012)* as well as being highly prevalent in DLB (*McKeith IG et al, 2017*). Therefore, we investigated the complex interplay between genetic risk factor for dementia in PD (APOE4), interaction beween APOE4 and PD with pRBD and its effect on cognitive decline in PD.

We fitted a multiple regression model adjusted for AAA and disease duration to investigate the effect of APOE4 genotype on clinical outcomes including cognitive performance in PD assessed by Montreal Cognitive Assessment (MoCA). Though we observed a trend in negative association of APOE4 and MoCA, the effect was not found significant (see Figure 5). Interestingly, we did not identify a higher level of motor impairment nor more pronounced cognitive decline in PD with pRBD vs PD non-pRBD in contrast to several previous studies (*Mao J et al, 2020*). This observation might be result of testing in idiopathic setting excluding all carriers of PD-linked mutations from our models identified via NeuroChip and GBA targeted sequencing. Equally, we did not detect any significant association between APOE4 genotype and pRBD in PD (see. Figure 5) well in line with the observation by *Gan-Or Z et al, 2017*. Based on our results, we imply that both APOE4 and pRBD in PD have an independent effect on cognitive performance without a synergic effect on cognitive decline in PD.

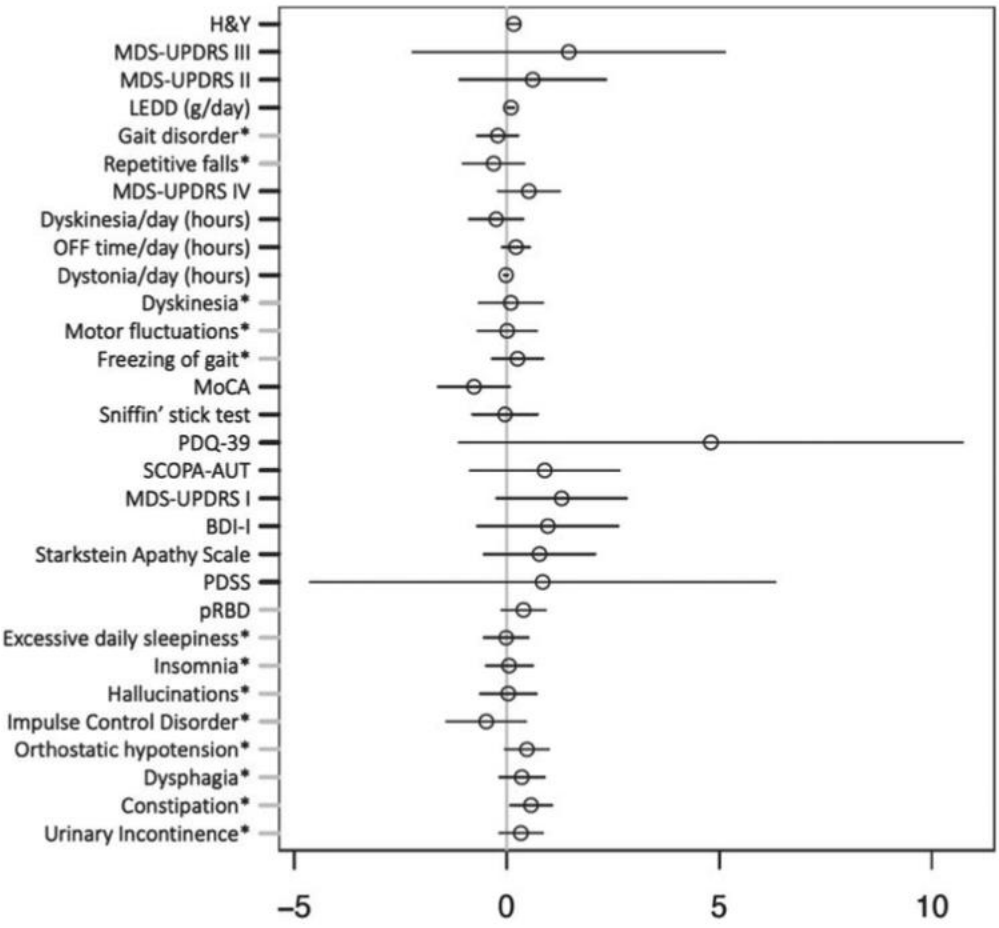

Figure 5. Multiple regression model investigating effect of APOE ε4 carrier status on clinical outcomes in idiopathic Parkinson's disease adjusted for age at assessment and disease duration. Forrest plot with estimated coefficients and corresponding confidence intervals (±1.96 x standard error) for APOE ε4 genotype, from linear/logistic regression of numerical/binary outcome on disease duration, age at assessment (AAA), and APOE (binary outcomes are annotated by asterisk). The color blue indicates significant negative effects of APOE ε4 genotype on the clinical outcome, and the color red indicates significant positive effects at the Bonferroni-adjusted 5% level (based on the published data and Figure 3 in Pavelka L et al, 2022, Body-First Subtype of Parkinson's Disease with Probable REM-Sleep Behavior Disorder Is Associated with Non-Motor Dominant Phenotype).

5. *How far the sex affects the clinical phenotype in PD? Is pRBD significantly associated with male sex in PD as reported in cohorts with RBD proven by polysomnography?*

The scientific reports focusing on the role of male vs female sex in PD vary enormously with generally low consensus across the studies (*Georgiev D et al, 2017*). Therefore, we applied a multiple regression model adjusting for AAA and disease duration in order to investigate association of male vs female sex with (i) pRBD and with (ii) the clinical phenotype in iPD. As shown in Figure 6, we did not determine a significant association between male sex and pRBD corresponding to similar studies using screening strategy for RBD on a large scale (*Sixel-Döring F et al, 2011; Bjørnarå KA et al; 2013, Baumann-Vogel H et al, 2020*). Additionally, previous studies investigating PSG-proven RBD have shown higher frequency of RBD in males than in females. However, caution must be taken in the interpretation of these finding due to a potential referral bias. Males were shown to have more violent presentation of dream enactment and thus higher probability of being referred to sleep laboratories (*Wong JC et al, 2016; Haba-Rubio J et al, 2018; Kang SH et al, 2013, Postuma RB et al, 2012*). While our study was free of referral bias to PSG laboratories, it added to the robustness of our findings.

