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## SHORT REPORT

# A multi-centre clinico-genetic analysis of the VPS35 gene in Parkinson disease indicates reduced penetrance for disease-associated variants

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## ABSTRACT

**Background** Two recent studies identified a mutation (p.Asp620Asn) in the vacuolar protein sorting 35 gene as a cause for an autosomal dominant form of Parkinson disease. Although additional missense variants were described, their pathogenic role yet remains inconclusive.

**Methods and results** We performed the largest multi-center study to ascertain the frequency and pathogenicity of the reported vacuolar protein sorting 35 gene variants in more than 15,000 individuals worldwide. p.Asp620Asn was detected in 5 familial and 2 sporadic PD cases and not in healthy controls, p.Leu774Met in 6 cases and 1 control, p.Gly51Ser in 3 cases and 2 controls. Overall analyses did not reveal any significant increased risk for p.Leu774Met and p.Gly51Ser in our cohort.

**Conclusions** Our study apart from identifying the p.Asp620Asn variant in familial cases also identified it in idiopathic Parkinson disease cases, and thus provides genetic evidence for a role of p.Asp620Asn in Parkinson disease in different populations worldwide.

## INTRODUCTION

There is increasing interest to try to identify uncommon and rare genetic variants that increase the risk of common diseases and that are difficult to identify using traditional genome-wide association studies (GWAS) approaches.<sup>1</sup> Rare variants which are not mapped by GWAS can be identified by using next generation sequencing, that is, exome sequencing in large families with multiple affected individuals.<sup>2</sup> Exome sequencing is now

routinely used to identify rare mutations in familial forms of disease in diverse phenotypes.<sup>2</sup>

Two recent studies independently performed exome sequencing in large families of Caucasian descent, and identified a mutation in the vacuolar protein sorting 35 (VPS35) gene as a possible cause for an autosomal dominant form of Parkinson disease (PD).<sup>3,4</sup> In addition, several non-synonymous base exchanges were identified, but their involvement in disease pathogenesis remains inconclusive. Furthermore, recently published studies provided conflicting results regarding the role of VPS35 in PD.<sup>5-8</sup> Here, we performed a large multi-centre study to determine the frequency and pathogenicity of VPS35 variants in PD in diverse populations worldwide.

## METHODS

### Consortium

Investigators from the Genetic Epidemiology of Parkinson disease Consortium were invited to participate in this study. A total of 23 sites representing 19 countries from four continents agreed to contribute DNA samples and clinical data for a total of 15 383 individuals (8870 cases and 6513 controls). Control individuals underwent neurological examination and were excluded from the study whenever there was clinical evidence for any extrapyramidal disorder.

### Genotyping

We selected seven non-synonymous variants exactly as they were proposed.<sup>3</sup> In addition, we selected tag single nucleotide polymorphisms

(SNPs) (HapMap Rel 28 phase II+III, Aug10, National Centre for Biotechnology Information. B36 dbSNP b126; <http://www.hapmap.org>) that cover the common genetic variants in the VPS35 gene using an  $r^2$  threshold of 0.8–1.0 to select tag SNPs for VPS35 gene. Using this strategy, we were able to capture 23 SNPs in a 40 kb region, including VPS35 ('chr16:46 693 589–46 723 144 based on hg 19'). Therefore, in total, 10 SNPs located in the VPS35 were genotyped (including seven rare non-synonymous and three common variants). Genotyping was performed by a central genotyping core. Genotyping was performed using a matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry on a MassArray system (Sequenom, San Diego, California, USA). Cleaned extension products were analysed by a mass spectrometer (Bruker Daltonic, Billerica, MA, USA) and peaks were identified using the MassArray Typer 4.0.2.5 software (Sequenom). Assays were designed by the AssayDesigner software 4.0 (Sequenom) with the default parameters for the iPLEX Gold chemistry and the Human GenoTyping Tools ProxSNP and PreXTEND (Sequenom). All variants were genotyped in one multiplex assay. The average call rate of the variants was >97%. The local Ethics Committee approved the study. All participants gave signed informed consent.

