Association of Glucocerebrosidase Mutations With Dementia With Lewy Bodies

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Background: Mutations in the glucocerebrosidase (GBA) gene are associated with Lewy body (LB) disorders.

Objective: To determine the relationship of GBA mutations and APOE4 genotype to LB and Alzheimer disease (AD) pathological findings.

Design: Case-control study.

Setting: Academic research.

Participants: The 187 subjects included patients with primary neuropathological diagnoses of LB disorders with or without AD changes (95 cases), randomly selected patients with AD (without significant LB pathological findings; 60 cases), and controls with neither LB nor AD pathological findings (32 cases).

Main Outcome Measures: GBA mutation status, APOE4 genotype, LB pathological findings (assessed according to the third report of the Dementia With Lewy Body Consortium), and Alzheimer plaque and tangle pathological findings (rated by criteria of Braak and Braak, the Consortium to Establish a Registry for Alzheimer Disease, and the National Institute on Aging–Reagan Institute).

Results: GBA mutations were found in 18% (34 of 187) of all subjects, including 28% (27 of 95) of those with primary LB pathological findings compared with 10% (6 of 60) of those with AD pathological findings and 3% (1 of 32) of those without AD or LB pathological findings (P=.001). GBA mutation status was significantly associated with the presence of cortical LBs (odds ratio, 6.48; 95% confidence interval, 2.45-17.16; P<.001), after adjusting for sex, age at death, and presence of APOE4. GBA mutation carriers were significantly less likely to meet AD pathological diagnostic (National Institute on Aging–Reagan Institute intermediate or high likelihood) criteria (odds ratio, 0.35; 95% confidence interval, 0.15-0.79; P=.01) after adjustment for sex, age at death, and APOE4.

Conclusion: GBA mutations may be associated with pathologically “purer” LB disorders, characterized by more extensive (cortical) LB, and less severe AD pathological findings and may be a useful marker for LB disorders.

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Molecular Genetic Analysis

Frozen cerebellar tissue was used to extract DNA by means of a kit (Genta Puregene; Qiagen, Valencia, California) following the manufacturer's instructions. All GBA exons were sequenced by means of polymerase chain reaction and sequencing primers described previously.22 Cycle sequencing in forward and reverse directions was performed on purified polymerase chain reaction products and run on a genetic analyzer (ABI 3700; Applied Biosystems, Foster City, California). Sequence chromatograms were viewed and genotypes determined by means of Sequencher (Gene Codes Corp, Ann Arbor, Michigan). APOE4 genotyping was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on the Sequenom, Inc (San Diego, California) platform as described previously.23

Statistical Analysis

Continuous and categorical variables were compared by t tests and χ² tests, respectively. Logistic regression models were constructed to examine the association of GBA mutation carrier status with the presence of each pathological diagnosis adjusting for sex, age at death, and the presence of APOE4.

Results

GBA Mutations

Table 1 lists all cases with mutations, together with age at onset of cognitive or extrapyramidal motor disorder, initial clinical presentation, and pathological diagnosis. GBA mutations were found in 34 of the 187 subjects (18%). We classified mutation types in Table 1 as having null, severe, mild, or unknown phenotypic effect according to previously published studies.12,24 Overall, 5 of 34 subjects with a GBA mutation (15%) had a null or severe mutation, although none of these was homozygous. Among the 17 different GBA mutations found in 34 individuals, 14 were missense mutations, 1 was an insertion mutation, 1 was a silent mutation (synonymous substitution), and 1 was a nucleotide substitution located in the noncoding region of exon 1 (g.1444A>G; -15 of the ATG start codon). With the exception of 5 variants (g.1444A>G, E388K, G389V, P171P, and N188R), all mutations have been reported previously.25 These 5 variants were not found in the 32 control brains reported on herein nor in an additional 179 controls described previously.26 Table 2 shows basic demographic and neuropathological information on carriers and noncarriers of GBA mutations. There was no difference in age at death or duration of symptoms in the carriers compared with noncarriers. Carriers were younger at diagnosis of dementia (63.2 vs 69.1 years; P = .01). There was no difference in ethnicity between carriers and noncarriers.

