Full-length Article

VPS35 mutation in Japanese patients with typical Parkinson disease

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Running head: VPS35 mutation in Japanese Parkinson disease

Key words: Parkinson disease, VPS35, autosomal dominant, hotspot, mutation.

Number of references: 25; Figures: 1; Tables: 2; Supplementary Figures: 0;
Supplementary Tables: 0.

Word count: abstract: 229 words; text: 1,950 words.

Financial disclosure related to research covered in this article:

The authors declare no conflict of interest.

This work was supported by Strategic Research Foundation Grant-in-Aid Project for Private Universities, Grants-in-Aid for Scientific Research (to NH, 80218510, and to HT, 21591098), Grant-in-Aid for Young Scientists (to MF, 22790829, and to YL, 23791003), Grant-in-Aid for Scientific Research on Innovative Areas (to NH,
23111003, and to MF 23129506) from the Japanese Ministry of Education, Culture, Sports, Science and Technology, and Grant-in-Aid from the Research Committee on Muro Disease (Kii ALS/PDC) (to YK: 21210301), the Ministry of Health, Labor, and Welfare, and JST, CREST.
ABSTRACT

Background Vacuolar protein sorting 35 (VPS35) was recently reported as a pathogenic gene for late-onset autosomal dominant Parkinson disease (ADPD) using exome sequencing. To date, VPS35 mutations have been detected only in Caucasians with Parkinson disease (PD). The aim of the present study was to determine the incidence and clinical features of Asian PD patients with VPS35 mutations.

Methods We screened seven reported nonsynonymous missense variants of VPS35, including p.D620N, known as potentially disease-associated variants of PD in 300 Japanese index patients with ADPD and 433 patients with sporadic PD (SPD) by direct sequencing or High Resolution Melting (HRM) analysis. In addition, we screened 579 controls for p.D620N mutation by HRM analysis.

Results The p.D620N mutation was detected in three patients with ADPD (1.0%), one patient with SPD (0.23%), and none in the control. None of the other reported variants of VPS35 were detected. Haplotype analysis suggested at least three independent founders for Japanese patients with p.D620N mutation. Patients with VPS35 mutation showed typical tremor-predominant PD.

Conclusion We reported Asian PD patients with VPS35 mutation. Although VPS35 mutations are uncommon in PD, the frequency of such mutation is relatively higher in Japanese than that reported in other populations. In VPS35, p.D620N substitution may be a mutational hotspot across different ethnic populations. Based on the clinical features, VPS35 should be analyzed in patients with PD, especially ADPD or tremor-predominant PD.
Introduction

Parkinson disease (PD) is a neurodegenerative disorder characterized by progressive motor disturbances manifested by tremor, rigidity, akinesia, and postural instability. Neuropathologically, PD is characterized by selective loss of dopaminergic neurons in the substantia nigra and presence of cytosolic inclusion called Lewy bodies (LBs) in the remaining neurons. The pathogenesis of PD is multifactorial, including genetic-environmental interaction. PD is a common disease in the elderly with an incidence of about 1-2% in individuals over 60 years. Among PD patients, approximately 5-10% have a positive family history of PD, and among these, the Mendelian forms of PD can contribute to the elucidation of the molecular pathways that lead to the degeneration and death of dopaminergic neurons.

Mutations in vacuolar protein sorting 35 (VPS35) have recently been identified in families with autosomal dominant late-onset PD (MIM 601501). Patients with VPS35 mutation present with tremor-predominant dopa-responsive parkinsonism. VPS35, a key component of the retromer cargo-recognition complex, is thought to associate with sorting cargos into the tubular endosomal network for retrieval to the trans-Golgi network. Therefore, pathogenic mutation of VPS35 may cause disruption of the retrograde transport system and contribute to the dopaminergic neuronal cell death in PD. One missense mutation has been reported to be pathogenic for PD. Mutation of c.1858G>A (p.D620N) was identified in three different Austrian families and one family each in Switzerland, USA, Tunisia, United Kingdom, and one family and one patient with sporadic PD (SPD) in Yemenite Jews from Israel. In addition, several variants, such as p.M57I, p.I241M, p.P316S, and p.R524W, have been
reported in Europe and the U.S. as potentially pathogenic for PD.\textsuperscript{3,4}

