Cerebral small vessel disease may not critically influence familial Parkinson's disease

Bigyan Marhat
Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt- Universität zu Berlin, Berlin Institute of Health
Malla Bimala
Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt- Universität zu Berlin, Berlin Institute of Health
Marco Foddis
Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health
Manuel Holtgrewe
Berlin Institute of Health, BIH
Dieter Beule
Berlin Institute of Health, BIH
Jose Bras
Michigan State University College of Human Medicine
Rita Guerreiro
Michigan State University College of Human Medicine
Vasilis Kola
Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health
Hans-Michael Schmitt
Werner Forsmann Hospital
Matthias Endres
Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health
Celeste Sassi
celeste.sassi@charite.de

Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health

Article

Keywords: Parkinson's disease (PD), vascular parkinsonism (VP), Parkinson Mendelian genes, cerebral small vessel disease (cSVD), white matter hyperintensities (WMH)

Posted Date: June 26th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4518069/v1

Additional Declarations: No competing interests reported.

Abstract

Familial Parkinson's disease (PD) and vascular parkinsonism (VP) overlap in their clinical, neuroradiologic and neuropathologic features. To investigate whether PD and VP may share a pathogenic link, we used the modified Scheltens scale and assessed the classic neuroradiological features of cerebral small vessel disease in the axial T2 MRI flair sequences in a cohort of 58 familial PD patients, 46 familial PD prodromal patients and 48 age-matched controls from the PPMI publicly available database. We next examined the protein coding variability in the main PD-causing genes and genetic risk factors in a cohort of 96 patients with familial cerebral small vessel disease (cSVD) and 243 elderly healthy individuals from the HEX database. Patients with familial and prodromal PD have a moderate but still significant burden of superficial white matter hyperintensities compared to age-matched controls (Wilcox Test p-value = 4.335e-07, OR = 4.1, 95% CI = 1.8–9.23), with moderate motor impairment and minimal and non-pathological cognitive decline (UPDRS and MoCa up to 25 and 26,respectively). In contrast, 100% of patients carrying *SNCA* p.A53T and 25% of patients carrying *LRRK2* p.G2019S, p.R1441C or *GBA* p.N409S, p.E365K and p.L483P had moderate to very severe dementia (average MoCa Score = 21) and mild motor impairment (mean UPDRS III score = 20) and only very modest white matter lesions. Finally, we report no known pathogenic coding variant in the PD genes studied in cSVD patients. Our study shows that familial PD and small vessel disease likely have distinct not necessarily mutually exclusive, pathogenic mechanisms.

INTRODUCTION

Familial Parkinson's disease (PD) and vascular parkinsonism (VP) present overlapping clinical (rigidity and bradykinesia, cognitive impairment to severe dementia) and to a different extent neuroradiological (white matter hyperintensities, PET-Scan decreased fluorodopa uptake in the basal ganglia) and neuropathological (depigmentation of the substantia nigra, cortical atrophy) features. Both conditions may co-exist particularly in elderly patients and are frequently misdiagnosed [1–2] (**Table S1**).

Vascular Parkinsonism is often caused by sporadic, severe, diffuse progressive cerebral small vessel disease (cSVD). Other less common causes of VP include mendelian cSVDs, such as Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL), and retinal vasculopathy with cerebral leukodystrophy (RVCL). More rarely, mendelian leukodystrophies such as hereditary diffuse leukoencephalopathy with spheroids (HDLS) leading to a reduction of thalamo-cortical drive can also cause VP [3,2] (https://www.omim.org/) (**Table S2**).

However, despite the comprehensive body of literature describing white matter macro- and microstructural changes in PD patients and correlating these to the progression of diverse clinical PD phenotypes, [4–5], a potential common pathogenic ground between familial PD and cSVD has not been extensively and systematically investigated.

Therefore, we used a modified Scheltens scale and screened the classical cSVD neuroradiological biomarkers (periventricular hyperintensities, lobar superficial white matter hyperintensities, lobar deep white matter hyperintensities, status cribrosus, lobar cortical small-microinfarcts, lacunar Infarcts) [6–7], **Table S3**) in T2 MRI Flair sequences of 58 familial PD and 46 familial PD prodromal patients and 48 age-matched controls from the PPMI publicly available database (www.ppmi-info.org/data) (Table 1, **Table S4).** Next, we performed exome sequencing on a cohort of 96 familial cSVD Caucasian patients from the US to investigate in this cohort protein coding variability in the most common PD mendelian genes (*VPS35*, *DJ1*, *PINK1*, *ATP13A2*, *PRKN*, *SNCA*, *LRRK2*) and risk factors (*LRRK2, GBA*, *MAPT*, *LAMP3*, *STK39*) (https://www.omim.org/) (Fig. 1).

Table 1

PPMI MRI cohort used in this study. Pheno., phenotype; PD, Parkinson's disease; Prod, prodromal; CTRLS, controls; N., number; pat, patients; AAO, age-at deviation; F, female; N, number; fam, familial; Hypert., hypertension; DMT 2, diabetes mellitus type 2; Hyperlipid, hyperlipidemia; AF, atrial fibrillation; CAD, co PFO, pervium foramen ovale; Hypercoag., hypercoagulability; * Data available for 33/104 (32%) Parkinson's disease and prodromal patier

									Cardiovascular risk factors (%)*						
Pheno.	**N. pat/ CTRLS**	**AAO (SD)**	**F (%)**	**N. fam. Cases (%)**	**LRRK2 (%)**	**GBA (%)**	**SNCA (%)**	***APOE* ε2 (%)**	**Hypert. (%)**	**DMT 2 (%)**	**Hyperlipid (%)**	**AF (%)**	**CAD (%)**	**Valve prolapse/ regurgitation**	**P**
PD	58	60 (32–82)	22 (38)	58 (100%)	42/104 (40)	30/104 (29)	2/104 (2)		8/14 (57)	4/14 (28)	7/14	4/14	1/14	0/14	1
Prod. patients	46	63 (34–77)	27 (59)	46 (100%)				16/104 (15%)	11/33 (61)	4/18 (22)	14/18	1/18	1/18	0/18	0
CTRLS	48	61 (32–81)	17 (35)	0				4/48 (8%)	4/18 (22)	2/18 (11)	6/18	1/18	0/18	3/18	0

MATERIAL AND METHODS

MRI Study

MRI Study cohort

Fifty-eight familial idiopathic Parkisnon's disease patients (females = 22, mean age-at onset 60 years [range: 32–82]), 46 familial prodromal PD patients (females = 27, mean age-at of onset 63 years [range: 34–77]) and 48 age-matched healthy controls (females = 17, mean age-at onset 61 years [range: 32–81]) were obtained from the Parkinson Progression Marker Initiative (PPMI) database (Table 1, **Table S4**). The PPMI study is well-described at ppmi-info.org. Briefly, PPMI is a comprehensive multi-center study designed to identify biomarkers of PD with the goal of improving evaluation of disease modifying therapeutics [8]. Healthy control subjects included in PPMI were above 30 years of age, had never been diagnosed with any major neurological disorder, had no first-degree relatives with idiopathic PD, and were not cognitively impaired based on a score of 26 or above on the Montreal Cognitive Assessment (MoCa). Familial PD and familial prodromal PD patients were defined as having at least one first-degree relative with idiopathic PD.