Relationship of GBA to Pathological Diagnoses

GBA mutations were found in 28% (27 of 95) of those with primary pathological diagnoses of LB disorders, compared with 10% (6 of 60) of cases with primary AD and 3% (1 of 32) of control cases containing neither AD nor LB pathological findings (P < .001). GBA mutations were not significantly more frequent among primary AD cases than among controls. GBA mutation carriers were significantly more likely to have cortical LBs (28 of 34 [82%]) than were nonmutation carriers (66 of 153 [43%]; P < .001). Presence of a GBA mutation appeared to relate more to the presence of cortical LBs than to LBs confined to the subcortical regions, but there were only 14 cases of the latter (Table 2).

In contrast to the greater likelihood of GBA mutation carriers to have LBs, these carriers were significantly less likely to meet NIA-RI pathological criteria for AD (13 of 34 [38%]) than were GBA mutation noncarriers (96 of 153 [63%]; P = .01) (Table 2). No significant difference was seen among carriers and noncarriers of APOE4 among cases with any LB pathological findings, cortical LB pathological findings, or presence of any AD pathological changes. There were significantly more APOE4 carriers who had AD pathological findings; more APOE4 carriers met NIA-RI criteria for AD (59 of 79 [75%]) than did non–APOE4 carriers (50 of 107 [47%]; P < .001). Examining both APOE4 and GBA mutation status conjointly among the 109 cases with pathological diagnosis of AD, the least frequent category was that of persons who were GBA carriers and APOE4 noncarriers (6 of 109 [6%]; Table 3).

Relationship of GBA Mutation Status to Cortical LB and AD Pathological Findings

In separate logistic models, GBA mutation carrier status was significantly associated with the presence of cortical LBs, not only adjusting for sex and age at death but also in models additionally including the...
presence of AD pathological findings, presence of APOE4, and a clinical diagnosis of dementia (Table 4). APOE4 was not independently associated with the presence of cortical LBs in any of the models. Analyses in which the dependent variable was the presence of any LB pathological finding, rather than just the presence of cortical LBs, gave similar results (because there were only 14 cases in which LB pathological findings were confined to the brainstem). As shown in Table 4, the presence of the GBA mutation was inversely associated with the presence of a pathological diagnosis of AD, even after adjustment for age at death, sex, and the presence of APOE4 (odds ratio, 0.35; 95% confidence interval, 0.15-0.79; P=.01), although APOE4 was significantly associated with AD pathological diagnosis in this model (3.97, 1.97-8.04; P<.001).

We have shown that carriers of GBA mutations are significantly more likely than noncarriers to have cortical LB pathological findings. This is true when adjusting for sex, age at death, the presence of AD pathological findings, APOE4, and clinical diagnosis of dementia. The presence of a GBA mutation is not associated with AD pathological findings, whereas APOE4 is independently associated with AD diagnostic pathological findings in the same model, suggesting that GBA mutation status may be a useful clinical marker in the accurate diagnosis of LB disorders.

GBA mutations are not exclusively present in cases with LBs, even in this autopsy series of elderly persons. One case with no significant AD or LB pathological findings

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**Table 1. Characteristics of Autopsy Cases With Identified GBA Mutations**