Although multi-population screenings for \textit{VPS35} mutation were preformed in recent reports, there is still no report of PD patients with \textit{VPS35} mutation from Asian ancestry.\textsuperscript{3,4,6-8} In the present study, we screened Japanese patients with autosomal-dominant PD (ADPD), SPD, and control subjects for mutations of \textit{VPS35}, with especial focus on seven reported nonsynonymous variants, which were found in patients with PD, including the p.D620N. Here we report three families and one SPD patient with p.D620N mutation in \textit{VPS35} and describe their clinical features.

\textbf{Subjects and Methods}

\textbf{Subjects.} The study was approved by the ethics committee of Juntendo University and all subjects gave written informed consent to participate in the genetic research. The study subjects were 308 Japanese patients (300 index patients) with ADPD [age at disease onset (AAO): 51.1±11.7 years, mean±SD, range: 8-83 years, female/male (F/M) ratio: 1.35] and 433 Japanese SPD patients (AAO: 47.2±12.9 years, range: 5-88 years, F/M ratio: 1.09) selected from the genebank of Juntendo University. Some of the selected subjects had been confirmed negative for \textit{SNCA}, \textit{PARK2}, \textit{PINK1}, \textit{PARK7}, \textit{LRRK2}, and \textit{PLA2G6} mutations.\textsuperscript{9-14} We also selected from the same genebank 579 healthy Japanese subjects without family history of parkinsonism (age at sampling: 58.0±9.3 years, range: 23-89 years, F/M ratio: 1.54). The criteria for the diagnosis of PD were adopted by the participating neurologists and were established based on the United Kingdom Parkinson’s Disease Society Brain Bank.\textsuperscript{15}
**Genetic analysis.** Genomic DNA was extracted from peripheral blood using a standard protocol. Patients with ADPD and SPD were examined for the following seven variants: p.M57I (exon 3), p.I241M (exon 7), p.P316S (exon 9), p.R524W (exon 13), p.D620N (exon 15), p.A737V (exon 16), and p.L774M (exon 17) of VPS35 (RefSeq accession number NM_018206.4). PCR-direct sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing kit and 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) or 3730 DNA Analyzer (Applied Biosystems). Additionally, SPD patients and control subjects were also genotyped for c.1858G>A (p.D620N) mutation by high resolution melting (HRM) analysis using LightScanner and LCGreen plus (Idaho Technology, Salt Lake City, ID). HRM analysis was performed using a previously described protocol and the following primer: forward; GAGGATGGTGGTCCTTGAA, reverse; TGCCAATGATCAAGGTGATG. All exons of VPS35 were also analyzed in patients with p.D620N mutation using the method described previously.

Haplotype analysis of the VPS35 flanking region was performed using 3130 Genetic analyzer and GeneMapper software (Applied Biosystems). To adjust the size of PCR products, we also genotyped Centre d'Étude du Polymorphisme Humain (CEPH) control samples (1331-01 and 1331-02) for comparison of haplotypes with previously reported patients carrying the p.D620N mutation. The sequences of the PCR primers were reported previously.

**Results**

*Detection of p.D620N mutation.* We detected heterozygous missense p.D620N
mutation in three unrelated patients with ADPD and one patient with SPD (Figure 1). The p.D620N has been reported previously as pathogenic mutation for familial PD.\textsuperscript{3,4,6} This mutation was not found in 1,158 control chromosomes. Patients carrying the p.D620N mutation did not have any other variants in all exons of \textit{VPS35}. In our population, the incidence of p.D620N mutation was 1.0\% (3/300) in ADPD and 0.23\% (1/433) in SPD. The remaining variants analyzed in this study were not identified in any of the patients.

