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Supplementary information

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Systematic rare variant analyses identify *RAB32* as a susceptibility gene for familial Parkinson's disease

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Supplementary Tables

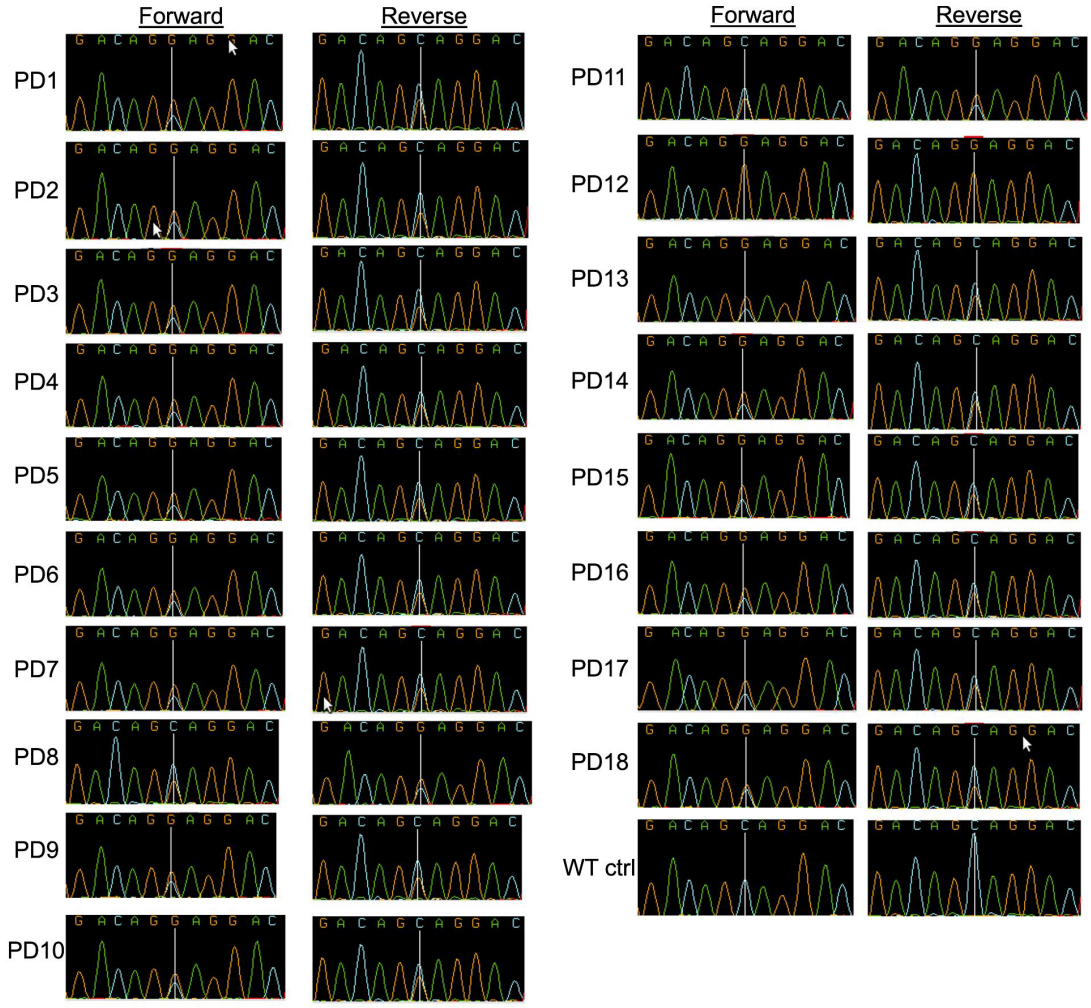
Supplementary Table 1. PCR and sequencing primers used for validation of the *RAB32* p.S71R variant.

PCR primers	**Sequence**
RAB32-exon1-Forward	ACCTCTTCAAGGTGCTGGTG
RAB32-exon1-Reverse	CCTGCTCCAACTCCTACTGC
RAB32-exon1a-Forward	CTCTTCTCCCAGCACTACCG
RAB32-exon1a-Reverse	GGATTGGGCCTCTCTCTTCT
M13 Sequencing Primers	
All reactions-Forward	AGTAAAACGACGGCCAGT
All reactions-Reverse	GCAGGAAACAGCTATGACC