Of the 104 PD and prodromal patients, 42 (40%) carried pathogenic *LRRK2* mutations, with 40/42 (95%) being heterozygous carriers for *LRRK2* p.G2019S, the most common risk factor for late-onset sporadic PD [9]. Thirty of the 104 patients (29%) carried pathogenic mutations in *GBA* in heterozygosity. A minority of patients 2/104 (2%) presented an autosomal dominant form of PD and carried the *SNCA* pathogenic mutation p.A53T in heterozygosity.

Information regarding cardiovascular risk factors was available only for a minority of cases (at least 32/104 [31%]) and controls (18/48 [38%]).

Among these, hyperlipidaemia was the most common risk factor, detected in 66% of cases and 33% of controls. 40% of cases and 22% of controls displayed hypertension and 16% of cases and 5% of controls presented atrial fibrillation. 16% of controls and none of the cases had a valve prolapse or regurgitation and a minority of cases (5%) and none of the controls presented coronary artery disease, patent foramen ovale (PFO) or hypercoagulability (Table 1)

Among the clinical features of the cohort, the motor impairment was measured using the Unified Parkinson's disease rating scale (UPDRS) III for motor skills, PD patients presented an average score of 28 (ranging from 5 to 64), prodromal patients an average score of 5 (ranging from 0 to 33) and the controls an average score of 4 (ranging from 0 to 4). Cognitive skills were measured using the MOCA Test and PD patients presented an average score of 26 (ranging from 9 to 30), the prodromal patients an average score of 28 (ranging from 20 to 30) and the controls an average score of 27 (ranging from 21 to 30).

For the PPMI cohort, the ethical approvals and commettees have been already described (https://www.ppmi-info.org/sites/default/files/docs/archives/Amendment-12.pdf). Informed consent was obtained. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

MRI T2 Flair

All the MRI scans were separately rated twice by two experienced neurologists (H-M.S and V.K) and by two trained experienced residents in neurology (B.M and C.S). All raters were experienced in grading white matter abnormalities on MRI. Each scan was rated using the modified Scheltens scale [10], representing a complement of the classical Fazekas scale, with the possibility of describing lobar distribution and rating with a score cortical and subcortical white matter hyperintensities (**Table S3**). Briefly, Scheltens and colleagues described 4 classes of white matter hyperintensities: periventricular hyperintensities, white matter hyperintensities, basal ganglia hyperintensities and infra-tentorial foci of hyperintensities.

We implemented this scale selecting 6 neuroradiological hallmarks for cerebral small vessel disease, based on the previous literature: periventricular hyperintensities [6], lobar superficial white matter hyperintensities, [11], lobar deep white matter hyperintensities [6], status cribrosus [12], lacunar infarcts [7] and lobar cortical small-microinfarcts [13] (**Table S3**, Fig. 1). Moreover, ischemic lesions such as cortical small-microinfarcts and lobar lacunes are a common feature of cerebral amyloid angiopathy (CAA) [14,13] and were considered as CAA hallmark (**Figure S2**).

Our rating scale provides 7 sum scores in a semiquantitative way, as explained in **Table S3**: periventricular hyperintensities (PVH), lobar superficial white matter hyperintensities (LSWMH), lobar deep white matter hyperintensities (LDWMH), deep white matter hyperintensities, status cribrosus, lobar cortical small-microinfarcts, lacunar infarcts. PVH were identified as continuous, confluent areas of high signal intensity adjacent to anterior or posterior horns of the lateral ventricles ("caps") and along the lateral ventricles ("bands"). LDWMH, located in the deep and subcortical white matter, were separately rated in the frontal, temporal, parietal and occipital regions; superficial white matter hyperintensities were defined as tracts originating within 5 mm of the cortical surface, as previously described [15]; status cribrosus describes the diffusely widened perivascular spaces (Virchow-Robin spaces) in the basal ganglia, especially in the corpus striatum on MRI [16,17]; cortical small and microinfarcts were defined as cortical hypointense lesions 15 mm and $\leq$ 4 mm in largest diameter respectively and distinct from perivascular spaces [13]; lacunar Infarcts were defined as lesions from 3 mm to < 15 mm in the supratentorial region, without cortical gray matter involvement [18].

Manual segmentation on T2-MRI Flair images was conducted using the publicly available IMAIOS Atlas (https://www.imaios.com/en/e-Anatomy/Brain/Brain-MRI-in-axial-slices).

Genetic Study

Patient Cohort

All the patients included in the genetic study were Caucasian non-Hispanic from the US (NINDS [National Institute of Neurological Disorders and Stroke]). DNA was extracted and collected at the NINDS Repository. All NINDS Repository Samples are collected only after an IRB-approved, signed informed consent is secured by the submitter.

Inclusion criteria comprised cerebral small vessel ischemic disease diagnosis based on TOAST classification, early age at onset (< 65 years [only 2 cases, whose age-at onset was 68 and 71 years old have been included in the study because of a positive family history]), absence of known pathogenic mutations in Mendelian small vessel disease genes (*HTRA1*, *NOTCH3*, *ACTA2* and *COL4A1*) and no enrichment for vascular risk factors except for hypertension, which generally plays a critical role in elderly people [19]. The mean age at disease onset was 51.5 years (range 34–71 years). 82.3% of the cases were male and 44.8% of the cases were positive for a familial history of cerebrovascular disorders. Among the comorbidities and possible risk factors for cSVID, hypertension was reported in 60.4% of the patients, diabetes type 2 in 30.2%, and myocardial infarction in 7.3%. The majority of the patients (at least 88.54%) were negative for atrial fibrillation (AF), which is among the most important risk factors for embolic small vessel occlusion [20]. In 4.1% and 7.3% of the patients the presence of AF was reported and unknown, respectively. Given the prevalent role of hypertension and type 2 diabetes in cSVID in the elderly people [19] and the young age at onset of the cohort, these patients were considered enriched for genetic risk factors (**Table S5**). Finally, 243 controls > 80 years of age were selected from 'HEALTHY EXOMES', HEX, a publicly available database, which collects exome sequencing data from elderly neuropathologically proven controls (https://www.alzforum.org/exomes/hex; [22]).

