Frequency of the D620N Mutation in VPS35 in Parkinson Disease

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Objective: To evaluate the frequency and clinical spectrum of the recently identified p.D620N mutation in the VPS35 gene in Parkinson disease (PD) in an international sample.

Design: Genetic analysis by DNA sequencing and detailed clinical and neuropsychiatric assessment as well as neuroimaging in mutation carriers.

Setting: Tertiary referral centers in Germany, Serbia, Chile, and the United States.

Patients: One thousand seven hundred seventy-four patients with PD.

Main Outcome Measure: Frequency of the p.D620N mutation.

Results: A single mutation carrier was identified. The mutation carrier was a 60-year-old German man who had tremor-dominant PD since the age of 45 years. Longitudinal follow-up over 13 years revealed a disease progression from Hoehn and Yahr stage 1 to 3. There was evidence of mild cognitive impairment on the Montreal Cognitive Assessment. No abnormalities were observed by multimodal neuroimaging. He had a family history consistent with autosomal dominant inheritance. An affected paternal aunt and 3 reportedly unaffected siblings were also found to be mutation carriers.

Conclusion: VPS35 mutations are a rare cause of PD in different populations. The clinical phenotype may be indistinguishable from idiopathic PD with the possible exception of an earlier age at onset. Genetic analysis of the extended family revealed incomplete penetrance of the p.D620N mutation.


RECENTLY, THE P.D620N MUTATION IN THE VACUOLAR PROTEIN SORTING 35 (VPS35) GENE WAS IDENTIFIED AS A NOVEL CAUSE OF AUTOSOMAL DOMINANT PARKINSON DISEASE (PD) IN 2 INDEPENDENT STUDIES IN A SWISS AND AN AUSTRIAN FAMILY USING EXOME SEQUENCING.1,2 SCREENING OF APPROXIMATELY 5200 UNRELATED PATIENTS AND APPROXIMATELY 5600 CONTROLS OF DIFFERENT ETHNIC BACKGROUNDS REVEALED 6 INDEX PATIENTS WITH THE P.D620N MUTATION, INCLUDING 1 PATIENT WITH SPORADIC PD.

Mutation carriers presented with tremor-predominant, asymmetric PD and levodopa responsiveness.3 Age at onset ranged from 40 to 68 years.1,2 To further elucidate the role of the VPS35 mutation, we screened an international sample of patients with PD for p.D620N and performed a comprehensive clinical assessment of mutation carriers.

METHODS

Unrelated patients with PD were consecutively collected from tertiary referral centers in Lübeck, Germany; Belgrade, Serbia; and Santiago, Chile. In addition, samples from the Neuroprotection Exploratory Trials in Parkinson’s Disease (United States) study were included (Table 1). All subjects gave written informed consent, underwent a neurological examination, and fulfilled UK Brain Bank diagnostic criteria (with the exception that a positive family history was not an exclusion criterion). The sponsors did not influence the design and conduct of the study.

Sequencing of exon 15 was performed as described.2 Screening of controls was not performed given that p.D620N has been previously excluded in more than 5000 controls (including 2248 German or Austrian controls).2 Haplotype analysis in the VPS35 region was carried out as reported.1 Prior to this study, the mutation carrier had tested negative for mutations in Parkin, DJ-1, and PINK1 as well as for selected mutations in SNCA and LRRK2.3

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Two neurological assessments (1998 and 2011) were performed in the mutation carrier including the United Parkinson’s Disease Rating Scale and Hoehn and Yahr Scale. Neuropsychiatric assessment consisted of the Mini-Mental State Examination (MMSE), Parkinson Neuropsychometric Dementia Assessment, Montreal Cognitive Assessment (MoCA), and Structured Clinical Interview for DSM-IV. Olfaction was tested using the University of Pennsylvania Smell Identification Test. Transcranial sonography was performed as described. Cerebral magnetic resonance imaging and an electroencephalogram were also obtained.

RESULTS

Of the 1774 patients screened, 1 German patient was identified with the p.D620N (c.1858G>A) mutation. No other variant was detected.

The mutation carrier was a 60-year-old man with the initial symptom of a tremor involving the toes of the left foot at the age of 45 years. Twelve months later, he commenced treatment with levodopa/benserazide, 300 mg/d, cabergoline, and selegiline, with a good clinical response. Left leg tremor continued to be his most troublesome concern for several years until it progressed to involve the upper limbs. By age 58 years, he had a rapid decline in his mobility with marked gait impairment that improved following self-dosing with levodopa above the earlier-prescribed doses. His current medication regimen includes a daily dose of a 6-mg rotigotine patch, 300 mg of tolcapone, and 1 g of levodopa (levodopa dosing about every 3 hours). At age 46 years, he also developed restless legs syndrome. He has had impulse control behaviors (hypersexuality and pathological gambling) over the last 5 years probably related to dopamine-agonist therapy.

