

Supplemental Materials and Methods

MRI Acquisition

MRI images for healthy controls, familial PD and prodromal patients were acquired with the MP-RAGE axial T2 flair MRI sequence (and when available axial T1-weighted on Siemens MRI scanners). MRI sequence information can be found in the MRI technical operations manual (<http://www.ppmi-info.org/wpcontent/uploads/2017/06/PPMI-MRI-Operations-Manual-V7.pdf>). PPMI image acquisition guidelines required slice thickness of 1.5mm or less and no interslice gap, with repetition (TR) and echo (TE) time varying according to suggested settings at each site. PPMI images were obtained from the PPMI imaging database on 1/04/2017.

Clinical features assessment

For the assessment of the clinical features, we used the UPDRS scale for the motoric function and particularly for the basal ganglia symptoms such as akinesia and rigidity, the scores of the finger tapping and rigidity test, as previously described ¹. We used the MoCa test for the cognitive function with a particular focus on executive and visuospatial function (trail making test, cube copy, or clock drawing) and memory impairment (delayed recall), which are critical in differentiating diverse dementing syndromes such as dementia with Lewy bodies, mostly associated to PD, and Alzheimer's disease (AD), which frequently co-exists with small vessel disease ^{2 3 4 5}

Exome sequencing in patients

We performed whole exome sequencing on a cohort of 96 independent familial and early-onset apparently sporadic cSVID cases. DNA was extracted from blood using standard protocols. Library preparation for next generation sequencing used 50 ng of DNA. Exome libraries were prepared using Nextera® Rapid Capture Exome Kit (4 rxn × 12 plex, FC-140-1002). The DNA library was then hybridized to an exome capture library (Nextera, Illumina Inc.) and precipitated

using streptavidin-coated magnetic beads (Nextera, Illumina). Exome-enriched libraries were PCR-amplified, and then DNA hybridized to paired-end flow cells using a cBot (Illumina, Inc.) cluster generation system. Samples were sequenced on the Illumina HiSeq™ 3000/4000 using 2x76 paired end reads cycles.

Bioinformatics, exome sequencing

The reads were aligned using BWA-MEM v0.7.15 to the reference GRCh37 (hs37d5.fa), separate read groups were assigned for all reads from one lane, and duplicates were masked using Samblaster v0.1.24⁶. Standard QC was performed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). The variants were then called using GATK UnifiedGenotyper v3.7⁷ and annotated using Jannovar v0.24⁸ using RefSeq v105 exons. Variants were identified as pathogenic based on 1) the combined annotation dependent depletion (CADD), where we used a cut-off 'C-score' ≥ 12.37 , which includes around 2% of the most damaging nucleotide changes in the genome, accordingly to a previous study⁹. CADD combines predictions from numerous bioinformatics algorithms into a single 'C-score' and grade all possible nucleotide changes in the genome based on potential to damage gene/protein function. We used clinvar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) to assess the potential pathogenicity of single variants. Moreover, variants were re-interpreted based on the American College of Medical Genetics and Genomics (ACMG) criteria¹⁰

All rare missense variants within the coding regions of 11 Mendelian or genome-wide association studies (GWASs) PD candidate genes (*VPS35* [ENSG00000069329], *DJ1* [ENSG00000116288], *PINK1* [ENSG00000158828], *ATP13A2* [ENSG00000159363], *PRKN* [ENSG00000185345], *SNCA* [ENSG00000145335], *LRRK2* [ENSG00000188906], *GBA* [ENSG00000177628], *MAPT* [ENSG00000186868], *LAMP3* [ENSG00000078081], *STK39* [ENSG00000198648] have been collected and analysed.

Statistical analysis and methods to prevent bias

This is an exploratory, descriptive study. Sample sizes were not based on a priori power calculations. To compare our results we have used Wilcoxon-Test and Fisher's exact Test in R version 3.3.2.

For the exome sequencing cohort, power calculations were performed with R statmod-package [v1.4.32](#). The study had 80% power for the detection of common variants MAF >5% with strong effect (OR <0.6 or >2), with a significance value of two-sided $\alpha = 0.05$. The genetic study had at least 80% power for the detection of common variants, MAF >5%, with strong effect (OR <0.6 or >2), with a significance value of two-sided $\alpha = 0.05$. Low frequency and rare variants were defined as having a $1\% < \text{MAF} < 5\%$ and $\text{MAF} < 1\%$, respectively, either in cases or controls. Minor allele frequency was based either on HEX database for elderly controls >70 years of age or ExAC database version 0.3.1 database (<http://exac.broadinstitute.org/>).