All methods were performed in accordance with the relevant guidelines and regulations.

The experimental protocols were approved by the PPI licensing committee (https://www.ppmi-info.org/sites/default/files/docs/archives/Amendment-12.pdf).

RESULTS

cSVID neuroimaging hallmarks in familial PD and control patients

To investigate the hypothesis that familial PD and cSVD may have shared pathogenic mechanisms we used a modified Scheltens Scale (**Table S3**) and screened the main 7 cSVD neuroradiological hallmarks (periventricular hyperintensities, lobar superficial white matter hyperintensities, lobar deep white matter hyperintensities, status cribrosus, lobar cortical small-microinfarcts, lacunar Infarcts) [6–7] in the MRI T2 Flair sequences in a cohort of 58 familial PD and 46 familial prodromal PD patients and 48 age-matched controls from the PPMI database (Fig. 1).

Cumulative cSVD neuroradiological biomarker analysis

When we considered the cSVD neuroradiological biomarker cumulative score, we did not identify any statistically significant difference between familial PD patients, familial prodromal PD patients and controls (p-value = 0.53, 95% CI -1.95-1.02). We report a linear and age-dependent increase of the cSVD neuroradiological hallmark cumulative score, particularly driven by periventricular and deep white matter hyperintensities both for familial PD and familial prodromal PD patients as well as for controls and more marked in the frontal lobe, representing 45% of the hemisphere [13] (Fig. 2A-C, **Table S6**)

The white matter hyperintensity cumulative score (WMHCS) was associated to a parallel and directly proportional and not statistically significant increase in UPDRS score (from 14 to 25, corresponding to a 10% increase of the total UPDRS III score and to a mild increase of the motoric impairment), as already reported in PD patients [23], and a to a very modest and not statistically significant decrease of MoCa score (from 28 to 26, corresponding to a 6% decrease of the total MoCa score, with no indications of cognitive impairment) (Fig. 4A)

Single cSVD neuroradiological biomarker analysis

Analyzing individual neuroradiological cSVD biomarkers, we found a statistically significant increased burden of superficial white matter hyperintensities in familial PD-prodromal PD patients (81/104, 77%) compared to age-matched controls (22/48, 45.8%) (Fig. 3A-B) (p-value = 0.0001538, Fisher Test and, Wilcox Test p-value = 4.335e-07, OR = 4.1, 95% CI = 1.8–9.23)

19% and 6.5% of familial PD and familial prodromal PD patients, respectively, presented several (> 6) superficial white matter hyperintensities and more than 10% of the patients presented lesions in multiple lobes, affecting in > 60% of these cases the frontal lobe (Fig. 3A-C).

Superficial white matter lesions clustered mostly in the superior frontal and inferior frontal gyrus, harboring the Broadmann areas 6 and 8 (Fig. 3C).

Analogously to the WMHCS, the increase of superficial white matter hyperintensity cumulative score (WMHCS) was paralleled by a mild and not statistically significant increase of the UPDRS score (from 14 to 25), as already reported in PD [22] and a very modest and not statistically significant decrease of MoCa score with no indications of cognitive impairment (from 28 to 26) (Fig. 4B)

Influence of white matter hyperintensities on PD outcome

Multiple bilateral lacunar infarcts in the basal ganglia, capsula interna and externa were detected both in elderly cases (familial PD patients [14/58, 24%] and prodromal patients [9/46, 19%]) and controls (14/48, 29%) without any statistically significant difference (Fisher Test, p-value = 0.41, 95% CI =-0.29-1.63). However, familial PD patients presented a burden of lacunar strokes particularly in the thalamus (relative frequency 9% in familial PD cases and 2% in age-matched controls), which were associated with typical basal ganglia symptoms such as moderate akinesia and rigidity (UPDRS average score 3 [Finger tapping] and 4 [rigidity of extremities], respectively) [24] (Fig. 3D-E).

We did not identify any lacunar infarct in the pons or mesencephalon.

Influence of PD causative and main genetic risk factors (SNCA, LRRK2 and GBA) and small vessel disease neuroradiological biomarkers on PD motor and cognitive outcome

Patients carrying pathogenic mutations in *SNCA* (p.A53T, 2/2 carriers) or significant risk factors in *GBA* (p.N409S, p.E365K and p.L483P, 7/29 [24%] carriers) and *LRRK2* (p. G2019S and p.R1441C, 7/41 [17%] carriers) displayed the lowest MoCa scores depicting a mild to a very severe dementia (from 25 to 9) characterized both by executive and visuospatial function and memory impairment (Trail Making Test, Cube Copy, or clock drawing). Importantly, we report for all these variants only a very modest WMHCS (average 1 [*GBA* p.L483P] to 14 [*LRRK2* p.R1441C]) (Fig. 4C). The phenotypical difference among carriers of *GBA* and *LRRK2* variants are likely due to a penetrance factor [25–26].

The carriers of *SNCA* p.A53T presented a very early onset (32y and 53y) and were characterized by very severe dementia (MoCa score 17 and 9, respectively) and marked motor impairment (UPDRS III 37 and 46, respectively) whereas the WMHCS was very mild (10 and 6, respectively). A rapidly progressive dementia was also associated to 6/33 (18%) of *LRRK2* p.G2019S carriers, and particularly in homozygous state associated to a statistically significant motor impairment (MoCa 18, UPDRS III 29). Analogously, *LRRK2* p.R1441C carriers displayed a mild dementia and severe motor impairment (MoCa 23 and UPDRS 33). Moreover, 7/22 (32%) carriers of *GBA* coding variants (p.N409S, p.E365K and p.L483P) presented a mild dementia (MoCa average 23.4) and only 4/29 (14%) *GBA* mutation carriers were characterized by very severe motor impairment (UPDRS 39) (Fig. 4C, **Table S4**).