Haplotype analysis demonstrated that the Japanese patients with p.D620N mutation had different genotypes from those of Caucasian patients with the same mutation.\textsuperscript{3} One disease allele was detected by analyzing patient AII-11 and his relatives. Patients AII-11 and BIII-8 in this study carried at least the same single allele of microsatellites in the flanking region of \textit{VPS35} (Table 1). On the other hand, patients CII-3 and D had a different genotype of D16S3105, with a locus mapped very close to \textit{VPS35}, compared with the disease allele of AII-11 (Table 1, bold face).

\textbf{Clinical presentation.} Table 2 summarizes the clinical features of the four \textit{VPS35} mutation-positive patients. Patient AII-11 was a 77-year-old man who developed right upper limb rest tremor at age 62. At age of 75, he underwent gastrostomy for progressive dysphagia, then developed cognitive dysfunction without hallucination. Single photon emission computed tomography of cerebral blood flow showed no reduction in blood flow in the basal ganglia. His father and 4 of 8 siblings were diagnosed with PD (Figure 1A) and presented levodopa-responsive typical parkinsonism; upper limb tremor and small-step gait. His nephew and niece were also
diagnosed with PD and the nephew developed parkinsonism in early fifties. Patient BIII-8 and patient CII-3 both developed upper limb rest tremor at age of 34 and 55, respectively. The mother and aunts of patient BIII-8 and father of patient CII-3 also developed PD (Figure 1 B and C). Patient D who developed upper limb rest tremor at 42 years of age had no family history of PD. She underwent subthalamic nucleus deep brain stimulation (STN-DBS) at 60 years of age because of disabling motor fluctuation and dyskinesia refractory to pharmacological treatment. All affected patients were born to nonconsanguineous parents.

Discussion

*VPS35* has been reported as the pathogenic gene for ADPD, and only one mutation; p.D620N, has been reported in several unrelated Caucasian families. To our knowledge, there are no reports of Asian PD patients with *VPS35* mutations. Based on this background, we set out this study to determine in the incidence of *VPS35* mutations in Japanese patients with PD. We detected heterozygous p.D620N mutation of *VPS35* in three ADPD families and one SPD patient with East Asian ancestry. On the other hand, we could not conclude the pathogenicity of six other variants that had been reported as potentially pathogenic for PD, since none of the variants was detected in our patients with PD.

The frequency of p.D620N mutation in Japanese patients was 1.0% in ADPD and 0.23% in SPD. Although the exact frequency among Caucasians is undetermined, the frequency is relatively higher in Japanese patients compared with that reported in previous studies (0-1.22%). Moreover, the frequency in Japanese patients also
differs greatly from those of even Asian populations such as Taiwanese patients and mainland Chinese patients (0%). Although, the mutation frequency was expected to be lower than that of other pathogenic genes for ADPD, such as multiplication of SNCA and point mutation of LRRK2, VPS35 may be one of the most important genes in Japanese PD. Since we screened for only seven reported variants, we cannot determine the exact frequency of VPS35 mutations in ADPD; we need to analyze all 17 exons of VPS35 in ADPD patients to screen for other variants and to assess the incidence of all disease-associated VPS35 mutations. Furthermore, we need to perform mutational analysis for SPD patients, in addition to ADPD, to identify Asian population-specific variants, such as LRRK2 p.G2385R, associated with susceptibility for PD.

