

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 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	Cognitive: cognitive decline Motor: Hemiparkinsonism/Parkinsonism	Cognitive: cognitive decline Motor: juvenile Parkinson disease retropulsion, dystonia of the feet, classic parkinsonism. Mild tremor, rigidity, and bradykinesia	Cognitive: psychiatric features, cognitive decline Motor: bradykinesia and rigidity, central hypoventilation, postural hypotension, bladder incontinence, myoclonus, pyramidal signs	Cognitive: No cognitive decline was noted in any of the patients even after long disease duration Motor: bradykinesia, rigidity, resting tremor, postural instability, and favorable response to levodopa. decreased olfactory function and hyposmia	https://www.omim.org/
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

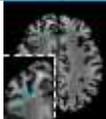
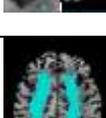
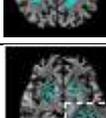
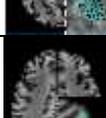
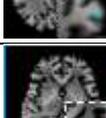
	Periventricular Hyperintensities (PVH 0-6)	Scores	Significance of the scores	References
	Capsule occipital	0/1/2	0 = absent	25
	Capsule frontal	0/1/2	1 = < 15mm	
	Bands lateral ventricles	0/1/2	2 = > 15 mm	
	Lobar Superficial White matter Hyperintensities (WMH 0-16)			
	Frontal	0/1/2/3/4	1 = < 15mm, 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 cribrosus (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

1. Lalvay L, Lara M, Mora A, et al. Quantitative Measurement of Akinesia in Parkinson's Disease. *Mov Disord Clin Pract*. 2017;4(3):316-322. doi:10.1002/mdc3.12410
2. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003;24(2):197-211. doi:10.1016/s0197-4580(02)00065-9
3. Yamamoto E, Mourany L, Colleran R, Whitman C, Touse B. Utility of Montreal Cognitive Assessment in Differentiating Dementia With Lewy Bodies From Alzheimer's Dementia. *Am J Alzheimers Dis Other Demen*. 2017;32(8):468-471. doi:10.1177/1533317517725811
4. Lim A, Tsuang D, Kukull W, et al. Clinico-neuropathological correlation of Alzheimer's disease in a community-based case series. *J Am Geriatr Soc*. 1999;47(5):564-569.
5. Langa KM, Foster NL, Larson EB. Mixed dementia: emerging concepts and therapeutic implications. *JAMA*. 2004;292(23):2901-2908. doi:10.1001/jama.292.23.2901
6. Faust GG, Hall IM. SAMBLASTER: fast duplicate marking and structural variant read extraction. *Bioinformatics*. 2014;30(17):2503-2505. doi:10.1093/bioinformatics/btu314
7. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011;43(5):491-498. doi:10.1038/ng.806
8. Jäger M, Wang K, Bauer S, Smedley D, Krawitz P, Robinson PN. Jannovar: a java library for exome annotation. *Hum Mutat*. 2014;35(5):548-555. doi:10.1002/humu.22531
9. Amendola LM, Dorschner MO, Robertson PD, et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res*. 2015;25(3):305-315. doi:10.1101/gr.183483.114
10. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30

11. Bruun M, Hjermind LE, Thomsen C, et al. Familial hemiplegic migraine type 1 associated with parkinsonism: a case report. *Case Rep Neurol*. 2015;7(1):84-89. doi:10.1159/000381827
12. Zhang L, Deng J, Pan Q, et al. Targeted methylation sequencing reveals dysregulated Wnt signaling in Parkinson disease. *J Genet Genomics*. 2016;43(10):587-592. doi:10.1016/j.jgg.2016.05.002
13. Gago MF, Azevedo O, Guimarães A, et al. Parkinson's Disease and Fabry Disease: Clinical, Biochemical and Neuroimaging Analysis of Three Pedigrees. *J Parkinsons Dis*. 2020;10(1):141-152. doi:10.3233/JPD-191704
14. Orimo S, Iwasaki T, Yoshino H, Arai M, Hiyamuta E. [An autopsied case of Fabry's disease presenting with parkinsonism and cardiomegaly as a cardinal clinical manifestation]. *Rinsho Shinkeigaku*. 1994;34(10):1003-1007.
15. Buechner S, De Cristofaro MTR, Ramat S, Borsini W. Parkinsonism and Anderson Fabry's disease: a case report. *Mov Disord*. 2006;21(1):103-107. doi:10.1002/mds.20675
16. Ragno M, Berbellini A, Cacchiò G, et al. Parkinsonism is a late, not rare, feature of CADASIL: a study on Italian patients carrying the R1006C mutation. *Stroke*. 2013;44(4):1147-1149. doi:10.1161/STROKEAHA.111.000458
17. Valenti R, Bianchi S, Pescini F, et al. First report of a pathogenic mutation on exon 24 of the NOTCH3 gene in a CADASIL family. *J Neurol*. 2011;258(9):1632-1636. doi:10.1007/s00415-011-5983-3
18. Chabriat H, Joutel A, Dichgans M, Tournier-Lasserre E, Bousser MG. Cadasil. *Lancet Neurol*. 2009;8(7):643-653. doi:10.1016/S1474-4422(09)70127-9
19. Singhal S, Bevan S, Barrick T, Rich P, Markus HS. The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. *Brain*. 2004;127(Pt 9):2031-2038. doi:10.1093/brain/awh223
20. Rufa A, De Stefano N, Dotti MT, et al. Acute Unilateral Visual Loss as the First Symptom of Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy. *Archives of Neurology*. 2004;61(4):577-580. doi:10.1001/archneur.61.4.577
21. Urbizu A, Canet-Pons J, Munoz-Marmol AM, et al. Cystatin C is differentially involved in multiple system atrophy phenotypes. *Neuropathol Appl Neurobiol*. 2015;41(4):507-519. doi:10.1111/nan.12134
22. Kanatani M, Adachi T, Sakata R, et al. Dravet syndrome with parkinsonian symptoms and intact dopaminergic neurons: A case report. *Brain Dev*. Published online November 13, 2020. doi:10.1016/j.braindev.2020.10.015

23. Ichikawa Y, Tanaka M, Kurita E, et al. Novel SLC20A2 variant in a Japanese patient with idiopathic basal ganglia calcification-1 (IBGC1) associated with dopa-responsive parkinsonism. *Hum Genome Var.* 2019;6:44. doi:10.1038/s41439-019-0073-7
24. J N, M S, S K, M P. A Corticobasal Syndrome Variant of Familial Creutzfeldt-Jakob Disease with Stroke-Like Onset. *Case reports in neurological medicine.* doi:10.1155/2016/4167391
25. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol.* 1987;149(2):351-356. doi:10.2214/ajr.149.2.351
26. Wang S, Zhang F, Huang P, et al. Superficial white matter microstructure affects processing speed in cerebral small vessel disease. Published online January 1, 2022:2021.12.30.474604. doi:10.1101/2021.12.30.474604
27. Ferrer I, Bella R, Serrano MT, 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.* 1990;98(1):37-50. doi:10.1016/0022-510x(90)90180-u
28. de Reuck J, Sieben G, de Coster W, vander Ecken H. Parkinsonism in patients with cerebral infarcts. *Clin Neurol Neurosurg.* 1980;82(3):177-185. doi:10.1016/0303-8467(80)90035-9
29. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. *The Lancet Neurology.* 2019;18(7):684-696. doi:10.1016/S1474-4422(19)30079-1