Finally, *APOE ε2* allele has been associated to increased severity of small vessel disease [27,28] however, we have not detected a statistically significant difference between PD and prodromal PD patients and controls as in our cohort 16/104 (15%) of patients and 4/48 (8%) of controls carried this allele in heterozygosity (Fisher Test p-value = 0.3059, 95% CI = 0.59–8.67, OR 1.9)(Table 1).

PD Mendelian genes and main GWAS loci genetic screening in familial SVID patients

To further investigate the hypothesis that PD Mendelian genes and main common risk factors are associated with cSVD, we screened protein coding variability in 11 PD causative genes (*VPS35*, *DJ1*, *PINK1*, *ATP13A2*, *PRKN*, *SNCA*, *LRRK2*) and genetic risk factors (*LRRK2, GBA*, *MAPT*, *LAMP3*, *STK39*) in 96 early-onset unrelated cerebral small-vessel disease cases and 243 elderly controls neuropathologically proven, from the UK (**Table S5**). We did not identify any known pathogenic variant in the studied genes in our cSVD cohort (**Table S7**).

None of the detected variants in heterozygosity has been reported as pathogenic or likely pathogenic in ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar).

Six variants (*ATP13A2* p.Q377R and p.G372R, *PINK1* p.R326C, *LRRK2* p.D41Y, p.M167L and p.P1661T) are not present in the ClinVar database.

PRKN p.G430D was found in heterozygosity in a male patient with very early-onset (41y) familial cSVD with a positive familial history (grand-mother affected) and hypertension treated with medications. Importantly, *PRKN* p.G430D in homozygosity causes autosomal recessive juvenile Parkinson whereas in heterozygosity it has not been associated to increased risk for PD [29].

Overall elderly controls displayed an higher relative frequency of variants in the studied genes (**Figure S1**). The only exception was in *SNCA*, presenting a relative frequency of 1% (1/96) and 0.4% (1/243) in cSVD patients and elderly controls, respectively.

Moreover, 10/96 (10%) early-onset cSVD cases and 82/243 (34%) elderly controls carried at least 1 rare coding variant in the studied genes. All the variants detected in the cSVD cohort were singletons. None of the cSVD patients carried any variant in homozygosity or compound heterozygosity. Moreover, in this cSVD cases we have not identified any rare coding variants in *GBA*, *LAMP3*, *MAPT*, *DJ1*, *STK39* and *VPS35* and we detected a total of 9 rare coding variants in *ATP13A2*, *PINK1, SNCA*, *PRKN* and *LRRK2*, absent in controls (**Table S7** and **Table S8**)

ATP13A2 and *PRKN*, *PINK1* and *SNCA* harbor the lowest and highest relative frequency of low-frequency and rare coding variants (mean 0.5 and 0.3 low-frequency-rare variants per kb of coding sequence) respectively. 90% of the variants were described as probably-damaging or possibly-damaging by *in-silico* prediction software (PolyPhen2).

Additionally, *LRRK2* p.G2019S, the most common cause of familial PD (5–6%) and a risk factor for sporadic PD (1%) was detected with a frequency of 4e-4 in the elderly controls and none of the cSVD familial cases [30] (**Table S8** and **Table S7**).

DISCUSSION

Using neuroradiology and genetics, we explored the hypothesis that familial PD, VP, and its hallmark, cSVD, which exhibit varying degrees of overlapping phenotype, may have been linked by a shared pathophysiology. To this end, we selected a cohort of 104 familial PD and familial PD prodromal patients from the PPMI database and used a modified Scheltens Scale to screen MRI T2 Flair sequences for the main cSVD neuroradiological biomarkers (ie, periventricular hyperintensities, lobar superficial white matter hyperintensities, lobar deep white matter hyperintensities, status cribrosus, lobar cortical small-microinfarcts, lacunar Infarcts) (Fig. 1).

Familial PD and prodromal PD patients presented a statistically significant enrichment for superficial white matter hyperintensities particularly in the frontal superior and inferior gyrus and to a lesser extent in the parietal lobe, mainly corresponding to the Brodmann area 8 and to the premotor cortex (Brodmann area 6) (Fig. 3A-C).

Importantly, superficial white matter represents a very vulnerable area, located beneath the infragranular layer of the cerebral cortex, containing the last fibers to myelinate and extensive cortico-cortical connections [15] and has been already associated to ischemic and hemorragic damage during Covid-19 infection and cititoxic lesions that are due deposition of A plaques, and may trigger epileptic seizures in patients with small vessel disease [11,31–32].

Although statistically significant, this white matter hyperintensity burden did not present a clinical correlate and was not associated with a parallel statistically significant worsening of the cognitive or motoric function and may likely be interpreted as a global neurodegenerative process. In line with this hypothesis and in concert with our findings, a growing body of evidence described microstructural damages in the frontal cortex patients involving Broadmann area 6 and 8 (premotor cortex, supplementary motor area, the presupplementary motor area) in the early stages of PD [33,34].

Accordingly, a burden of superficial white matter hyperintensity has been associated to different neurodegenerative disorders such as AD [35]

In addition, we report an enrichment for lacunar infarcts in the basal ganglia and especially in thalamus in the elderly familial PD patients, corresponding to a mild although not statistically significant increase of the UPDRS scores.

Importantly, white matter hyperintensities in the frontal and parietal lobe and lacunar infarcts in the basal ganglia and particularly in the thalamus have been linked to an increased risk for mild parkinsonism, likely driven by the interruption of the basal ganglia–thalamofrontal cortical circuits leading to a reduction in the thalamocortical drive [3].

Thus suggesting that the statistically significant enrichment for superficial white matter hyperintensities particularly clustering in the frontal lobe and lacunar infarcts in the thalamus may shape familial PD endophenotypes mostly influencing the progression of classical basal ganglia symptoms such as akinesia and rigidity.

By contrast, mutations in PD genes were associated to a severe and rapidly progressive dementia and moderate worsening of the motoric function.

We next tested the hypothesis that the main PD genetic causative and risk factors may have explained the enrichment for cSVD neuroradiological biomarkers such as superficial white matter hyperintensities and lacunar infarcts in familial PD and prodromal patients, screening protein coding variability in *VPS35*, *DJ1*, *PINK1*, *ATP13A2*, *PRKN*, *SNCA*, *GBA*, *MAPT*, *LAMP3*, *STK39* in a cohort of familial 96 small vessel disease patients. We did not report any pathogenic variant in the studied genes in this cohort, arguing for a non-critical role of the main PD genes for the development and progression of cSVD.