On clinical examination at the age of 60 years, the patient had evidence of rigidity, rest tremor, bradykinesia, and postural instability. Motor fluctuations were apparent with peak-dose dyskinesias while subjectively “on” and marked gait impairment and freezing during the subjective “off” state. Profuse hyperhidrosis was present with no other features of dysautonomia. There were no ocu-lomotor, cerebellar, or corticospinal tract signs. Although his MMSE scores remained normal, his MoCA score revealed a cognitive deficit chiefly affecting memory. Smell was also impaired (Table 2).

Transcranial sonography did not reveal substantia nigra hyperechogenicity on the left side, while the bone window of the right side was insufficient. The cerebral magnetic resonance imaging and electroencephalogram were normal.

The patient has 6 siblings, all reportedly unaffected. His father (II:1) developed PD in his late 40s and dementia in his late 70s. His paternal aunt (II:4) had PD with disease onset as lower limb tremor in her 60s. She received deep brain stimulation, which gave little benefit but was complicated by dysarthria. His paternal uncle (II:3) and grandfather (I:1) have also received a diagnosis of PD. Genetic analysis of the extended family revealed that the affected paternal aunt and 3 of the ap-

### Table 1. Demographics of the PD Sample Screened for the p.D620N Variant in VPS35

<table>
<thead>
<tr>
<th>PD Population</th>
<th>Sample Size</th>
<th>Sex M:F</th>
<th>Mean (SD), y</th>
<th>Age at Onset</th>
<th>Family History of PD, No. (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>692</td>
<td>1:0.7</td>
<td>65.1 (11.1)</td>
<td>51.5 (14.2)</td>
<td>276 (31.5)</td>
</tr>
<tr>
<td>Serbia</td>
<td>418</td>
<td>1:0.6</td>
<td>59.9 (10.6)</td>
<td>50.8 (11.9)</td>
<td>71 (46.5)</td>
</tr>
<tr>
<td>United States</td>
<td>441</td>
<td>1:0.6</td>
<td>66.0 (8.6)</td>
<td>59.1 (10.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Chile</td>
<td>223</td>
<td>1:0.9</td>
<td>67.3 (11.2)</td>
<td>60.3 (10.8)</td>
<td>192 (22.4)</td>
</tr>
<tr>
<td>Total</td>
<td>1774</td>
<td>1:0.7</td>
<td>64.4 (11.6)</td>
<td>54.3 (12.5)</td>
<td>539 (30.2)</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; PD, Parkinson disease.  
*a Number of patients for whom these data are accessible.

### Table 2. Longitudinal Comparison of Clinical Findings and Rating Scales

<table>
<thead>
<tr>
<th></th>
<th>2 Years Postsymptom Onset, Age 47 y</th>
<th>15 Years Postsymptom Onset, Age 60 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinesia/tremor/rigidity/postural instability</td>
<td>Yes/yes/no</td>
<td>Yes/yes/yes</td>
</tr>
<tr>
<td>Motor fluctuations</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>UPDRS-III/IV scores</td>
<td>11/2</td>
<td>60/6</td>
</tr>
<tr>
<td>H&amp;Y score</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Schwab and England Scale score</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>MMSE score</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>PANDA score/possible total score</td>
<td>Not performed</td>
<td>17/30</td>
</tr>
<tr>
<td>MoCA score/possible total score</td>
<td>Not performed</td>
<td>23/30</td>
</tr>
<tr>
<td>SCID</td>
<td>Not performed</td>
<td>No psychiatric diagnosis</td>
</tr>
<tr>
<td>UPSIT percentile/interpretation</td>
<td>Not performed</td>
<td>10th/Severe hyposmia</td>
</tr>
</tbody>
</table>

Abbreviations: H&Y, Hoehn and Yahr Scale; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PANDA, Parkinson Neuropsychometric Dementia Assessment; Schwab and England, Schwab and England Activities of Daily Living Scale; SCID, Structured Clinical Interview for DSM-IV; UPDRS, United Parkinson’s Disease Rating Scale; UPSIT, University of Pennsylvania Smell Identification Test.
parently unaffected siblings also carried the mutation. The proband’s relatives were not available for clinical assessment. The mutation-bearing haplotype was identified (Figure).

**COMMENT**

VPS35 is a component of the retromer complex, which mediates retrograde transport of transmembrane proteins from endosomes to the trans-Golgi network. Mutations in VPS35 may result in impaired cargo recognition and binding, leading to defective receptor recycling.