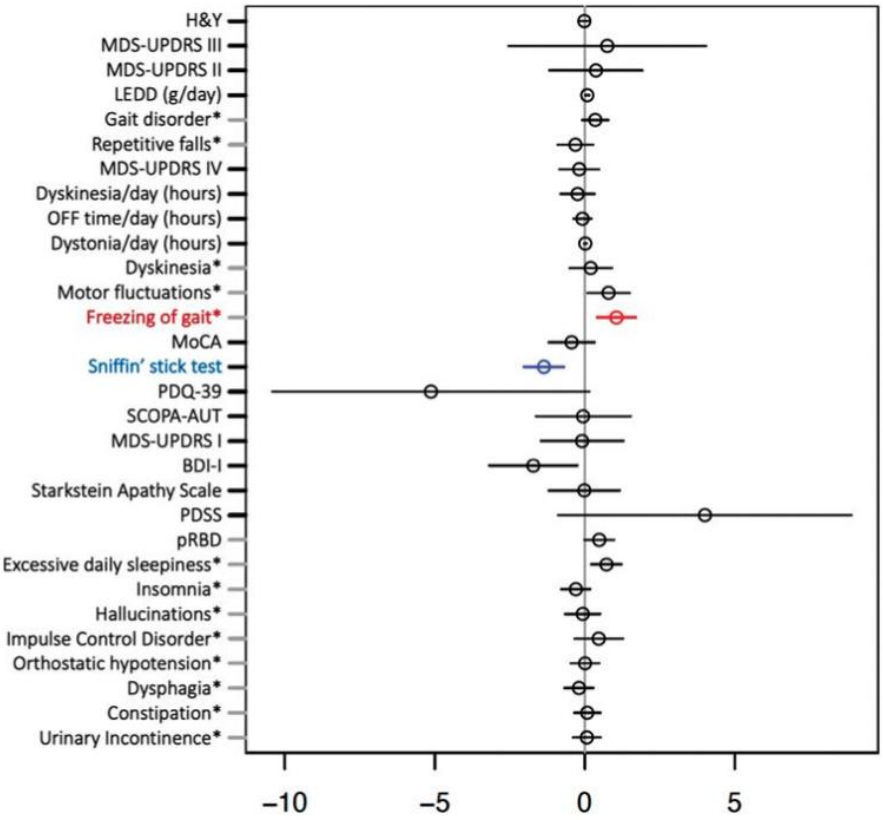

Figure 6. Multiple regression model investigating effect of sex on clinical outcomes in idiopathic Parkinson's disease adjusted for age at assessment and disease duration. Forrest plot with estimated coefficients and corresponding confidence intervals (±1.96 x Standard error) for sex, from linear/logistic regression of numerical/binary outcome on disease duration, AAA, and sex (binary outcomes are annotated by asterisk). The color blue indicates significant negative effects of male vs. female sex on the clinical outcome, and the color red indicates significant positive effects at the Bonferroni-adjusted 5% level (based on the published data and Figure 4 in Pavelka L et al, 2022, Body-First Subtype of Parkinson's Disease with Probable REM-Sleep Behavior Disorder Is Associated with Non-Motor Dominant Phenotype).

When looking into the effect of sex on the overall clinical profile, we found olfactory dysfunction (lower scores on Sniffin' Stick test indicate lower olfactory performance) to be significantly positively associated with male vs female sex in line with previous studies in early-stage and drug-naïve PD patients (*Liu et al, 2015; Picillo M et al, 2013*). Furthermore, male sex was significantly positively associated with FOG in our study in agreement with a large study in 6620 PD patients performed by *Macht M et al 2007*, but contradictory to the recent large meta-analysis showing no significant sex predilection of FOG (*Tosserams A et al, 2021*). The strength of our study is that the classification of FOG vs non-FOG was based on semi-structured interview with study physician rather than based on self-reported questionnaires as in majority of the previous studies.

Additionally, the methodological differences in capturing of FOG vs non-FOG across the studies included in the meta-analysis further weakens the robustness of their findings.

The underlying preponderance for PD of males vs females has been ascribed to a neuroprotective effect of different hormonal profile, notably estrogen (*Maioli S et al, 2021*). In terms of underlying pathological mechanism behind the sex-related differences in PD, females vs males have been shown to differ in the gene expression located in dopaminergic neurons, having inter-sex differences in representation of dopaminergic receptor subclasses and manifesting various response to oxidative stress and neuroinflammation (*Cerri S et al, 2019).*
Nevertheless, we did not include sex as confounding factor in the above-mentioned regression models (investigating the effect of AAO, pRBD and APOE4 genotype on clinical phenotype in iPD) given the low effect of sex on the investigated clinical outcomes in our study.

To conclude, we present two studies focusing on the potential key players determining the PD phenotype that were published in highly ranked peer-reviewed scientific journals and are attached as integral part of the thesis. Based on our results using the unique monocentric deep-phenotyped and genotyped dataset of Luxembourg Parkinson's Study, we provided a(n)

(i) novel approach in disentangling a potential effect of con-current ageing process when investigating the effect of AAO on clinical phenotype in PD,
(ii) insight into the relationship between polygenic background, its effect on AAO and severity of PD,
(iii) contribution to an open debate on potential effect of male vs female sex on clinical profile and progression of PD,
(iv) insight into the complex interplay between pRBD, APOE4 genotype and clinical phenotype of PD and

(v) contribution to the recently established paradigm of *body-first* vs *brain first* subtype of PD highlighting non-motor dominant endophenotype of PD in association with pRBD.