The burden of superficial white matter as well as white matter cumulative score were not associated to a parallel clinical motoric and cognitive worsening and, on the contrary, Mendelian mutations corresponded to a severe dementia and to a lesser extent motoric impairment, suggesting that white matter degeneration may shape the familial PD phenotype and likely influence the variant penetrance but do not play a critical role for the progression of PD. Moreover, PD Mendelian mutations were associated to modest increase of white matter hyperintensity cumulative scores implying that these genes are not likely to influence white matter burden.

On the other hand, cSVD may facilitate the penetrance of some pathogenic mutations such as *LRRK2* p.G2019S [30] and prime the α-synuclein protein misfolding and lead to Lewy body formation, as already described for other neurodegenerative disorders such Alzheimer's disease [36].

We conclude that small vessel disease may not crucially influence familial PD severity and progression and are not critically linked to PD genetic main causative and risk factors.

Our findings should foster a validation in a bigger cohort of familial PD and cSVD patients.

Declarations

Acknowledgements

Prof. Ulrich Dirnagl from the Center for Stroke Research Berlin (CSB), Charité , Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität and Berlin Institute of Health, for the supervision, Dr. Zocholl Dario from Institute of Biometry and Clinical Epidemiology, Berlin, who provided the statistical advice.

Data used in the preparation of this article were obtained [22nd December 2021] from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/access-data-specimens/download-data), RRID:SCR_006431. For up-to-date information on the study, visit www.ppmi-info.org. Funding: PPMI – a public-private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including 4D Pharma, Abbvie, AcureX, Allergan, Amathus Therapeutics, Aligning Science Across Parkinson's, AskBio, Avid Radiopharmaceuticals, BIAL, Biogen, Biohaven, BioLegend, BlueRock Therapeutics, Bristol-Myers Squibb, Calico Labs, Celgene, Cerevel Therapeutics, Coave Therapeutics, DaCapo Brainscience, Denali, Edmond J. Safra Foundation, Eli Lilly, Gain Therapeutics, GE HealthCare, Genentech, GSK, Golub Capital, Handl Therapeutics, Insitro, Janssen Neuroscience, Lundbeck, Merck, Meso Scale Discovery, Mission Therapeutics, Neurocrine Biosciences, Pfizer, Piramal, Prevail Therapeutics, Roche, Sanofi, Servier, Sun Pharma Advanced Research Company, Takeda, Teva, UCB, Vanqua Bio, Verily, Voyager Therapeutics, the Weston Family Foundation and Yumanity Therapeutics."

DNA panels from the NINDS Repository were used in this study, as well as clinical data. Data used in the preparation of this article were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). For up-to-date information on the study, visit www.ppmi-info.org. PPMI, a public private partnership, was funded by the Michael J. Fox Foundation (MJFF) for Parkinson's Research and funding partners, including Abbvie, Avid Radiopharmaceuticals, Biogen, Britsol-Myers Squibb, Covance, GE Healthcare, Genetech, GlaxoSmithKline, Lilly, Lundbeck, Merck, Meso Scale Discovery,Pfizer, Piramal, Roche, Servier, and UCB. Neither the funding agency nor any of the sponsors of the PPMI were involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

DISCLOSURES

FUNDING SOURCES AND CONFLICT OF INTEREST

This study was supported by NeuroCure, Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Alexander von Humboldt Fellowship (to Celeste Sassi).

All the authors declare that there are no conflicts of interest relevant to this work.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

References

1. Kalra, S., Grosset, D. G. & Benamer, H. T. S. Differentiating vascular parkinsonism from idiopathic Parkinson's disease: a systematic review. Mov Disord 25, 149–156 (2010).
2. Peralta, C. *et al.* Parkinsonism following striatal infarcts: incidence in a prospective stroke unit cohort. J Neural Transm (Vienna) 111, 1473–1483 (2004).
3. de Laat, K. F. *et al.* Cerebral white matter lesions and lacunar infarcts contribute to the presence of mild parkinsonian signs. Stroke 43, 2574–2579 (2012).
4. Bohnen, N. I. & Albin, R. L. White matter lesions in Parkinson disease. Nat Rev Neurol 7, 229–236 (2011).
5. Gattellaro, G. *et al.* White matter involvement in idiopathic Parkinson disease: a diffusion tensor imaging study. AJNR Am J Neuroradiol 30, 1222–1226 (2009).
6. Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I. & Zimmerman, R. A. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am J Roentgenol 149, 351–356 (1987).
7. Wardlaw, J. M., Smith, C. & Dichgans, M. Small vessel disease: mechanisms and clinical implications. The Lancet Neurology 18, 684–696 (2019).
8. Parkinson Progression Marker Initiative. The Parkinson Progression Marker Initiative (PPMI). Prog Neurobiol 95, 629–635 (2011).
9. Paisán-Ruiz, C., Lewis, P. A. & Singleton, A. B. LRRK2: Cause, Risk, and Mechanism. J Parkinsons Dis 3, 85–103 (2013).
10. Scheltens, P. *et al.* A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. J Neurol Sci 114, 7–12 (1993).
11. Wang, S. *et al.* Superficial white matter microstructure affects processing speed in cerebral small vessel disease. 2021.12.30.474604 Preprint at https://doi.org/10.1101/2021.12.30.474604 (2022).
12. Ferrer, I., Bella, R., Serrano, M. T., Martí, E. & Guionnet, N. Arteriolosclerotic leucoencephalopathy in the elderly and its relation to white matter lesions in Binswanger's disease, multi-infarct encephalopathy and Alzheimer's disease. J Neurol Sci 98, 37–50 (1990).
13. Xiong, L. *et al.* Cerebral Cortical Microinfarcts on Magnetic Resonance Imaging and Their Association With Cognition in Cerebral Amyloid Angiopathy. Stroke 49, 2330–2336 (2018).
14. Reijmer, Y. D., van Veluw, S. J. & Greenberg, S. M. Ischemic brain injury in cerebral amyloid angiopathy. J Cereb Blood Flow Metab 36, 40–54 (2016).
15. Reginold, W. *et al.* Altered Superficial White Matter on Tractography MRI in Alzheimer's Disease. Dement Geriatr Cogn Dis Extra 6, 233–241 (2016).
16. de Reuck, J., Sieben, G., de Coster, W. & vander Ecken, H. Parkinsonism in patients with cerebral infarcts. Clin Neurol Neurosurg 82, 177–185 (1980).
17. Poirier, J. & Derouesné, C. [The concept of cerebral lacunae from 1838 to the present]. Rev Neurol (Paris) 141, 3–17 (1985).
18. Riba-Llena, I. *et al.* Small cortical infarcts: prevalence, determinants, and cognitive correlates in the general population. Int J Stroke 10 Suppl A100, 18–24 (2015).
19. Abraham, H. M. A. *et al.* Cardiovascular risk factors and small vessel disease of the brain: Blood pressure, white matter lesions, and functional decline in older persons. J. Cereb. Blood Flow Metab. 36, 132–142 (2016).
20. de Leeuw, F. E. *et al.* Atrial fibrillation and the risk of cerebral white matter lesions. Neurology 54, 1795–1801 (2000).
21. Guerreiro, R. *et al.* A comprehensive assessment of benign genetic variability for neurodegenerative disorders. bioRxiv 270686 (2018) doi:10.1101/270686.
22. Jeong, S. H. *et al.* White Matter Hyperintensities, Dopamine Loss, and Motor Deficits in De Novo Parkinson's Disease. Mov Disord 36, 1411–1419 (2021).
23. Shulman, L. M. *et al.* The clinically important difference on the unified Parkinson's disease rating scale. Arch Neurol 67, 64–70 (2010).
24. Lalvay, L. *et al.* Quantitative Measurement of Akinesia in Parkinson's Disease. Mov Disord Clin Pract 4, 316–322 (2017).
25. Blauwendraat, C. *et al.* Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. Brain 143, 234–248 (2020).
26. Lee, A. J. *et al.* Penetrance estimate of LRRK2 p.G2019S mutation in individuals of non-Ashkenazi Jewish ancestry. Mov Disord 32, 1432–1438 (2017).
27. Groot, C. *et al.* Clinical phenotype, atrophy, and small vessel disease in APOEε2 carriers with Alzheimer disease. Neurology 91, e1851–e1859 (2018).
28. Gesierich, B. *et al.* APOE 2 is associated with white matter hyperintensity volume in CADASIL. J. Cereb. Blood Flow Metab. 36, 199–203 (2016).
29. Kay, D. M. *et al.* Heterozygous parkin point mutations are as common in control subjects as in Parkinson's patients. Ann Neurol 61, 47–54 (2007).
30. Tan, M. M. X. *et al.* Genetic analysis of Mendelian mutations in a large UK population-based Parkinson's disease study. Brain 142, 2828–2844 (2019).