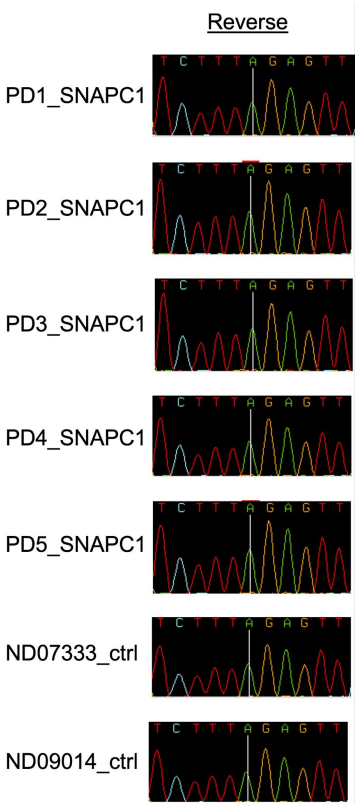
Supplementary Table 2. PCR and sequencing primers used for validation of the *SNAPC1* c.1073-2A>T variant.

PCR primers	**Sequence**
SNAPC1-forward	GCACCTGGCCGATAGTATGT
SNAPC1-reverse	CTGGCAGTCTTCCTGTTCAA
M13 Sequencing Primers	
All reactions-Forward	agtaaaacgacggccagt
All reactions-Reverse	gcaggaaacagctatgacc

Supplementary Figures



Supplementary Figure 1. Sanger sequencing of 18 *RAB32* p.S71R carriers and 1 WT control. Primers are listed in Supplementary Table 1.



Supplementary Figure 2. Sanger sequencing of the *SNAPC1* c.1073-2A>T variant. Primers are listed in Supplementary Table 2.

Supplementary Note

Case reports

Patient 10

In her medical history, this patient had diabetes, uveitis, and hypertension.

The PD symptoms began at the age of 62, characterized by a right resting tremor along with depression and hyposmia. Treatment with L-DOPA started a year later, followed by dopamine-agonist therapy 3 years afterward, which showed a positive response. Six years into the disease, she started experiencing freezing of gait (FOG), falls, and non-motor symptoms such as constipation and rapid eye movement sleep behavior disorder (RBD). After 7 years, dysautonomic symptoms, including urge incontinence, emerged. Motor fluctuations and dyskinesias appeared after a decade, and dysphagia developed after 12 years, leading to relapses and aspiration pneumonia. Hallucinations manifested 14 years into the PD. Ultimately, she passed away from an ischemic stroke after 16 years of battling PD at the age of 78.

A brain MRI conducted a decade after the onset of the disease revealed chronic vasculopathy and an ischemic stroke in the left nucleo-capsular region.
As for her family: her mother was affected by PD, and passed away at the age of 82. Additionally, one uncle experienced parkinsonism.

Patient 11

In her medical history, this patient had rheumatic polymyalgia, hypertension, diabetes, and Hashimoto's thyroiditis. Additionally, a few years before the onset of PD, she experienced hyposmia and depression.

PD emerged at the age of 70, marked by bilateral bradykinesia. She initiated treatment with dopamine agonists and L-DOPA, which yielded a positive response but also resulted in mild impulsive control disorders (ICD). Motor fluctuations and dyskinesias arose after 5 years, necessitating treatment with ICOMT (entacapone).

After another 5 years, a brain MRI revealed moderate cortical fronto-parietal bilateral atrophy and mild subcortical atrophy, while a DATSCAN showed severe asymmetric (right to left) dopaminergic striatal uptake reduction.

The last neurological examination, conducted 9 years after the onset, indicated dysautonomic dysfunction (constipation, urge incontinence, hypotension), falls, and dysphagia.

The patient passed away 19 years after the onset of PD at the age of 89, the cause of death remaining unknown.

As for her family: her mother and one brother affected by PD (no other news on family).

Patient 12

In his medical history, this patient had a gastric ulcer and lung cancer.

The onset of PD occurred at the age of 62, characterized by a right resting tremor and micrographia. Treatment with L-DOPA commenced a year later, followed by dopamine agonist therapy 2 years afterward, which yielded a positive response. Motor fluctuations and dyskinesias emerged 9 years after the onset, with falls and mild cognitive impairment appearing after 10 and 11 years, respectively.

The patient passed away 12 years after the onset of PD at the age of 74, the cause of death remaining unknown.

As for his family: the mother was affected by uncertain parkinsonism.

Patient 13

In his medical history, this patient experienced only hyposmia. PD onset occurred at the age of 42, marked by a left resting tremor and bradykinesia.

Dopamine agonists were initially used as the first-line treatment, leading to mild ICD. After 3 years, L-DOPA was added with an excellent response. Motor fluctuations and dyskinesias emerged 4 years after the onset and progressively worsened over the course of ten years.