Based on haplotype analysis reported in previous studies, the substitution of VPS35 c.1858G>A (p.D620N) occurs from independent mutational events. We were able to determine the chromosomal phase in only patient AII-11 (family A). The p.D620N mutation possibly shared a common founder between Japanese ADPD families A and B, however it was inconclusive because the phase of patient BIII-8 was undetermined. On the other hand, the same p.D620N mutation probably occurred independently in patient CII-3 (family C) and patient D. By genotyping of D16S3105, which is located approximately 1.5kb centromeric of VPS35, there were at least three different haplotypes in Japanese because families A and C and patient D (SPD) did not have the same alleles for this microsatellite. To determine the chromosomal phase of families B and C, detailed genetic analyses of other family members are needed in future studies. These results suggest the existence of three or more founders in
Japanese patients, in addition to the reported Caucasian patients with p.D620N mutation or de novo mutation, indicating that the p.D620N mutation site is a mutational hotspot in VPS35 across different ethnic populations.

According to previous reports, the average AAO of patients with VPS35 mutation was 50-60 years (50.6±7.3 years), with a distinctive feature of a slightly younger AAO compared to patients with idiopathic PD. In our study, the AAO was nonspecific with a wide range between 30-70 years. Since the family history of patient D was unknown, she was categorized as SPD. With regard to VPS35 mutation penetrance, it is incomplete from the results of a previous report. Therefore, although the frequency is low, patients with p.D620N mutation could be found among SPD patients.

The clinical symptoms of our patients with VPS35 mutation closely resembled the idiopathic PD form, with tremor-dominant dopa-responsive parkinsonism. Psychiatric problems are inconspicuous; however, dementia may occur in patients with a long disease course, similar to patient AII-11 who had PD for 15 years. Our patients with VPS35 mutation had normal brain MRI and cardiac MIBG scintigraphy. There have been no definite pathological mutations of VPS35 in the spectrum of LB disorders. Based on these results, patients with VPS35 mutation could show comparatively benign disease course without widespread LBs pathology.

VPS35 assembles into the retromer cargo-recognition complex that associates with the cytosolic face of the endosomes. The retromer mediates the retrograde transport of transmembrane cargo from the endosomes to the trans-Golgi network. The p.D620N mutation of VPS35 might cause impairment of interaction with other
components of the retromer complex and impaired retrograde trafficking of recycling proteins, similar to α-synuclein and LRRK2, which are involved in vesicle trafficking. Mutations in familial PD genes, including VPS35, may cause disruption of intracellular trafficking and lead to neurodegeneration. These findings suggest that impairment of intracellular trafficking systems is associated with the pathogenesis of PD. Although the association between p.D620N mutation of VPS35 and PD remains unknown, further functional studies might shed light on the pathogenesis of VPS35 mutation and the effects of interaction with other known pathogenic gene products on PD.

In conclusion, we reported Asian PD patients with VPS35 p.D620N mutation. The p.D620N substitution may be a mutational hotspot across different ethnic populations. The frequency of VPS35 mutation was low in ADPD; however, it is relatively high in Japanese patients compared with that reported in other populations. Based on the clinical features of patients with VPS35 mutation, VPS35 should be analyzed in patients with PD, especially ADPD or tremor-predominant PD.
Acknowledgments

The authors thank all the participants in this study.

 Authors' Roles

1. Research project: A. Conception, B. Organization, C. Execution
3. Manuscript: A. Writing of the first draft, B. Review and Critique

### Full financial disclosure of all authors for the past year

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YES¹: MF: Grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Young Scientists (22790829) and Grant-in-Aid for Scientific Research on Innovative Areas (23129506). Grant from Juntendo University School of Medicine, Project Research Grants-in-Aid (2334).

YES²: YL: Grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Young Scientists (23791003). Grant from Juntendo University School of Medicine, Project Research Grants-in-Aid (2333).

YES³: YK: Grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Scientific Research (23591242), and Grant-in-Aid of the Research Committee of Muro disease (Kii ALS/PDC) (21210301).
YES: TH: Grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Young Scientists (23700389).

YES: HT: Grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Scientific Research (21591098), and Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan (22140901).

References


Figure Legends

**Figure 1. Pedigrees of families with VPS35 p.D620N mutation.**