31. Kirschenbaum, D. *et al.* Intracerebral endotheliitis and microbleeds are neuropathological features of COVID-19. Neuropathol Appl Neurobiol 47, 454–459 (2021).
32. Stösser, S., Böckler, S., Ludolph, A. C., Kassubek, J. & Neugebauer, H. Juxtacortical lesions are associated with seizures in cerebral small vessel disease. J Neurol 266, 1230–1235 (2019).
33. Karagulle Kendi, A. T., Lehericy, S., Luciana, M., Ugurbil, K. & Tuite, P. Altered diffusion in the frontal lobe in Parkinson disease. AJNR Am J Neuroradiol 29, 501–505 (2008).
34. Yoshikawa, K., Nakata, Y., Yamada, K. & Nakagawa, M. Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI. J Neurol Neurosurg Psychiatry 75, 481–484 (2004).
35. Veale, T. *et al.* Loss and dispersion of superficial white matter in Alzheimer's disease: a diffusion MRI study. Brain Commun 3, fcab272 (2021).
36. Raz, L., Knoefel, J. & Bhaskar, K. The neuropathology and cerebrovascular mechanisms of dementia. J Cereb Blood Flow Metab 36, 172–186 (2016).

Figures

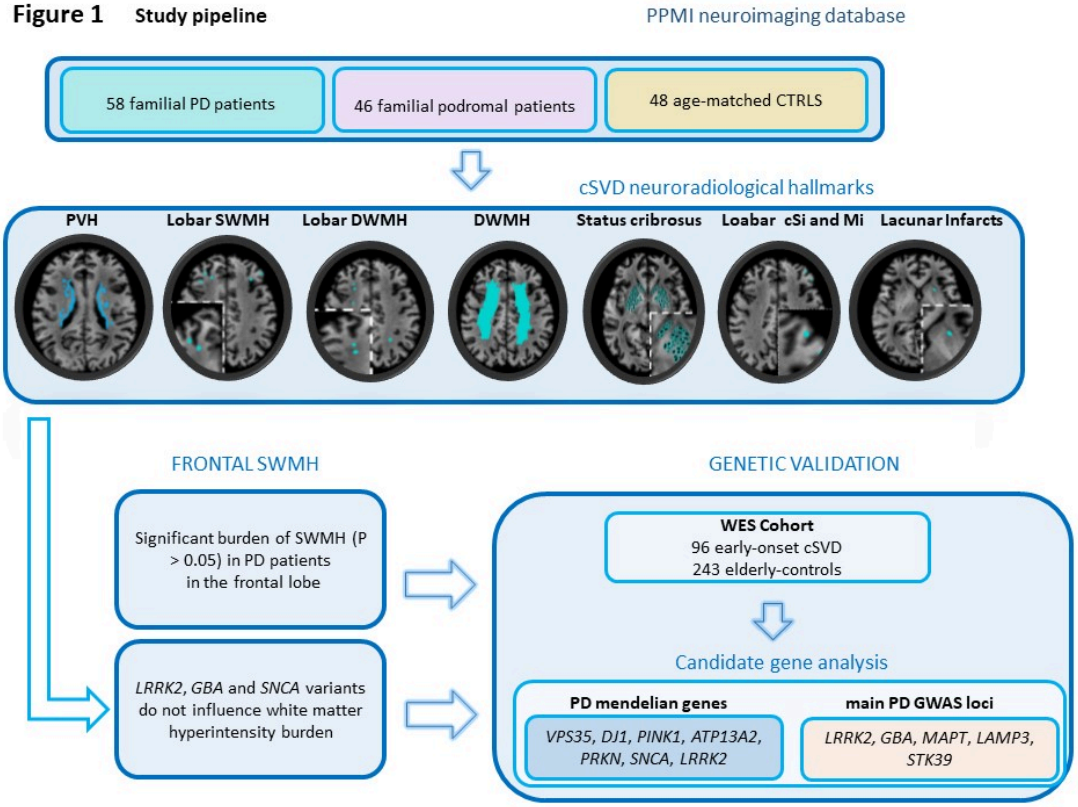

Figure 1

Study pipeline. T2 MRI Flair scans of a cohort of 152 individuals (58 familial PD patients, 46 familial prodromal PD patients and 48 age-matched controls) from the Parkinson's Progression Markers Initiative (PPMI) database, were screened for the main cerebral small vessel disease neuroradiological hallmarks: PVH, peri-ventricular hyperintensities; lobar SWMH, superficial white matter hyperintensities; lobar