Glucocerebrosidase mutations are an important risk factor for Lewy body disorders

O. Goker-Alpan, MD; B.I. Giasson, PhD; M.J. Eblan, BA; J. Nguyen, BA; H.I. Hurtig, MD; V.M.-Y. Lee, PhD; J.Q. Trojanowski, MD, PhD; and E. Sidransky, MD

The synucleinopathies are clinically diverse neurodegenerative disorders, including Parkinson disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), characterized by aberrant fibrillization of α-synuclein in neuronal and glial cell populations. PD presents with motor dysfunction, and pathologic features that include the loss of dopaminergic neurons in the substantia nigra and the presence of neuronal α-synuclein inclusions known as Lewy bodies and Lewy neurites. DLB is a neurodegenerative dementia with parkinsonian symptoms, widespread cortical and brainstem inclusions, and sometimes senile plaques and neurofibrillary tangles. MSA presents with parkinsonism, ataxia, and autonomic dysfunction with α-synuclein inclusions in oligodendrocytes.1

While mutations that increase the propensity of α-synuclein to aggregate or increase the expression of wild-type α-synuclein result in rare, inherited parkinsonian syndromes, the etiology of most synucleinopathies is unknown. Recent clinical observations and neuropathologic data suggest that alterations in the glucocerebrosidase gene (GBA) may contribute to a vulnerability for the development of parkinsonism. Parkinsonian manifestations have been noted in a subset of patients with Gaucher disease, the inherited deficiency of glucocerebrosidase, and there is evidence that parkinsonism is more frequent among carrier relatives of subjects with Gaucher disease.2,3

In complementary studies, mutations in the GBA gene have been encountered with increased frequency in several cohorts of subjects with parkinsonism, suggesting their role as a risk factor for the development of parkinsonian symptoms.4,7 In one study using brain bank samples, we demonstrated that eight of 57 subjects with a diagnosis of PD (14%) harbored GBA mutations.6 Two other studies in clinically defined Ashkenazi Jewish cohorts identified GBA mutations in between 10% and 31% of their subjects with PD.4,5

Methods. To investigate the role of GBA in specific autopsy-confirmed disease entities, all 11 exons and flanking intronic regions of GBA were sequenced as previously described4 in a unique cohort of 75 autopsied subjects with synucleinopathies pathologically characterized at the University of Pennsylvania Center for Neurodegenerative Disease Research. The subjects were of mixed ethnicity (71 Caucasians, 2 Asians, and 2 African-Americans) but unknown ancestry, with a mean age at death of 76 years. This small sample included 35 cases with the pathologic diagnosis of DBL, 29 with PD, and 12 with MSA. The subjects were also screened for another mutation associated with parkinsonism and pleomorphic pathology, LRRK2 G2019S, using a Taqman Assay-by-Design SNP strategy.8

Results. Nine of the 75 subjects (12%) were heterozygous for GBA mutations (table 1). This incidence is significantly greater than the background carrier frequency for Gaucher mutations in the general population (0.006) or in the high-risk Ashkenazi Jewish population (0.0343).6 Moreover, it is markedly higher than the mutation rate encountered among screened non-Parkinson controls.4,5,7 Although this brain bank did not collect control tissues, in our previous study we did not observe any GBA mutations among a small cohort of 44 control autopsy samples (23 males and 21 females) from subjects of mixed ancestry (28 Caucasians, 1 Asian, 1 Hispanic, 5 African-Americans, and 9 unknown) with a mean age at death of 71 years.9

Among the 9 subjects carrying GBA mutations, 5 had the common N370S mutation, 2 carried rare mutations (R120W and A359X), and 2 had novel alterations (T267I and I161N). Each novel mutation was confirmed by amplification with alternate PCR primers, and neither has been found in more than 500 previously genotyped Gaucher alleles, 400 alleles from subjects with PD, and 300 alleles from screened adult controls. Both amino acids are found

This article was previously published in electronic format as an Expedited E-Pub at www.neurology.org.

From the Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD (O.G.-A., M.J.E., J.N., E.S.); and the Departments of Pharmacology (B.I.G.), Neurology (H.I.H.), and Pathology and Laboratory Medicine (V.M.-Y.L., J.Q.T.), University of Pennsylvania School of Medicine, Philadelphia, PA.

This research was supported by the Intramural Research Program of the NIH, NHGRI, and NIMH and by NIA AG09215.

Disclosure: The authors report no conflicts of interest.

Received November 14, 2005. Accepted in final form May 4, 2006.

Address correspondence and reprint requests to Dr. Ellen Sidransky, Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, Building 35, Room 1A100, 35 Convent Drive, MSC 3708, Bethesda, MD 20892-3708; e-mail: sidrans@nih.gov

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in Domain III of glucocerebrosidase, the region forming the catalytic domain. Six of the 75 subjects carried E326K, and 1 carried T369M, both alterations that have been identified in control alleles and are likely to be polymorphisms.