4. SUMMARY

Parkinson's disease (PD) is the fastest growing neurodegenerative disorder. However, the variability of its clinical presentation and underlying mechanisms is still a challenge for both, clinicians as well as researchers in the field. In this work, we aimed at disentangling the key elements determining the clinical profile and severity of PD. Using data from unique deep-phenotyped and -genotyped cohort of the monocentric longitudinal Luxembourg Parkinson's Study, we performed analysis of over 1000 individuals excluding known PD-linked genetic mutations for analyzing idiopathic PD. Based on our results, we provide a new perspective on how far the observed PD phenotype based on age at onset (AAO) is determined by parallel ageing process rather than due to a different dynamics of neurodegeneration. Surprisingly, our data showed that most of the non-motor symptoms were accounted to the ageing process rather than to the neurodegenerative process in PD. Therefore we suggest that physiological ageing is most likely responsible for the previously described association of non-motor symptoms with an older AAO. Additionally, we addressed a potential contribution of multiple common genetic variants with low effect size translated into the polygenic risk score (PRS). We explored a potential effect of PRS on AAO in PD, and subsequently inquired whether PRS may have an impact on severity of PD phenotype and found a significant inverse correlation of PRS and AAO. However, the polygenic risk score had no significant effect on the severity of PD phenotype. Furthermore, we applied the emerging concept of stratification of PD to *body-first* and *brain-first subtype* suggesting the REM-Sleep Behaviour Disorder (RBD) to be part of the *body-first* subtype with specific phenotype. Based on our analysis we identified PD with comorbid probable RBD (pRBD) to be significantly associated with a non-motor dominant clinical phenotype showing higher burden of autonomic dysfunction and depression in line with the concept of *body-first* PD subtype. Moreover, we investigated a potential genetic association of the APOE4 carrier status with pRBD as well as an overall effect of APOE4 on the clinical profile in PD without significant findings in both cases. Finally, we analysed the potential effect of sex in PD reporting only significant

positive effect of male sex on freezing of gait as well as negative effect on olfactory capacity.

This work contributes to the current body of evidence using stratification strategy that allowed to gain insight into a (i) complex interplay between age, ageing and polygenic risk score (PRS) on one side, as well as between (ii) sex, probable RBD, APOE4, all determining the phenotypic variability of PD to certain degree. Incorporation of these stratifiers into the future setting of clinical trials will play a key role in determining a disease-modifying interventions in PD.

5. ZUSSAMENFASSUNG

Die Parkinson-Krankheit (PK) ist die zweithäufigste neurodegenerative Störung. Die Variabilität des klinischen Erscheinungsbildes verblüfft jedoch seit Jahrzehnten sowohl Kliniker als auch Forscher. In dieser Arbeit haben wir uns bemüht, die Schlüsselelemente zu entschlüsseln, die das klinische Profil und den Schweregrad von Morbus Parkinson bestimmen. Unter Verwendung eines einzigartigen, tiefgreifend phänotypisierten und genotypisierten Datensatzes aus der monozentrischen Beobachtungsstudie in Luxemburg haben wir eine Analyse von über 1000 Personen nach dem Ausschluss von PK-assoziierten Mutationen ausschließt durchgeführt. Auf der Grundlage unserer Ergebnisse bieten wir eine neue Perspektive darauf, inwieweit der beobachtete PK-Phänotyp, der auf dem Alter bei Krankheitsbeginn (AKB) basiert, durch einen parallelen Alterungsprozess bestimmt wird und nicht durch eine andere Dynamik der Neurodegeneration. Überraschenderweise zeigten unsere Daten, dass die meisten nicht-motorischen Symptome eher auf den Alterungsprozess als auf den neurodegenerativen Prozess bei PK zurückzuführen sind. Wir vermuten, dass die physiologische Alterung für die meisten der nicht-motorischen Symptome verantwortlich ist, die in früheren Studien mit AKB in Verbindung gebracht wurden. Darüber hinaus untersuchten wir einen möglichen Beitrag mehrerer häufiger genetischer Varianten mit geringer Effektgröße, die in den polygenen Risikoscore (PRS) einfließen. Wir untersuchten eine mögliche Störwirkung des PRS auf die AKB bei PK und fragten anschließend, ob der PRS einen Einfluss auf den Schweregrad des Phänotyps haben könnte. Wir fanden eine signifikante negative Korrelation zwischen PRS und AKB, jedoch hatte der polygene Risikoscore keine signifikante Auswirkung auf den Schweregrad des PD-Phänotyps. Darüber hinaus folgten wir dem Konzept der Stratifizierung von PK auf *Body-First-* und *Brain-First-Modell*, was darauf hindeutet, dass die REM-Schlaf-Verhaltensstörung (verkürzt RBD aus englischem REM-Sleep behaviour disorder) Teil des *Body-First-Modell* mit spezifischem Phänotyp ist. Basiert auf unsere Modelle konnten wir feststellen, dass PK mit einer komorbiden wahrscheinlichen Schlafstörung (wRBD) signifikant mit einem nicht-motorisch dominanten klinischen Phänotyp assoziiert ist, der eine höhere Belastung durch