DWMH, deep white matter hyperintensities; status cribrosus; lobar cortical Si and Mi, small-and micro-infarcts and lacunar infarcts. Familial PD patients and familial PD prodromal patients displayed a statistically significant burden of cortical frontal superficial white matter hyperintensities (p-value = 0.0001538, Fisher Test and, Wilcox Test p-value= 4.335e-07, OR = 4.1, 95% CI = 1.8-9.23) compared to controls. To investigate the hypothesis that PD main genetic causative and risk factors may have played a critical role for the development of these SWMH and likely in cSVD, we performed exome sequencing on a cohort of 96 early-onset familial small vessel disease Caucasian from the US and in 243 elderly and neuropathologically proven controls from the publicly available HEX Database (https://www.alzforum.org/exomes/hex) and screened protein coding rare genetic variability in the main PD causative genes and GWAS loci.

Figure 2

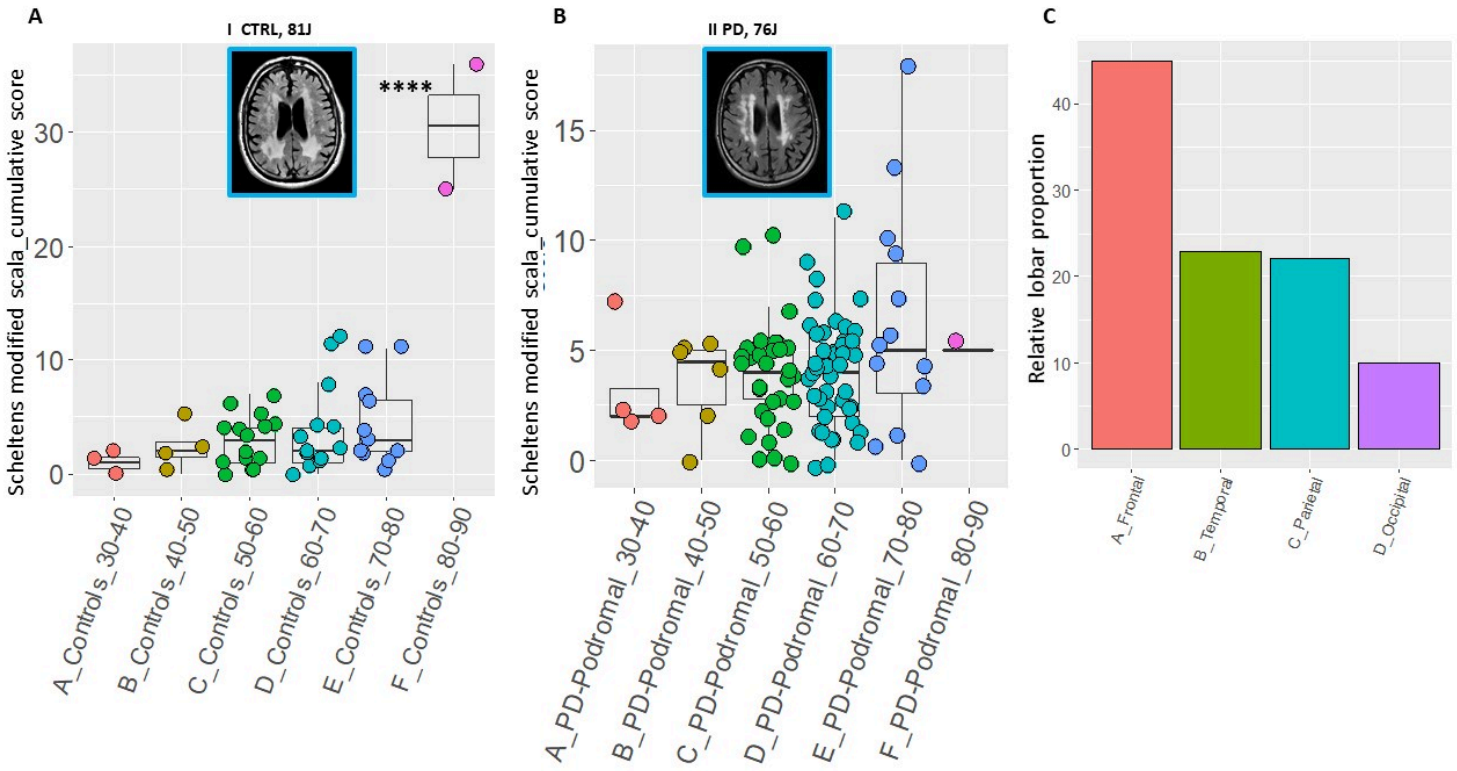

Figure 2

Modified cumulative Scheltens score obtained from the sum of the 7 cSVD neuroradiological hallmarks, showing a strongly linear and age-dependent increase, both for controls (**2A**) and PD and prodromal PD patients (**2B**) particularly driven by periventricular and deep white matter scores (**2AI** and **2BI**) and with a predilection for the frontal lobe, representing 45% of the whole lobar hemisphere (**2C**). PD, familial Parkinson's disease patients.