**Discussion.** The vast majority of GBA mutations were identified among subjects with DLB, where the mutation rate was 23% (table 1). Only 1 of 28 subjects with classic PD (4%) had a GBA mutation, and no mutations were found among 12 subjects with MSA. In contrast with earlier reports, the parkinsonian subjects harboring GBA mutations did not have an earlier age at onset, although their disease progression may have been faster. The clinical and pathologic features of the 9 subjects with GBA mutations are summarized in table 2. Of note, all 9 identified heterozygotes were Caucasian males, and all developed dementia. The reason for the male preponderance is not clear, but the subjects with GBA mutations typically result in decreased catalytic function, identified were missense. Although missense mutations are not available; C = Caucasian; PD = Parkinson disease.

**Table 2 Clinical and pathological features of patients with GBA mutations**

<table>
<thead>
<tr>
<th>Subject</th>
<th>GBA genotype</th>
<th>Sex/ethnicity</th>
<th>Age at onset, y</th>
<th>Age at death, y</th>
<th>Initial clinical presentation</th>
<th>1-Dopa response</th>
<th>Dementia</th>
<th>Neuropathologic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N370S/wt</td>
<td>M/C</td>
<td>78</td>
<td>90</td>
<td>Cognitive impairment</td>
<td>Not given</td>
<td>Yes</td>
<td>DLB/AD</td>
</tr>
<tr>
<td>2</td>
<td>T267I + E326K/wt</td>
<td>M/C</td>
<td>54</td>
<td>75</td>
<td>Parkinsonism</td>
<td>Yes</td>
<td>Yes</td>
<td>DLB</td>
</tr>
<tr>
<td>3</td>
<td>I161N/wt</td>
<td>M/C</td>
<td>72</td>
<td>81</td>
<td>Cognitive impairment</td>
<td>Yes</td>
<td>Yes</td>
<td>DLB/AD</td>
</tr>
<tr>
<td>4</td>
<td>A350X/wt</td>
<td>M/C</td>
<td>37</td>
<td>58</td>
<td>Parkinsonism</td>
<td>Yes</td>
<td>Yes</td>
<td>DLB</td>
</tr>
<tr>
<td>5</td>
<td>N370S/wt</td>
<td>M/C</td>
<td>NA</td>
<td>77</td>
<td>Difficulties with word retrieval</td>
<td>Not given</td>
<td>Yes</td>
<td>DLB/AD</td>
</tr>
<tr>
<td>6</td>
<td>R120W/wt</td>
<td>M/C</td>
<td>73</td>
<td>79</td>
<td>Parkinsonism</td>
<td>NA</td>
<td>Yes</td>
<td>DLB/AD</td>
</tr>
<tr>
<td>7</td>
<td>N370S/wt</td>
<td>M/C</td>
<td>81</td>
<td>86</td>
<td>Parkinsonism</td>
<td>Yes</td>
<td>Yes</td>
<td>PD</td>
</tr>
<tr>
<td>8</td>
<td>N370S/wt</td>
<td>M/C</td>
<td>62</td>
<td>66</td>
<td>Parkinsonism</td>
<td>Yes</td>
<td>Yes</td>
<td>DLB/AD</td>
</tr>
<tr>
<td>9</td>
<td>N370S/wt</td>
<td>M/C</td>
<td>70</td>
<td>78</td>
<td>Parkinsonism</td>
<td>Yes</td>
<td>Yes</td>
<td>DLB/AD</td>
</tr>
</tbody>
</table>

All patients developed Parkinsonism and presented with Lewy bodies in dopaminergic neurons of the substantia nigra pars compacta. Patients characterized as dementia with Lewy bodies (DLB) presented with widespread cortical and brainstem Lewy pathology consistent with consensus criteria. A concomitant diagnosis of Alzheimer disease (AD) was based on consensus criteria for the presence of senile plaques and neurofibrillary tangles.
phagy in lysosomes,10 where mutant glucocerebro-
side could disrupt lysosomal function and interfere
with synuclein clearance. The absence of GBA muta-
tions among the subjects with MSA, a disorder
where α-synuclein is found predominantly in oligo-
dendrocytes, and the specificity of brain pathology in
Gaucher disease may implicate a specific contribu-
tion of mutant GBA to intraneuronal pathology. GBA
mutations also seem to be associated with the more
widespread neuronal aggregation of α-synuclein
characteristic of DLB rather than the more regional
distribution typical of PD.

The observation that certain synucleinopathies
are associated with mutations in GBA both in het-
erozygotes and in homozygotes suggests that the
enzyme’s substrate, glucocerebroside, is unlikely to be
the culprit. This serves as an example of how an
enzyme, when mutated, may take on a totally differ-
ent and unexpected role unrelated to its primary
function and contribute to the pathogenesis of a com-
mon complex disorder.

The identification of GBA mutations in DLB is
particularly significant because this is among the
first examples of a genetic change associated with
this diagnosis. By unraveling the relationship be-
tween altered glucocerebrosidase and α-synuclein,
we may advance our understanding of the mecha-
nisms and etiologies involved in the pathobiology of
specific synucleinopathies.

Acknowledgment
The authors thank Mary E. LaMarca for her editorial assistance.

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