VPS35 mutations are a rare cause of PD. Combining our data with previous studies gives a carrier frequency for the p.D620N mutation of 0.001 (0.1%) among patients with PD. The p.D620N is rare across multiple ethnic groups including Eastern European and South American populations. Compared with other causes of

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**Figure.** Pedigree of the mutation carrier. Filled symbols indicate individuals with Parkinson disease; a dot within the symbol marks reportedly unaffected mutation carriers; an arrow indicates the proband; and slashed symbols mark deceased relatives. M indicates mutation and wt, wild type. The age of family members at the time of genetic analysis is also listed. Respective haplotypes are given below the symbols with allele sizes in base pairs according to CEPH (Centre d’Etudes du Polymorphisme Humaine) standards. The mutation-bearing haplotype is boxed.
autosomal dominant PD, the p.D620N mutation of VPS35 has a lower frequency than LRRK2 mutations\(^1\) and probably has a similar frequency to missense mutations in SNCA.\(^8\) Screening of the remaining exons of VPS35 could also be considered. The p.D620N mutation seems to be a mutational hotspot and may be the only disease-associated variant in VPS35 since screening of all exons of VPS35 in more than 1000 patients did not reveal any other mutation with clear pathogenicity.\(^1,2\)

To our knowledge, the index patient is the first identified p.D620N mutation carrier from Germany and had young-onset (<50 years) PD. The phenotype resembled the idiopathic form of the disorder with asymmetry and levodopa responsiveness. However, leg tremor as the initial symptom (that was also found in his aunt) occurs in only 2% of patients with pathologically confirmed idiopathic PD but may also be more frequent among carriers of LRRK2 mutations.\(^9\) The progression of the disease in the German patient appears to be consistent with idiopathic PD.\(^10\) Genetic testing of the proband’s family confirmed that the mutation cosegregates with disease in affected family members. The identification of several apparently unaffected mutation carriers (age range, 54–62 years) is consistent with previous reports that the mutation has incomplete, age-associated penetrance.\(^1,2\) Haplotype analysis underlines recurrent mutational events of this mutation since there was no shared haplotype with previously reported cases\(^1\) (Figure).

Evidence has emerged of a link between retromer complex dysfunction and Alzheimer disease etiology,\(^7\) prompting interest in the cognitive function of VPS35 mutation carriers. Interestingly, in the Swiss kindred, 2 affected subjects developed dementia several years after disease onset.\(^3\) The German patient’s MoCA score would be consistent with mild cognitive impairment. Although the MMSE score was normal, MoCA is superior to MMSE in discriminating between patients with PD who have mild cognitive impairment and those who have normal cognition.\(^11\) Notably, about 25% of patients with idiopathic PD without dementia can be classified as having mild cognitive impairment.\(^12\) Further studies are necessary to determine whether dementia is more prominent among patients with PD and VPS35 mutations.

It is curious that the transcranial sonography did not reveal abnormal echogenicity of the substantia nigra, as this is found in 90% of patients with idiopathic PD.\(^13\) Similarly, hyperechogenicity was also absent in patients with PD and mutations in ATP13A2.\(^6\) Given that the nature of the pathological substrate leading to the abnormal echogenicity is unknown, it is difficult to draw any pathophysiological inferences from this finding.

In conclusion, there is compelling evidence that mutations in the VPS35 gene are a rare cause of PD with a phenotype resembling the idiopathic form of this disorder. Pathological and functional studies are now required to determine the role of VPS35 mutations in the pathogenesis of PD and to further elucidate the link with Alzheimer disease.

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Author Contributions: Dr Lohmann had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Kumar, Weissbach, Klein, and Lohmann. Acquisition of data: Kumar, Weissbach, Heldmann, Kasten, Tunc, Svetel, Kostić, Segura-Aguilar, Vieregge, Münte, Hagenah, and Klein. Analysis and interpretation of data: Kumar, Weissbach, Heldmann, Kasten, Sue, Ramirez, Simon, Münte, and Lohmann. Drafting of the manuscript: Kumar and Münte. Critical revision of the manuscript for important intellectual content: Kumar, Weissbach, Heldmann, Kasten, Tunc, Sue, Svetel, Kostić, Segura-Aguilar, Ramirez, Simon, Vieregge, Münte, Hagenah, Klein, and Lohmann. Statistical analysis: Kumar, Kasten, and Münte. Obtained funding: Weissbach, Svetel, Münte, and Klein. Administrative, technical, and material support: Weissbach, Tunc, Sue, Ramirez, Simon, Vieregge, Münte, and Klein. Study supervision: Weissbach, Sue, Klein, and Lohmann.

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References