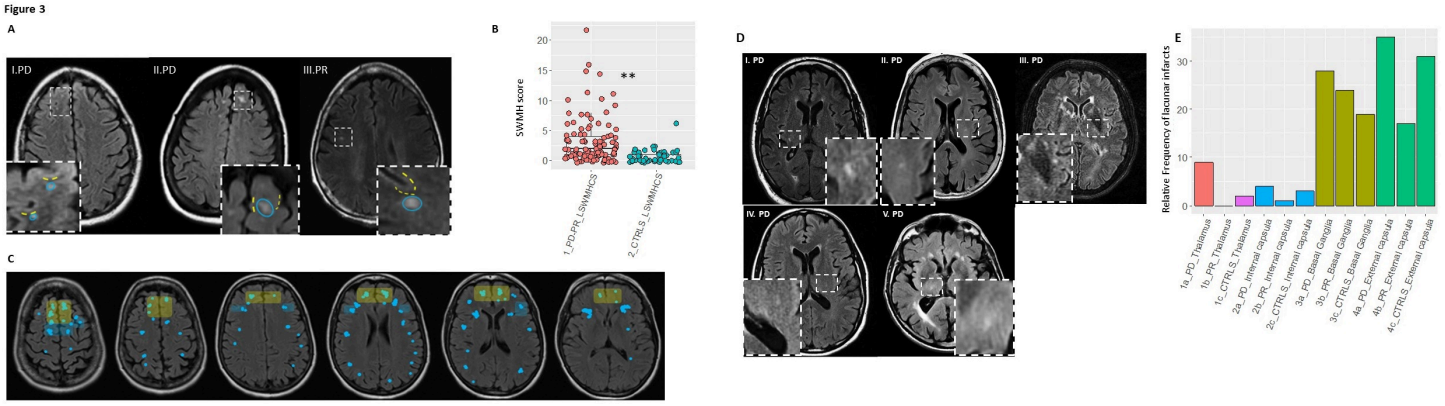

Figure 3

Superficial white matter hyperintensities in PD and prodromal PD patients particularly clustering in the superior frontal gyrus and framed within a dashed line (**3A I**, **II**, **III**). **3B** Familial PD patients and familial PD prodromal patients displayed a statistically significant burden of cortical frontal superficial white matter hyperintensities (p-value = 0.0001538, Fisher Test and, Wilcox Test p-value= 4.335e-07, OR = 4.1, 95% CI = 1.8-9.23). **3C** Collective representation of the superficial white matter hyperintensities in the 104 PD and prodromal PD patients analysed in the study, clustering in the frontal superior and to a lesser extent inferior gyrus, corresponding to the Brodmann area 8 (in yellow) and Brodmann area 6 (in light blue) . Each blue dot represents a single superficial white matter hyperintensity. PD, familial Parkinson's disease patients; PR, familial prodromal Parkinson's disease patients; CTRLS, controls; **, p-value > 0.00005. **3 D-E.** Lacunar infarcts detected in 58 familial PD patients, 46 familial PD prodromal patients and 48 age-matched controls. **3D I-V**, Thalamus infarcts detected in 5 late-onset familial PD patients, framed within a dashed line. **3E.** Relative frequency of different lacunar infarcts (thalamus, basal ganglia, capsula interna and capsula externa) detected in our cohort. PD, familial Parkinson's disease patients; PR, familial prodromal Parkinson's disease patients; CTRLS, controls.

Figure 4

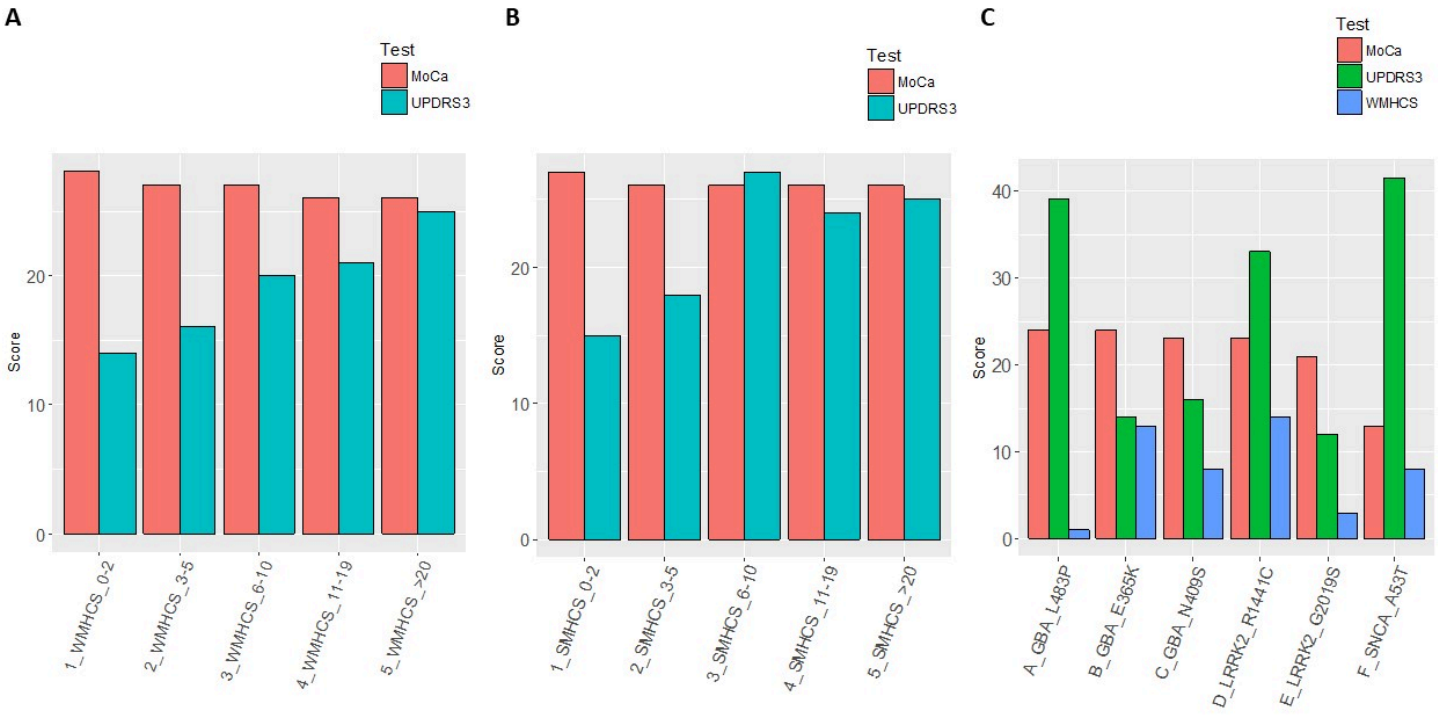

Figure 4

4A-C. Effect of white matter hyperintensities and *SNCA*, *GBA* and *LRRK2* mutations on Parkinson's disease outcome. **A-B** Bar plot showing an increasing white matter hyperintensity cumulative score (**A**) and superficial white matter cumulative score (**B**) and a parallel progressive mild decrease of MoCa scores and marked increase of UPDRS III scores. **C** Bar plot describing a mild to very severe dementia triggered by *GBA*, *LRRK2* and particularly *SNCA* mutations and a concomitant increase of UPDRS motoric scores, paralleled by only a modest increase of white matter cumulative scores. WMHCS, white matter hyperintensity cumulative score.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.jpg
- FigureS2.jpg
- SVDPDSupplementaryTables02.06.xlsx
- SuppelmentaryMaterialsandMethodsTables.05.06.pdf
- SupplementaryTableslegends.docx