### Statistical analysis

Logistic regression was used to test the association between VPS35 and PD in our overall cohort. For common variants (minor allele frequency >5%), we synthesised the effect estimates using fixed and random effects models. Fixed effect models assume that the genetic effect is the same in populations from different sites and that observed differences are due to chance alone. For associations showing between-study heterogeneity, fixed effect estimates yield narrower CIs and smaller *p* values as compared with random effects models, which incorporate between-study heterogeneity.<sup>9–10</sup> Random effects models allow the genetic effects might be different due to genuine heterogeneity that may exist across different sites. Random effects calculations take into account the estimated between-study heterogeneity. Cochran's Q test of homogeneity and the  $I^2$  metric were used to evaluate the between-site heterogeneity. The  $I^2$  metric ranges from 0% to 100% and measures the proportion of variability that is beyond chance. Typically, estimates of  $I^2$  <25% are considered to reflect little or no heterogeneity, 25%–50% moderate heterogeneity, 50%–75% large heterogeneity and >75% very large heterogeneity. The overall main analysis considered all sites and populations irrespective of ancestry. For variants with minor allele frequency <1%, an exact test was used to compare the frequency differences between cases and controls combining data across all 21 sites.

## RESULTS

### Characteristics of sites and overall database

Overall, 23 sites contributed a total of 8870 cases and 6513 controls. Characteristics of all participating sites are shown in table 1. Most sites contributed participants of Caucasian ancestry (N=19); four sites included participants of Asian ancestry. The proportion of men and women ranged from 42% to 58% across different participating sites (table 1). The median age at onset of PD in our studied population was 61 years.

### Rare variants

Overall, we observed p.Asp620Asn in seven cases, p.Leu774Met in six cases and one control, p.Gly51Ser in three cases and two

controls. Details per site are shown in table 2. The controls subjects carrying p.Leu774Met (P-13) and p.Gly51Ser (P-2 and P-16) at the time of study sampling were 81, 84 and 76 years, respectively. In Caucasian populations, the number of carriers in cases and controls for the three variants were 5 versus 0 (p.Asp620Asn), 4 versus 1 (p.Leu774Met) and 3 versus 1 (p.Gly51Ser), respectively. In Asian descent populations, the respective numbers were 2 versus 0 (p.Asp620Asn), 2 versus 0 (p.Leu774Met) and 0 versus 1 (p.Gly51Ser). Most interestingly, two out of seven patients carrying the p.Asp620Asn variant presented without any family history for PD. This represents the first evidence for reduced penetrance of the respective variant initially attributed to autosomal dominant familial PD. We did not observe any carriers for one variant (p.Arg524Trp) in our cohort. Two non-synonymous variants (p.Met57Ile, p.Thr82Arg) failed genotyping. By collapsing the rare variants across different sites, we did not observe statistically significant increased risk for p.Leu774Met and p.Gly51Ser in our cohort (see online supplementary table S1).

### Overall data synthesis for common variants

Out of three tag SNPs, one SNP (rs3218745) failed genotyping. We did not observe significant association for any of common variants with PD either with either fixed effect or random effect models (see online supplementary table S2). The OR ranged from 0.96 to 0.99 and tight 95% CIs excluded modest association effects. We observed no substantial heterogeneity for the two genotyped SNPs, and also the Q test was non-statistically significant for common SNPs. Moreover, examining the Caucasian or Asian populations separately did not change our results (data not shown).

### Clinical features

All PD patients who carried potential pathogenic variants (p.Asp620Asn, p.Gly51Ser, p.Leu774Met) were clinically diagnosed with PD (Online supplementary clinical analysis data). A few of these (0.2%) affected individuals also have a positive family history. Affected individuals exhibited classical symptoms of PD (resting tremor, bradykinesia, rigidity) (table 2). The clinical diagnosis of PD was made by movement disorder specialists who used UK brain bank criteria for PD. Non-motor symptoms were present in the majority of PD patients carrying a pathogenic variant (table 2). Interestingly, hallucinations and dementia were also observed in one asymptomatic carrier suggesting clinical heterogeneity associated with VPS35. The identified healthy carriers have not shown any sign of PD as yet (table 2).