<table>
<thead>
<tr>
<th>GBA Mutation/ Severity</th>
<th>cDNA Nucleotide Substitution</th>
<th>Exon</th>
<th>Zygosity</th>
<th>Age, y</th>
<th>Symptom</th>
<th>Primary Pathological Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>c.84dupG</td>
<td>2</td>
<td>Heterozygous</td>
<td>66</td>
<td>Memory problems</td>
<td>DLB</td>
</tr>
<tr>
<td>Severe</td>
<td>c.882T&gt;G</td>
<td>7</td>
<td>Heterozygous</td>
<td>55</td>
<td>Behavior change</td>
<td>DLB</td>
</tr>
<tr>
<td>H255Q</td>
<td>c.1342G&gt;C</td>
<td>9</td>
<td>Heterozygous</td>
<td>71</td>
<td>Memory problems</td>
<td>DLB</td>
</tr>
<tr>
<td>L444P</td>
<td>c.1504C&gt;T</td>
<td>10</td>
<td>Heterozygous</td>
<td>55</td>
<td>Parkinsonism</td>
<td>DLB</td>
</tr>
<tr>
<td>Mild</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>68</td>
<td>Memory problems</td>
<td>LBV-AD</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>62</td>
<td>Parkinsonism</td>
<td>DLB</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>58</td>
<td>Parkinsonism</td>
<td>DLB</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>74</td>
<td>Parkinsonism</td>
<td>DLB</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>69</td>
<td>Parkinsonism</td>
<td>DLB</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>NA</td>
<td>NA</td>
<td>DLB</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>54</td>
<td>Parkinsonism</td>
<td>DLB</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>55</td>
<td>Memory problems</td>
<td>DLB</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>69</td>
<td>Memory problems</td>
<td>LBV-AD</td>
</tr>
<tr>
<td>N370S</td>
<td>c.1226A&gt;G</td>
<td>9</td>
<td>Heterozygous</td>
<td>NA</td>
<td>NA</td>
<td>DLB</td>
</tr>
<tr>
<td>R496H</td>
<td>c.1604G&gt;A</td>
<td>11</td>
<td>Heterozygous</td>
<td>74</td>
<td>Parkinsonism</td>
<td>DLB</td>
</tr>
</tbody>
</table>

**Abbreviations:** AD, Alzheimer disease; cDNA, complementary DNA; DLB, dementia with Lewy bodies; LBV-AD, Lewy body variant of Alzheimer disease; NA, not available; PD, Parkinson disease.

*a* Mutations are classified as having null, severe, mild, and unknown effect on the expected clinical phenotype according to Beutler et al.*

*b* Genomic nucleotide position is based on the accession file GenBank J03059.1, and GBA cDNA nucleotides are numbered according to the GenBank sequence NM_000157.2.
and 10 cases with autopsy-proved primary AD (4 of which lacked any LB pathological findings) nonetheless had GBA mutations. A previous report analyzing GBA status in a clinic-based series of 74 Ashkenazi patients with AD genotyped for only 6 mutations (N370S, L444P, 84GG, IVS+1, V394L, and R496H) found that 4% (3 of 74) carried a GBA mutation. This is not dissimilar from our observed mutation frequency of 10% (6 of 60) in cases with primary pathological diagnoses of AD; 2 of these cases had rare LBs, but 4 had complete absence of any discernible LBs. The mutations in these 6 cases were all of the mild or unknown function type.

Two previously published studies have examined GBA genotype in neuropathologically confirmed DLB cases from autopsy series. 17,18 Mata et al 18 examined 57 cases with DLB (54 had autopsies) and found a mutation frequency of only 3.5%, but they sequenced for only 2 GBA mutations (N370S and L444P). Goker-Alpan et al 17 examined GBA genotype in a clinic-based series of 74 Ashkenazi patients with AD genotyped for only 6 mutations (N370S, L444P, 84GG, IVS+1, V394L, and R496H) found that 4% (3 of 74) carried a GBA mutation. This is not dissimilar from our observed mutation frequency of 10% (6 of 60) in cases with primary pathological diagnoses of AD; 2 of these cases had rare LBs, but 4 had complete absence of any discernible LBs. The mutations in these 6 cases were all of the mild or unknown function type.