Due to the worsening symptoms, 3 years later, the patient underwent deep brain stimulation (DBS) without any pathological findings on brain MRI and CT-PET. The procedure resulted in remarkable motor improvement and a significant reduction in dopamine replacement therapy. However, the patient experienced worsened ICD and

impulsivity, culminating in an attempted suicide. As a result, antipsychotic drugs were incorporated into the therapy regimen.

Motor benefits have been maintained up to the present, although there has been a mild onset of FOG 4 years post-DBS, along with mild camptocormia affecting posture.

As for his family: his father was affected by PD.

Patient 14

In his family history, there is a record of prostatic cancer and hypertension. Parkinson's disease (PD) onset was observed at the age of 65, characterized by right bradykinesia. Two years later, he initiated dopamine agonist therapy, followed by the introduction of L-DOPA treatment, which showed a positive response.

Unfortunately, we lack any further neurological evaluations beyond the last one conducted in 2002.

No familiarity for PD.

Patient 15

In his medical history, this patient had Hashimoto's thyroiditis. Parkinson's disease (PD) onset was observed at the age of 70, marked by deteriorating walking, postural deficits, and falls. He commenced L-DOPA treatment, which showed a positive response.

Two years later, hallucinations and dysautonomic dysfunctions emerged, prompting his admission to our ward for further evaluation. Tests including DATSCAN exam (revealing bilateral dopaminergic putaminal and caudate uptake reduction), CT-PET (showing bilateral central gyrus uptake reduction), and neuropsychological examination (which returned normal results) were conducted.

Our most recent neurological evaluation dates back to 2012.

As for his family: one out of 4 sisters was affected by PD.

Patient 16

The onset of PD occurred in this male patient at the age of 54, characterized by left bradykinesia. Treatment with L-DOPA commenced a year later, followed by dopamine-agonist therapy 5 years afterward, resulting in a positive response.

Motor fluctuations and dyskinesias manifested 5 years after the onset, followed by the emergence of FOG, falls, and postural instability a few months later. There were no reports of dementia or hallucinations.

Throughout the course of PD, he also battled multiple myeloma, which was managed with chemotherapy and bone marrow auto-graft, along with prostate cancer.

The patient passed away 12 years after the onset of PD due to myeloma recurrence.

As for his family: one uncle from father's side suffered from PD.

Patient 17

The onset of PD in this male patient occurred at the age of 39, characterized by a right resting tremor. There were no reported symptoms of depression or hyposmia.

Treatment with dopamine agonist commenced one year later, followed by subsequent therapy with L-DOPA. Motor fluctuations appeared 5 years after the onset of PD. Throughout the years of the disease, he also experienced ICD.

Our most recent neurological evaluation took place in 2009.

As for his family: one grand mother form mother side suffered from PD.

Patient 18

This 61-year-old Mayo Clinic female patient developed her first symptoms of dominant right-hand tremor a year earlier. She was last seen at the Mayo Clinic two years later at age 63. Phenotype was of classic PD, tremor predominant subtype with good response to low dose of levodopa in combination with low dose of amantadine and rasagiline. Over the period of two-year observation, she tolerated her therapy well without dyskinesias, fluctuations in motor performance, on and off symptoms, or dystonia. Three years into her illness, she was fully independent bothered only by intermittent rest

tremor in her right hand. On examination she had all features of PD, rest tremor, bradykinesia, and rigidity predominantly affecting her right, dominant side. No balance troubles. The non-motor symptoms included difficulties with nocturnal sleep that was disturbed by frequent awakenings but no features that would be consistent with REM-sleep behavior disorder (polysomnography was not done and melatonin was offered). She also reported leg paresthesias that were responded to low dose of gabapentin. EMG was normal. Brain MRI done a year after the symptomatic disease onset was normal. DaT scan was done about six months into the illness and demonstrated an asymmetrical basal ganglia tracer uptake worse on the left side supported the diagnosis of PD. Her uncle was diagnosed with Parkinson's disease at age 85. Apparently died at age 93. She has no children. She denies history of head trauma, encephalitis, meningitis, or exposure to industrial toxins. Her other illnesses include chronic low back pain since age 57. She also reported some anxiety for which she was taken Xanax on as needed basis only. In the past she was diagnosed with fibromyalgia requiring treatment with tramadol also on as needed basis. She used to work as an office employee. She smoked Tabaco for 25 years but quit about 15 years prior to seen at the Mayo Clinic. She denied alcohol consumption.

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