Feature	Vascular Parkinsonism		Familial Parkinson's disease			References
	Chronic familial cSVID (22%)	Acute sporadic subacute cSVID (2%)	AR	AD	Risk Factor	
Genetics	<i>COL4A1, FOXC1, GLA, HTRA1, NOTCH3, TREX1, CST3, SCN1A, SLC20A2</i> mutation <i>EIF2B</i> -related disorder	Hypertension	<i>DJ1</i> and <i>PARK2</i>	<i>SNCA</i>	<i>LRRK2</i> G2019S	https://www.omim.org/
Age at onset	4–10 years later compared to patients with idiopathic Parkinson disease		Very early-onset mean age of onset was 27 years	Early-onset, mean 46.5 years, and rapid progression	Late-onset mean age of onset 65 years	https://www.omim.org/
Clinic	Cognitive: dementia, cognitive impairment	Cognitive: cognitive decline	Cognitive: cognitive decline	Cognitive: psychiatric features, cognitive decline	Cognitive: No cognitive decline was noted in any of the patients even after long disease duration	https://www.omim.org/
	Motor: (Bilateral) -rapid course, -akinesia, rigidity, -rarely bradykinesia of the upper limbs, almost absence of the resting tremor, postural tremor, -postural instability, falls -pyramidal signs, pseudobulbar palsy, incontinence	Motor Hemiparkinsonism/Parkinsonism	Motor: juvenile Parkinson disease retropulsion, dystonia of the feet, classic parkinsonism. Mild tremor, rigidity, and bradykinesia	Motor: bradykinesia and rigidity, central hypoventilation, postural hypotension, bladder incontinence, myoclonus, pyramidal signs	Motor: bradykinesia, rigidity, resting tremor, postural instability, and favorable response to levodopa. decreased olfactory function and hyposmia	
Neuroimaging (MRI/CT)	MRI/CT: periventricular and subcortical white matter lesions (75–90%) multiple territory infarcts (90-95%), basal ganglia ischemic lesions (38–44%). -DAT-SPECT and IBZM-SPECT activity normal or little reduced	- Infarcts affecting left caudate, putamen and internal capsule - Lacunar infarct affecting the substantia nigra	MRI:atrophy of the cerebellar hemisphere and vermis, as well as high intensity areas in both pyramidal tracts. PET-Scan: significantly decreased fluorodopa uptake in the caudate, putamen, and ventral and dorsal midbrain	normal brain MRI SPECT scan showed decreased blood flow in the language region.	PET-Scan: progressive dopaminergic dysfunction affecting transport and uptake	https://www.omim.org/
Neuropathology	Patchy areas of neuronal degeneration and loss, with free-lying nigral pigment and mild gliosis in the substantia nigra. Around the dilated perivascular space of the paramedian perforating branches, free-lying pigment and moderate proliferation of astroglial cells. Perivascular pallor, gliosis, hyaline thickening, and enlargement of perivascular spaces Status cribrosus of the lateral striatal arteries in the putamina and substantia nigra bilateral		depigmentation of the substantia nigra, with severe loss of pigmented neurons in the pars compacta, deposition of extraneuronal melanin, and mild gliosis. No Lewy bodies or neurofibrillary tangles	depigmentation of the substantia nigra, severe cell loss and gliosis in the brainstem, and multiple alpha-synuclein-immunopositive Lewy neurites. Cortical neuritic changes associated with tissue vacuolization were present, mostly in the medial temporal regions.	pure nigral degeneration without Lewy bodies or neurofibrillary tangles	https://www.omim.org/

Table S1.