### Table 2. Demographic and Neuropathological Characteristics of Subjects Examined at Autopsy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No GBA Mutation (n=153)</th>
<th>GBA Mutation (n=34)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No. (%) male</td>
<td>79 (52)</td>
<td>21 (62)</td>
<td>.34</td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td></td>
<td></td>
<td>.26</td>
</tr>
<tr>
<td>White</td>
<td>123 (80)</td>
<td>32 (94)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>7 (5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2 (1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>21 (14)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td>Age at death, mean (SD), y (n = 187)</td>
<td>77.8 (11)</td>
<td>76.0 (6.7)</td>
<td>.21</td>
</tr>
<tr>
<td>Age at dementia (total), mean (SD), y (n=113)</td>
<td>69.1 (10)</td>
<td>63.2 (6.7)</td>
<td>.01</td>
</tr>
<tr>
<td>Duration from onset of dementia or parkinsonism to death, mean (SD), y (n=113)</td>
<td>10.0 (8)</td>
<td>12.6 (5.8)</td>
<td>.07</td>
</tr>
<tr>
<td>No. (%) with Lewy bodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>79 (52)</td>
<td>29 (85)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cortical</td>
<td>66 (43)</td>
<td>28 (82)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Subcortical</td>
<td>13 (8)</td>
<td>1 (3)</td>
<td>.24</td>
</tr>
<tr>
<td>No. (%) with pathological findings of AD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>123 (80)</td>
<td>29 (85)</td>
<td>.35</td>
</tr>
<tr>
<td>Diagnosis (NIA-RI)</td>
<td>96 (63)</td>
<td>13 (38)</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; NIA-RI, National Institute on Aging–Reagan Institute.

### Table 3. Distribution of GBA and APOE4 by Pathological Diagnosis

<table>
<thead>
<tr>
<th>Pathological Findings, No. (%)</th>
<th>Lewy Body</th>
<th>Alzheimer Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brainstem Only (n=13)</td>
<td>Cortical (n=85)</td>
</tr>
<tr>
<td>GBA+</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>APOE4−</td>
<td>19</td>
<td>1 (8)</td>
</tr>
<tr>
<td>APOE4+</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>GBA−</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>APOE4−</td>
<td>88</td>
<td>11 (85)</td>
</tr>
<tr>
<td>APOE4+</td>
<td>64</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>

*One subject did not have an APOE genotype.

### Table 4. Association of GBA Mutation With the Presence of Cortical Lewy Bodies or AD Pathological Diagnosis

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>P Value</th>
<th>Covariates in Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cortical Lewy Bodies</td>
</tr>
<tr>
<td>7.24 (2.69-19.48)</td>
<td>&lt;.001</td>
<td>Age at death, sex, AD pathological diagnosis</td>
</tr>
<tr>
<td>6.62 (2.41-18.27)</td>
<td>&lt;.001</td>
<td>Age at death, sex, any AD pathological findings</td>
</tr>
<tr>
<td>6.48 (2.45-17.16)</td>
<td>&lt;.001</td>
<td>Age at death, sex, APOE4</td>
</tr>
<tr>
<td>5.91 (2.14-16.33)</td>
<td>&lt;.001</td>
<td>Age at death, sex, APOE4, dementia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD Pathological Diagnosis</td>
</tr>
<tr>
<td>0.40 (0.18-0.89)</td>
<td>.03</td>
<td>Age at death, sex</td>
</tr>
<tr>
<td>0.35 (0.15-0.79)</td>
<td>.01</td>
<td>Age at death, sex, APOE4 (for APOE4: OR, 3.97; 95% CI, 1.97-8.04; P&lt;.001)</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; CI, confidence interval; OR, odds ratio.
perform full genotyping of GBA in 63 cases with LB pathological findings including 35 with cortical LBs (DLB or LBV-AD) and 28 with pure PD. They found an overall GBA mutation frequency of 14% (9 of 63) among the LB cases but 29% (8 of 28) among the cases with cortical LB. Thus, the frequency of GBA mutations of 28% that we found in a set of LB cases (of which few had pure PD pathological findings) is similar to that observed in the previous studies. Our study, which examined not only individuals with LB pathological findings but also those with AD and some with neither AD nor LB pathological findings and included genotyping for APOE, shows that GBA is a marker for cortical LB pathological findings, independent of AD pathological features, and is unrelated to APOE genotype.