## DISCUSSION

We performed the first multi-centre study to define the role of the VPS35 gene (PARK17) in PD by assessing the frequency of the reported non-synonymous variants in familial and sporadic PD patients from different populations worldwide. Among 15 383 subjects genotyped, we found a pathogenic relevance for p.Asp620Asn in different populations. Most interestingly, out of seven subjects who carry p.Asp620Asn, two have a negative family history. Therefore, our results provide additional evidence that VPS35 is a rare cause of familial as well as the common sporadic form of PD. In total, about 0.4% of PD cases in diverse population were due to disease-associated variant in the VPS35 gene. Our lack of supporting the role of common variants of the VPS35 gene in PD is consistent with recently published GWAS and also meta-analyses of GWAS of PD, as none of these highlighted the role of common variability in VPS35 gene as a risk factor for PD.<sup>11–15</sup> The p.Asp620Asn

**Table 1** Description of datasets contributed by each study site

Site	Country	N	Case	Control	Male (%)	Female (%)	Mean AAO	Mean Age at study	Diagnostic criteria
Annesi	Italy	394	197	197	204 (51.7%)	190 (48.2%)	61.5	63.7	UKPDBB
Brice	France	505	272	233	302 (59.8%)	203 (40.1%)	47.6	57.8	UKPDBB
Bozi	Greece	222	114	108	107 (48.1%)	115 (51.8%)	69.9	74.5	UKPDBB
Wszolek	USA	1518	692	826	794 (52.3%)	724 (47.6%)	64.4	71.7	UKPDBB
Garraux	Belgium	82	68	14	45 (54.8)	37 (45.1%)	62.1	69.6	UKPDBB
Hadjigeorgiou	Greece	714	357	357	379 (53.0%)	335 (46.9%)	63.4	63.7	UKPDBB
Jeon	Korea	749	408	341	314 (41.9%)	435 (58.0%)	57.6	NA	UKPDBB
Opala	Poland	629	352	277	340 (54.0%)	288 (45.7%)	50.2	68.1	UKPDBB
Lynch	Ireland	740	368	372	340 (45.9%)	400 (54.0%)	50.5	70.7	UKPDBB
Lin	Taiwan	320	160	160	160 (50%)	160 (50%)	62.0	70.8	UKPDBB
Facheris	Italy	181	114	67	86 (47.5%)	95 (52.4%)	63.0	NA	UKPDBB
Maraganore	USA	1024	801	223	600 (58.5%)	361 (35.3%)	59	74.7	Bower
Mellick	Australia	2024	1012	1012	1042 (51.4%)	981 (48.4%)	59	72.2	Bower
Morrison	England	1120	766	354	606 (54.1%)	514 (45.8%)	66.1	NA	UKPDBB
Mok	China	436	260	176	264 (60.5%)	170 (38.9%)	NA	63.5	UKPDBB
Aasly	Norway	1278	656	622	721 (56.4%)	557 (43.5%)	58.8	72.9	UKPDBB
Wirdefeldt	Sweden	299	83	216	147 (49.1%)	152 (50.8%)	65.8	71.4	Gelb
Van Broeckhoven	Belgium	1010	501	509	500 (49.5%)	509 (50.3%)	60.5	66.3	Pals/Gelb
Rogaeva	Canada	560	387	173	303 (54.1%)	257 (45.8%)	49.7	64.2	UKPDBB
Tan	Singapore	391	194	197	244 (62.4%)	147 (37.5%)	59.7	54.0	UKPDBB
Hattori	Japan	121	121	0	62 (51.2%)	59 (48.7%)	NA	NA	UKPDBB
Gasser/Sharma	Germany	760	760	0	479 (63.3%)	281 (36.9%)	58.9	NA	UKPDBB
Toda	Japan	306	227	79	161 (52.6%)	145 (47.3%)	57.8	65.1	UKPDBB
Total		<b>15 383</b>	<b>8870</b>	<b>6513</b>			<b>59.5</b>	<b>67.6</b>	

AAO, Age at onset; NA: Not applicable.

variant is located in the C-terminal region of the VPS35 protein pointing that subtle structural changes might influence the disease pathogenesis.<sup>3</sup>