Gene	cSVD (OMIM)	Inh	Main clinical features	Parkinsonism	CT-MRI	Reference
CACNA1A	Migraine, familial hemiplegic, 1 (141500)	AD		rigidity, bradykinesia and a resting tremor, progressive gait instability, hypomimia, finger tapping and hand diadochokinesia	normal substantia nigra, bilateral loss of substance in the basal ganglia	¹¹
FOXC1	Axenfeld-Rieger Syndrome (602482)	AD		Parkinson		¹²
GLA	Fabry disease (301500)	XL	Transient ischemic attacks (TIAs), strokes [2] and brain white matter lesions (WML) [3] are common central nervous system (CNS) manifestations. In recent years there has been growing body of evidence supporting a link between the pathogenesis of PD and lysosomal dysfunction, namely in Gaucher disease (GD) and FD.	Atypical Parkinsonism with axial signs (gait and postural instability), mild symmetrical rigidity and pyramidal signs (mild right hemiparesis, generalized hyperreflexia and positive Babinski sign on both sides	T2 Hyperintensities in the basal ganglia and deep white matter regions; Leukoencephalopathy with multifocal ischemic lesions including the head of the right caudate nucleus deficiency on 18F-DOPA PET	^{13, 14, 15}
HTRA1	CARASIL syndrome (only SVD) (600142)	AR AD		Extrapyramidal signs, Gait disturbance, Rigidity	Diffuse white matter abnormalities, Subcortical focal lacunae	https://www.omim.org/
NOTCH3	Cadasil (only SVD) (125310) - p.R1006C - p.Cys1315Tyr	AD	migraine, recurrent transient ischemic attacks or stroke, cognitive decline, psychiatric manifestations, Less frequent manifestations of the disease are epilepsy, transient disturbances of consciousness, visual impairment, and hemorrhagic strokes and parkinsonism moderate cognitive impairment (MMSE = 17/30)	slowly progressive parkinsonism, not responsive to L-dopa: akinesia, rigidity, and postural instability, symmetry of parkinsonism, Cognitive impairment from moderate to severe, Apraxia, Pseudobulbar signs, Urinary incontinence	T2-weighted images or FLAIR showed widespread ischemic lesions in the periventricular white matter, the internal and external capsules, the basal ganglia, and thalami. Multiple subcortical lacunar infarcts and leukoencephalopathy extended to the external capsule on cerebral MRI suggested the presence of CADASIL.	^{16, 17, 18, 19, 20,}
TREX1	Cadasil (only SVD) (192315)	AD		extrapyramidal signs		https://www.omim.org/
CST3	Cerebral amyloid angiopathy (105150)	AD		Associated to MSAp type		²¹
SCN1A	Migraine, familial hemiplegic, 3 (609634)	AD		masked face and bradykinesia, resting tremor, hypertonia, antecollis, crouch gait, and bradykinesia, variable response to L-Dopa		²²
SLC20A2	Basal ganglia calcification, idiopathic, 1 (213600)	AD		severe bradykinesia, postural instability, mild symmetric rigidity without tremor, levodopa response	CT calcifications in the bilateral basal ganglia, thalami, and dentate nuclei. Dopamine transporter (DAT) single photon emission CT shows diffusely decreased DAT density in the bilateral striatum.	²³
PRNP	Cerebral amyloid angiopathy, PRNP-related (137440)	AD		Parkinsonism		²⁴ , https://www.omim.org/entry/137440

Table S2


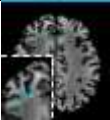
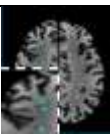

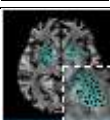
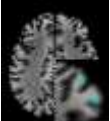
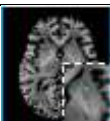
	Periventricular Hyperintensities (PVH 0-6)	Scores	Significance of the scores	References
	Capsule occipital	0/1/2	0 = absent	25
	Capsule frontal	0/1/2	1 = < 15mm	
	Bands lateral ventricles	0/1/2	2 = > 15 mm	
	Lobar Superficial White matter Hyperintensities (WMH 0-16)			
	Frontal	0/1/2/3/4	1 = < 15mm, n ≤ 5	26
	Parietal	0/1/2/3/4	2 = < 15 mm, n > 6	
	Occipital	0/1/2/3/4	3 = > 15 mm, n ≤ 5	
	Temporal	0/1/2/3/4	4 = > 15 mm, n > 6	
	Lobar Deep White matter Hyperintensities (WMH 0-20)			
	Frontal	0/1/2/3/4/5	1 = < 15mm, n ≤ 5	25
	Parietal	0/1/2/3/4/5	2 = < 15 mm, n > 6	
	Occipital	0/1/2/3/4/5	3 = > 15 mm, n ≤ 5	
	Temporal	0/1/2/3/4/5	4 = > 15 mm, n > 6	
			5 = confluent	
	Deep White matter Hyperintensities (WMH 0-3)			
		0	0 = absent	25
		1	1 = punctuate foci	
		2	2 = beginning confluence	
		3	3 = severe (large confluent areas)	
	Status cribrus (SC 0-2)			
		0	0 = Absent	27
		1	1 = Mild/moderate	28
		2	2 = Severe	
	Lobar cortical small-microinfarcts (CMI 0-16)			
	Frontal	0/1/2/3/4	1 = < 15mm, n ≤ 5	(Xiong et al., 2018)
	Parietal	0/1/2/3/4	2 = < 15 mm, n > 6	
	Occipital	0/1/2/3/4	3 = > 15 mm, n ≤ 5	
	Temporal	0/1/2/3/4	4 = > 15 mm, n > 6	
	Lacunar Infarcts (LI. 0-1)			
		0	0 = Absent	29
		1	1 = Present	