We, like Goker-Alpan et al., observed a higher frequency of GBA mutation carriers among those with cortical LBs than among those with only brainstem LBs (pure PD), although we had proportionately few cases with pure PD. If this finding should be further confirmed, there are several possible explanations, including that GBA relates specifically to cortical LB degeneration, as differentiated from pure PD, or that GBA relates to some combination of age at onset, rapidity of disease progression, and mortality. We are currently expanding our studies to distinguish between these possibilities.

It is unclear whether specific mutations in the GBA gene are more likely to be associated with specific phenotypic responses. In the study of Goker-Alpan et al., 9 of 63 subjects with LBs (14%) carried a GBA mutation including N370S (n = 5), R120W (n = 1), A359X (n = 1), T267I (n = 1), and I161N (n = 1). We also found that N370S was the most frequent mutation in subjects with LBs; this mutation was found in 29% (10 of 34) of our GBA mutation carriers. We also observed additional mutations that have been previously reported in PD cases and that are reported to be pathogenic in cases of Gaucher disease. Five mutations identified in our study are novel. Three of these are missense mutations; 1 is a silent mutation (synonymous substitution), and 1 nucleotide substitution is located in the noncoding promoter region of exon 1. Currently, the pathogenicity of these mutations is unknown, and functional studies will be needed to determine their effects on the GBA protein. The mechanism by which GBA mutations might increase the likelihood of LB disease, such as DLB, is unclear. The mutations are nearly exclusively heterozygous and many are deemed “mild” for Gaucher disease even if homozygous, so it is unlikely but possible that gene product insufficiency might be the predisposing factor. More likely, alterations in GBA might affect lysosomal protein degradational processes, increasing the likelihood of aberrant α-synuclein processing, and LB neurodegeneration.

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Author Contributions: Study concept and design: Clark, Karstaklis, Mayeux, Honig, and Marder. Acquisition of data: Clark, Karstaklis, Wolf Gilbert, Dorado, Ross, Kisselev, Verbitsky, Mejia-Santana, Cote, Andrews, Vonsattel, Fahn, and Honig. Analysis and interpretation of data: Clark, Karstaklis, Wolf Gilbert, Dorado, Meja-Santana, Mayeux, and Honig. Drafting of the manuscript: Clark, Karstaklis, Wolf Gilbert, Dorado, Ross, Verbitsky, Mejia-Santana, Vonsattel, Honig, and Marder. Critical revision of the manuscript for important intellectual content: Clark, Kisselev, Cote, Andrews, Fahn, Mayeux, and Honig. Statistical analysis: Karstaklis, Andrews, and Honig. Obtained funding: Clark, Wolf Gilbert, Cote, and Honig. Administrative, technical, and material support: Clark, Ross, Verbitsky, Andrews, Vonsattel, Honig, and Marder. Study supervision: Clark, Verbitsky, Mejia-Santana, and Mayeux. Neuropathological evaluation: Vonsattel.

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REFERENCES

are associated with Parkinson’s disease in southern Italy. Mov Disord. 2008;23(3):460-463.

Announcement

Trial Registration Required. In concert with the International Committee of Medical Journal Editors (ICMJE), Archives of Neurology will require, as a condition of consideration for publication, registration of all trials in a public trials registry (such as http://ClinicalTrials.gov). Trials must be registered at or before the onset of patient enrollment. This policy applies to any clinical trial starting enrollment after July 1, 2005. For trials that began enrollment before this date, registration will be required by September 13, 2003, before considering the trial for publication. The trial registration number should be supplied at the time of submission.

For details about this new policy, and for information on how the ICMJE defines a clinical trial, see the editorial by DeAngelis et al in the January issue of Archives of Dermatology (2005;141:76-77). Also see the Instructions to Authors on our Web site: www.archneurol.com.