The spectrum of proteins involved in PD aetiology has grown considerably. This includes proteins that are related to mitochondrial quality control (Parkin, PINK1 and DJ1), proteins involved in protein aggregation (SNCA) (Synuclein, MAPT) Microtubule associated protein Tau), and proteins

which are involved in sorting and degradation within endocytic and autophagy pathways ((VDAC) Voltage dependent anion channel, (GBA) Glucocerebrosidase gene, VPS35).<sup>16 17</sup> So far, very little is known about the specific role of VPS35 in PD, except that it is hypothesised that it is involved in cargo recognition as part of a retrograde complex recycling membrane proteins from endosomes to the trans-Golgi network.<sup>3 4</sup> Indeed, in vitro and in vivo studies strongly implicate the role of VPS35

**Table 2** Clinical description of carriers of non-synonymous variants of vacuolar protein sorting 35 gene

Id	Ethnicity	Rare variant	Age at onset	Clinical signs	Bradykinesia	Rigidity	Tremor	Postural instability	L-dopa responsive	Non-motor symptoms	Family history
P-1	Caucasian	p.Asp620Asn	59	Classical PD	+	+	+	+	+	Negative	Negative
P-2	Caucasian	p.Gly51Ser	NA	Control	-	-	-	-	-	Negative	Negative
P-3	Caucasian	p.Gly51Ser	NAV	Classical PD	+	+	+	+	+	Dementia, visual hallucinations	Negative
P-4	Caucasian	p.Gly51Ser	55	Classical PD	+	+	+	+	+	Negative	Negative
P-5	Caucasian	p.Gly51Ser	49	Classical PD	+	+	+	+	+	Negative	Negative
P-6	Caucasian	p.Asp620Asn	37	Classical PD	+	+	+	+	+	Negative	Positive
P-7	Caucasian	p.Asp620Asn	59	Classical PD	+	+	+	+	+	Negative	Positive
P-8	Caucasian	p.Asp620Asn	55	Classical PD	+	+	+	+	+	Negative	Positive
P-9	Caucasian	p.Asp620Asn	66	Classical PD	+	+	+	+	+	Negative	Positive
P-10	Caucasian	p.Leu774Met	41	Classical PD	+	+	+	+	+	Negative	Positive
P-11	Caucasian	p.Leu774Met	65	Classical PD	+	+	+	+	+	Negative	Positive
P-12	Caucasian	p.Leu774Met	65	Classical PD	+	+	+	+	+	Disturbance of gait and balance	Positive
P-13	Caucasian	p.Leu774Met	NA	Control	-	-	-	-	NA	-	Negative
P-14	Caucasian	p.Leu774Met	44	Classical PD	+	+	+ Rest 1st sx	+	+	Autonomic dysfunction	Positive
P-15	Asian	p.Asp620Asn,p.Leu774Met	52	Classical PD	+	+	+	+	+	Negative	Negative
P-16	Asian	p.Gly51Ser		Control	-	-	-	-	-	-	Negative
P-17	Asian	p.Leu774Met	75	Classical PD	+	+	+	+	+	Negative	Negative
P-18	Asian	p.Asp620Asn	43	Classical PD	+	+	+	+	+	Mild cognitive impairment	Positive

NA, not applicable; NAV, not available; PD, Parkinson disease; +positive; -negative.

gene in neurodegeneration. For example, reduced levels of VPS35 have been found in affected brain regions of Alzheimer disease (AD) patients<sup>18</sup> and loss of VPS35 function has been shown to increase the levels of amyloid  $\beta$  and cause synaptic impairment in a mouse model of AD. Furthermore, variants in another member of the VPS family and substrate of retromer complex, SORL1, have been implicated in AD.<sup>19</sup>

In this study, we have focused only on non-synonymous variants identified by Zimprich and colleagues.<sup>3</sup> Of note, we confirmed the pathogenic relevance of the p.Asp620Asn variant which was identified by both studies for familial cases and in sporadic PD. Recently, published studies also identified p.Asp620Asn mutations in PD,<sup>6, 20</sup> thus providing support to the role of p.Asp620Asn in PD. In our study, clinically, the symptomatic carriers showed a broad spectrum of clinical phenotypes ranging from typical PD to (DLB) Dementia with Lewy body, so longitudinal evaluation of carriers at risk will provide unique information on the natural course of the disease caused by VPS35. Even though our data support the role of p.Asp620Asn variant in PD, given the fact that the frequency in diverse population is far below <1%, it is likely to be a rare cause of PD worldwide. Nevertheless, sequencing of families is encouraged for identifying additional missense variants which may provide mechanistic insight into the causes of PD.