Table S3.

Country of origin	N	Disease	AAO (SD)/Age	M:F	N cases with family history (%)	Hypertension (%)	Diabetes (%)	N cases with migraine (%)	N cases with heart comorbidities (MI, AF) (%)	N cases with Hypercholesterolemia (%)
US	96	cSVID	51.5 (8.1)y	0.82	43 (44.7)	58 (60.4)	29 (30.2)	NA	11 (11.4)	2 (2)
GB	243	CTRLS	➤ 80y		NA	NA	NA	NA	NA	NA

Table S5.

Gene	Inh.	Position	rsID	Ref/Alt	cDNA	Aa	Gen	ExAC	ACMG classification	ClinVar	SVID (%)	Gender	AAO	PD	Family history	Vascular risk factors
<i>ATP13A2</i>	AR	chr1: 17323565	rs756955409	T/C	c.1130A>G	p.Q377R	Het	0.0	UNK [PM2,PP3]	Not present	1/96 (1)	F	60y	no	absent	Smoking (40 years)
<i>ATP13A2</i>	AR	chr1: 17323581	rs886395233	C/T	c.1114G>A	p.G372R	Het	0.0	Likely pathogenic [PP3(Strong),PM2]	Not present	1/96 (1)	M	49y	no	absent	Former smoker (34 years)
<i>PINK1</i>	AR	chr1: 20971032	rs778009684	C/T	c.826C>T	p.R276W	Het	4.118e-05	UNK [PM2,PP3(Supporting)]	Uncertain significance	1/96 (1)	M	48y	no	present (father with MI at 77y)	Positive familial history
<i>PINK1</i>	AR	chr1: 20972069	rs376323248	C/T	c.976C>T	p.R326C	Het	1.647e-05	UNK[PM2]	Not present	1/96 (1)	M	51y	no	absent	Smoking (35y), hypertension
<i>SNCA</i>	AD	chr4: 90650386	rs145138372	G/T	c.349C>A	p.P117T	Het	4.118e-05	UNK[PM2]	Uncertain significance	1/96 (1)	M	42y	no	Present (mother, CVA)	hypertension
<i>PRKN</i>	AR	chr6: 161771240	rs191486604	C/T	c.1289G>A	p.G430D	Het	9.885e-05	Pathogenic[PP5(Strong),PP3(Strong),PM1(Strong)]	Pathogenic/Likely pathogenic (in homozygosity)	1/96 (1)	M	41y	no	Present (Grandmother)	Hypertension treated with medications
<i>PRKN</i>	AR	chr6: 161771243	rs760223151	C/T	c.1286G>A	p.G429E	Het	1.648e-05	Likely Pathogenic [PM1(Strong),PM2,PP3]	Not present	1/96 (1)	F	62y	no	Present (mother)	Smoking (25y), MMSE 28
<i>LRRK2</i>	AD, risk factor	chr12: 40619054	rs1422672946	G/T	c.121G>T	p.D41Y	Het	0.0	UNK[PM2]	Not present	1/96 (1)	M	42y	no	absent	Smoking (25 years), hypertension treated with medications
<i>LRRK2</i>	AD, risk factor	chr12: 40631833	rs1465061766	A/T	c.499A>T	p.M167L	Het	0.0	UNK[PM2,BP4(supporting)]	Not present	1/96 (1)	M	64y	no	absent	Former smoker
<i>LRRK2</i>	AD, risk factor	chr12: 40713943	rs766206415	C/A	c.4981C>A	p.P1661T	Het	4.119e-05	UNK[PM2]	Not present	1/96 (1)	M	43y	no	Present (mother)	Current smoker (21 years)

Table S7

References

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