autonome Funktionsstörungen und Depressionen aufweist, was dem Konzept des *Body-First-Modells* entspricht. Darüber hinaus untersuchten wir eine mögliche genetische Assoziation des APOE4-Trägerstatus mit wRBD sowie die Auswirkungen von APOE4 auf das gesamte klinische Profil bei PK, ohne dass in beiden Fällen signifikante Ergebnisse erzielt wurden. Schließlich untersuchten wir die potenziellen Auswirkungen des Geschlechts bei Morbus Parkinson und stellten fest, dass das männliche Geschlecht nur einen signifikanten positiven Effekt auf das ‚Freezing of Gait' sowie einen negativen Effekt auf das Riechvermögen hat.

Diese Arbeit ist ein Beitrag zu den aktuellen Erkenntnissen über die Stratifizierungsstrategie, die einen Einblick in das komplexe Zusammenspiel zwischen (i) Alter, Alterung und PRS auf der einen Seite und (ii) Geschlecht, wRBD und APOE4 auf der anderen Seite ermöglicht, die alle die phänotypische Variabilität von Parkinson bestimmen können. Die Einbeziehung dieser Stratifikatoren in künftige klinische Studien wird eine Schlüsselrolle bei der Festlegung krankheitsmodifizierender Maßnahmen bei PK spielen.

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7. DECLARATION OF OWN CONTRIBUTION

The following two declarations of own contribution represent the contribution of the first author with regard to the published peer-reviewed articles listed (article n.1 and article n.2) as an integral part of this cumulative thesis.

1. **Pavelka L**, Rauschenberger A, Landoulsi Z, Pachchek S, May P, Glaab E, Krüger R; NCER-PD Consortium. Age at onset as stratifier in idiopathic Parkinson's disease - effect of ageing and polygenic risk score on clinical phenotypes. NPJ Parkinsons Dis. 2022 Aug 9;8(1):102. doi: 10.1038/s41531-022-00342-7. Erratum in: NPJ Parkinsons Dis. 2022 Sep 2;8(1):112.

L.P.: Conceived, organised, and executed the research project; co-executed the statistical analysis and interpretation of results; wrote the manuscript; substantially participated in data collection, data exportation and data curation. R.K.: Conceived, organised, and co-executed the research project; participated in interpretation of results; critically revised the manuscript. A.R.: Executed the statistical analysis and interpretation, critically revised the manuscript, substantially participated in data curation. E.G.: Co-executed the research project; participated in interpretation of results; critically revised the manuscript. P.M.: Executed the genetic analysis; participated in interpretation of results; critically revised the manuscript. S.P.: Contributed genetic data; co-executed the research project; critically revised the manuscript. Z.L.: Executed the genetic analysis; participated in interpretation of results; critically revised the manuscript.

2. **Pavelka L**, Rauschenberger A, Landoulsi Z, Pachchek S, Marques T, Gomes CPC, Glaab E, May P, Krüger R; NCER-PD Consortium. Body-First Subtype of Parkinson's Disease with Probable REM-Sleep Behavior Disorder Is Associated with Non-Motor Dominant Phenotype. J Parkinsons Dis. 2022;12(8):2561-2573. doi: 10.3233/JPD-223511.

LP: Conceived, organized, and executed the research project; co-executed the statistical analysis and interpretation of results; wrote the manuscript; substantially participated in data collection, data exportation and data curation.
RK: Conceived, organized, and co-executed the research project; participated in interpretation of results; critically revised the manuscript.
AR: Executed the statistical analysis and interpretation, critically revised the manuscript, substantially participated in data curation.
EG: Co-executed the research project; participated in interpretation of results; critically revised the manuscript.
PM: Executed the genetic analysis; participated in interpretation of results; critically revised the manuscript.
SP: Contributed genetic data; co-executed the research project; critically revised the manuscript.
ZL: Executed the genetic analysis; participated in interpretation of results; critically revised the manuscript.
TM: Participated in interpretation of results; critically revised the manuscript.
GCPC: Participated in interpretation of results; critically revised the manuscript.