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## Correction

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### *Clinical Analysis*

**P-1:** This patient showed no family history of neurological or psychiatric disease. The first signs of the disease appeared with bradykinesia in the left arm. Subsequently, the subject developed a classic phenotype of PD (resting tremor, rigidity, bradykinesia and good response to-L-Dopa treatment). The clinical diagnosis of PD was made by movement disorder specialists who used UK brain bank criteria for PD.

**P-2:** This subject previously of good health, was included in the study as a control. The subject had no family history for Parkinson's disease, (PD). A few years later the subject was diagnosed with atrial fibrillation and suffered a large cerebral hematoma from which this patient died a week later. Prior to stroke the patient had no signs of a movement disorder and mental functions were intact

**P-3:** This patient was diagnosed with PD. The subject had no family history for PD but tested positive for the LRRK2 G2019S mutation. The subject responded fairly well to levodopa. Later- on the subject gradually developed dementia of the PDD type. In addition to that the subject had mild visual hallucinations, but no severe psychiatric symptoms.

**P- 4.** The first symptoms of this subject were right sided bradykinesia and tremor. The patient had no family history for PD, and responded well to levodopa. Later-on, the subject showed gradual cognitive decline and developed severe psychiatric symptoms. The situation deteriorated and eventually requiring nursing home placement with akinetic mutism and with feeding through a percutaneous endoscopic gastroscopy, (PEG), tube.



**P-5:** This patient has no family history for PD. The patient developed axial bradykinesia and left-sided rest tremor, and responded well to levodopa. The patient developed severe psychiatric symptoms and was admitted to a psychiatric hospital. Since that time PD has been fluctuating with on and off periods. The patient needed support from home healthcare services and mental status has been unstable with periods of aggression. In parallel the patient has also developed renal failure needing dialysis 3 times a week.

**P-6:** This patient was diagnosed with Parkinson disease. The clinical symptoms included gait dysfunction in the absence of tremor (typical postural instability gait disorder phenotype). The patient responded well to levodopa. The patient reported multiple affected members with PD.

**P-7:** This patient was diagnosed with PD after approximately one year of muscular skeletal symptoms (rigidity) and tremor. The patient responded well to levodopa. The patient reported family history of PD.

**P-8:** This patient developed left hand kinetic tremor. The initial symptoms were mild without impairment of daily living functions. At later stages the patient exhibited bilateral rest tremor in both lower extremities symmetrically. Five years later the patient was diagnosed with PD. At that time patient had postural tremor in both upper extremities. However, the rest tremor in both hands followed later. Other stigmata of parkinsonism including rigidity and bradykinesia were also present. The patient responded positively to carbidopa/levodopa therapy. With progression of illness and disability, the bilateral subthalamic nucleus deep brain stimulation surgery was performed. The patient died at age 67 years. Autopsy was not performed. There was a positive family history of PD.

**P-9:** This patient was evaluated and diagnosed with Parkinson's disease. Onset had been 1.5 years before, initially manifesting as left lower limb rest tremor. The patient reported positive family history. The neurologic exam was remarkable only for left hemiparkinsonism and the MRI brain scan was essentially normal.

**P-10:** Parkinsonism onset was first noted with tremor in the left lower limb and “dragging” of that leg. Symptoms progressive and patient were diagnosed with Parkinson's disease the same year; a brain MRI scan was unremarkable. She proved levodopa responsive, but complicated by a short-duration response (wearing-off), plus levodopa-dyskinesia. The patient reported positive family history.

