Glucocerebrosidase mutations are an important risk factor for Lewy body disorders

Abstract—The synucleinopathies are neurodegenerative disorders defined by inclusions composed of aberrantly fibrillized α-synuclein, but factors contributing to this process remain largely unknown. The authors examined the glucocerebrosidase gene in 75 autopsy specimens with different synucleinopathies and identified mutations in 23% of cases of dementia with Lewy bodies, expanding on previous findings in subjects with Parkinson disease. Mutations in this lysosomal protein may interfere with the clearance or promote aggregation of α-synuclein.

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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 described[6] 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 DLB, 29 with PD, and 12 with MSA. The subjects were also screened for another mutation associated with parkinsonism and pleiomorphic 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.[6]

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

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Table 1 *Distribution of GBA mutations and clinical features by diagnosis in 75 subjects with synucleinopathies*

	Parkinson disease (n = 28)		Dementia with Lewy bodies (n = 35)		Multiple system atrophy (n = 12)	
Diagnosis (total cases) GBA mutation	Mutation present	No mutation	Mutation present	No mutation	Mutation present	No mutation
No. of cases (% of n)	1 M:0 F (4)	17 M:10 F (96)	8 M:0 F (23)	15 M:12 F (77)	0	7 M:5 F (100)
No. of cases with progressive dementia	1	6	8	27	—	2
Mean age at onset (range), y	81	62 (38–87)	65 (37–78)	63 (49–79)	—	58 (39–74)
Mean duration (range), y	5	16 (4–31)	11 (4–21)	16 (4–26)	—	8 (4–20)

The number of subjects with and without GBA mutations is indicated for each diagnosis, followed by the percentage of total cases with that diagnosis. Within each group, the sex distribution, number of subjects presenting with dementia, mean age at onset, and mean time until death are indicated.

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 in our previous series were also male.[6] However, the vast majority of the subjects in both cohorts were male, which may reflect sex differences in brain donation or in the frequency of different synucleinopathies.

This study greatly expands on previous reports because of the use of autopsy tissue from subjects with different synucleinopathies, confirmed by detailed pathologic characterization. This implicates the contribution of mutant GBA in different disease entities characterized by neuronal inclusions. Given the clinical diagnostic difficulties in distinguishing different parkinsonian syndromes, only pathologic diagnoses, provided by a single team of pathologists using current criteria, were used in this work. Taken in the context of other published studies regarding GBA mutations in subjects with classic PD,[4-7] it is clear that the association encompasses a spectrum of synuclein-related disorders. Although the entire cohort of 75 cases was not screened for mutations in all PD-related genes, none of the GBA mutation carriers had the LRRK2 G2019S alteration.

Studies of brains from subjects with Gaucher disease alone demonstrate unique pathology, with astrogliosis and neuronal loss specifically involving the hippocampal CA2–4 regions.[9] In patients with Gaucher disease and parkinsonism, synuclein-positive inclusions were identified in hippocampal CA2–4 neurons, whereas others had cortical pathology resembling DLB.[3,9] DLB is one of few disorders that selectively target the hippocampal CA2–3 region. Further studies in replicate samples are needed to verify the relationship between GBA and DLB.

As in previous studies, all of the GBA mutations identified were missense. Although missense mutations typically result in decreased catalytic function, they may also cause a conformational change in the protein that might enhance protein aggregation. Furthermore, recent evidence indicates that α-synuclein is degraded by chaperone-mediated au-

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

Subject	GBA genotype	Sex/ethnicity	Age at onset, y	Age at death, y	Initial clinical presentation	L-Dopa response	Dementia	Neuropathologic diagnosis
1	N370S/wt	M/C	78	90	Cognitive impairment	Not given	Yes	DLB/AD
2	T267I+E326K/wt	M/C	54	75	Parkinsonism	Yes	Yes	DLB
3	I161N/wt	M/C	72	81	Cognitive impairment	Yes	Yes	DLB/AD
4	A359X/wt	M/C	37	58	Parkinsonism	Yes	Yes	DLB
5	N370S/wt	M/C	NA	77	Difficulties with word retrieval	Not given	Yes	DLB/AD
6	R120W/wt	M/C	73	79	Parkinsonism	NA	Yes	DLB/AD
7	N370S/wt	M/C	81	86	Parkinsonism	Yes	Yes	PD
8	N370S/wt	M/C	62	66	Parkinsonism	Yes	Yes	DLB/AD
9	N370S/wt	M/C	70	78	Parkinsonism	Yes	Yes	DLB/AD

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.

wt = wild-type; NA = not available; C = Caucasian; PD = Parkinson disease.

tophagy in lysosomes,[10] where mutant glucocerebrosidase could disrupt lysosomal function and interfere with synuclein clearance. The absence of GBA mutations among the subjects with MSA, a disorder where α-synuclein is found predominantly in oligodendrocytes, and the specificity of brain pathology in Gaucher disease may implicate a specific contribution 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 heterozygotes 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 different and unexpected role unrelated to its primary function and contribute to the pathogenesis of a common 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 between altered glucocerebrosidase and α-synuclein, we may advance our understanding of the mechanisms and etiologies involved in the pathobiology of specific synucleinopathies.

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References

1. Galvin JE, Lee VM, Trojanowski JQ. Synucleinopathies: clinical and pathological implications. Arch Neurol 2001;58:186–190.
2. Goker-Alpan O, Schiffmann R, LaMarca ME, Nussbaum RL, McInerney-Leo A, Sidransky E. Parkinsonism among Gaucher disease carriers. J Med Genet 2004;41:937–940.
3. Tayebi N, Walker J, Stubblefield B, et al. Gaucher disease with parkinsonian manifestations: does glucocerebrosidase deficiency contribute to a vulnerability to parkinsonism? Mol Genet Metab 2003;79:104–109.
4. Aharon-Peretz J, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. N Engl J Med 2004;351:1972–1977.
5. Clark LN, Nicolai A, Afridi S, et al. Pilot association study of the beta-glucocerebrosidase N370S allele and Parkinson's disease in subjects of Jewish ethnicity. Mov Disord 2005;20:100–103.
6. Lwin A, Orvisky E, Goker-Alpan O, LaMarca ME, Sidransky E. Glucocerebrosidase mutations in subjects with parkinsonism. Mol Genet Metab 2004;81:70–73.
7. Sato C, Morgan A, Lang AE, et al. Analysis of the glucocerebrosidase gene in Parkinson's disease. Mov Disord 2005;20:367–370.
8. Nichols WC, Pankratz N, Hernandez D, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet 2005;365:410–412.
9. Wong K, Sidransky E, Verma A, et al. Neuropathology provides clues to the pathophysiology of Gaucher disease. Mol Genet Metab 2004;82:192–207.
10. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 2004;305:1292–1295.