**P-11:** PD symptoms first developed insidiously consisting of slowness and clumsiness of the left upper limb. The patient was diagnosed with Parkinson's disease by a movement specialist and treated with carbidopa/levodopa with control of symptoms. Depression was first noted around that same time. Subsequently patient developed motor fluctuations and dyskinesias, further treated with adjunctive drugs. Later-on patient developed dream enactment behavior. About 15 years after parkinsonism onset, mild cognitive impairment was noted. The patient reported positive family history.

**P-12:** This patient developed the classical signs of PD and was followed at the Movement Disorders clinic when the subject was in early 70s. At that time in addition to PD, patient also exhibited signs of cerebellar ataxia, disturbance of gait and balance. The contact with the patient was lost and recent attempt to re-establish it failed.

**P-13:** This subject was enrolled in ongoing genetic study of PD as a control subject. The subject had no family history of PD. The neurological examination including motor and cognitive functions was entirely normal. The blood specimen was collected approximately 5 years ago and re-examination of this subject to confirm unaffected status for this study has not been possible.

**P-14:** This patient developed right hand tremor and was followed by asymmetrical bradykinesia and rigidity. The patient benefited from therapy with levodopa/carbidopa and after 12 years of treatment developed motor fluctuations and dyskinesias. He retired 16 years after the onset of his symptoms. One year after his retirement, the patient demonstrated classical features of PD with H&Y stage in “ON” of 3, and in “OFF” of 4, and UPDRS total score in “ON” of 61 and in “OFF” of 70. His MMSE is 30/30 but on this treatment patient reports some autonomic dysfunction (orthostatic hypotension).

**P-15:** This patient presented with truncal rigidity, shuffling gait and resting tremor affecting the left leg. She has no family history of Parkinson’s disease (PD). Physical examination showed a typical mask face, generalized bradykinesia and mild rigidity, with left side more severe than right side, and a mild resting tremor of the left leg. The subject had no postural instability. This patient presented with features compatible with idiopathic PD and symptoms were responsive to anti-PD medications.

**P-16:** The neurological examination is normal. The subject did not have a family history of parkinsonism or dementia.

**P-17:** This patient developed progressive motor disturbances. The patient developed progressive decrease in speech output, dysarthria, bradykinesia, bradyphrenia and

progressive gait disturbance. Initiation difficulty was prominent with shuffling and festinant gait. The patient subsequently developed slurred monotonous speech with drooling. Autonomic dysfunction with urinary frequency, retention sense and orthostatic hypotension was present. The MMSE score was 21/30. Resting tremor was absent but postural tremor was present in both hands worse on the left side. Cogwheel rigidity was worse on the left side. Posture was stooped. The patient walked in short steps and festination. Postural instability was severe. Arm swing was decreased more on the left. Symptoms were responsive to levodopa treatment. The patient was lost on follow-up and died at age 84. The patient did not have a family history of parkinsonism or dementia.

**P-18:** This patient developed the classical signs of PD, including mild cognitive impairment. The patient had a positive family history for PD.

Supplementary Table 1: Assessment of non-synonymous variants of VPS35 gene in PD. Analysis is performed irrespective of ethnicity. Two variants, p.Arg524Trp and p.Ile241Met are monomorphic and hence not shown in the table.

Gene	Non-synonymous variant	Minor/Major allele	MAF in carriers	MAF in controls	Odds ratio (95%CI)	p-value
VPS35	p.Gly51Ser	T/C	0.0002	0.0001	1.30 (0.21-7.84)	0.629
VPS35	p.Leu774Met	T/G	0.0004	0.00008	5.35(0.65-43.56)	0.116

MAF: minor allele frequency, CI: confidence interval,

Supplementary Table 2: Overall analysis irrespective of ethnicity and influence of between-study heterogeneity

	SNP	Q test p-value	I <sup>2</sup> (95%CI)	Odds ratio (95%CI) by random effects	Fixed effects p-value	Random effects p value
Gene						
VPS35	rs4966616	0.26	17(0-53)	0.96 (0.88-1.05)	0.629	0.466
VPS35	rs33994299	0.65	0(0-46)	0.99(0.93-1.05)	0.878	0.878

CI: confidence interval