The link between the GBA gene and parkinsonism

Ellen Sidransky, Grisel Lopez

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Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA (E Sidransky MD, G Lopez MD)

Correspondence to: Dr Ellen Sidransky, Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Building 35, Room 1A213, 35 Convent Drive, MSC 3708, Bethesda, MD 20892-3708, USA sidranse@mail.nih.gov Mutations in the glucocerebrosidase (*GBA*) gene, which encodes the lysosomal enzyme that is deficient in Gaucher's disease, are important and common risk factors for Parkinson's disease and related disorders. This association was first recognised in the clinic, where parkinsonism was noted, albeit rarely, in patients with Gaucher's disease and more frequently in relatives who were obligate carriers. Subsequently, findings from large studies showed that patients with Parkinson's disease and associated Lewy body disorders had an increased frequency of *GBA* mutations when compared with control individuals. Patients with *GBA*-associated parkinsonism exhibit varying parkinsonian phenotypes but tend to have an earlier age of onset and more associated cognitive changes than patients with parkinsonism without *GBA* mutations. Hypotheses proposed to explain this association include a gain-of-function due to mutations in glucocerebrosidase that promotes α -synuclein aggregation; substrate accumulation due to enzymatic loss-of-function, which affects α -synuclein processing and clearance; and a bidirectional feedback loop. Identification of the pathological mechanisms underlying *GBA*-associated parkinsonism will improve our understanding of the genetics, pathophysiology, and treatment for both rare and common neurological diseases.

Introduction

Recent progress in human genetics has resulted in dramatic advances in our understanding of Parkinson's disease and related disorders. Different genetic techniques have proven useful in unravelling the complexity of these multifactorial diseases. In some instances, the analysis of a pedigree with several affected members has led to the identification of linkage, and ultimately to a causative gene. Whole-genome investigations, such as genome-wide association studies of large cohorts, have directed investigators to important candidate genes. Recently, whole-exome and whole-genome sequencing have resulted in the identification of unanticipated genes and pathways involved in the cause of disease. However, in the case of Parkinson's disease, the most common genetic risk factor identified to date came about from an unanticipated clinical finding made in the genetics clinic during studies of patients with the rare lysosomal storage disorder Gaucher's disease. In most populations with Parkinson's disease, mutations in the glucocerebrosidase (GBA) gene are more frequent than in other implicated genes including dardarin (LRKK2), α-synuclein (SNCA), and parkin (PARK2). In this Review, we detail how glucocerebrosidase was identified as a risk factor for parkinsonism, discuss the clinical relevance of this association, and describe new areas for research and treatment that have resulted from this discovery.

Gaucher's disease

Gaucher's disease, the inherited deficiency of the enzyme glucocerebrosidase, is the most common lysosomal storage disorder. First described by Philippe Gaucher in 1882,¹ Gaucher's disease is an autosomal recessive disorder that primarily affects the mononuclear phagocyte system where lysosomes within cells of the macrophage lineage become engorged with stored lipid. Patients typically manifest with hepatosplenomegaly, anaemia, thrombocytopenia, and bony involvement.^{2,3} A subgroup of patients with neuronopathic forms of Gaucher's disease also have brain involvement of varying severity

(panel 1). Although the disorder is panethnic, it is more frequent in the Ashkenazi Jewish population.

The gene encoding glucocerebrosidase, GBA, is located in a gene-rich region on chromosome 1q21.45 A nearby pseudogene, which shares 96% exonic sequence homology with GBA, complicates sequencing and detection of mutations. So far, about 300 different mutations have been identified in GBA, including point mutations, frameshift mutations, splice-site alterations, and recombinant alleles that encompass segments of the pseudogene sequence.67 Eight mutant alleles frequently occur in patients of Ashkenazi Jewish ancestry, the most common of which is N370S. Among Ashkenazi Jews, one in 14-18 individuals harbour GBA mutations, and N370S accounts for 70% of the mutant alleles.^{8,9} The carrier frequency in other ethnic groups is less than 1%, with a vast range of GBA mutations reported. Although some phenotypic predictions can be made from the identified genotype, genotype-phenotype correlation in this disorder is incomplete. Differing clinical presentations in patients and even siblings sharing the same genotype suggest a role for disease modifiers.^{10,11}

Because of the wide spectrum of phenotypes encountered, Gaucher's disease is typically divided into three types (panel 1) on the basis of the absence (type 1) or rate of progression (types 2 and 3) of neuronopathic involvement.^{2,3,12} However, the associated phenotypes can also be considered a continuum, ranging from asymptomatic individuals identified inadvertently through family screenings to infants who have Gaucher's disease-associated hydrops fetalis *in utero*.^{13,14} The major distinction between these types is CNS involvement, which, in some cases, results in progressive neurodegeneration.

Gaucher's disease with parkinsonism

Among the more atypical and rare Gaucher phenotypes described are patients who develop progressive parkinsonian features, dementia, or both. Initially, case reports of patients were documented, although in 1996 a small series of patients from Italy and Israel was described,¹⁵ and additional reports were subsequently published.¹⁶⁻¹⁸ In 2003, a group of 17 patients with Gaucher's disease and parkinsonism who were of different ethnic origins, including Ashkenazi Jewish patients, were described.¹⁹ The parkinsonian manifestations were similar to those noted in sporadic Parkinson's disease, although many had an age of onset in their 40s and most, although not all, responded favourably to levodopa.¹⁹ Sequencing of *GBA* was done in these 17 patients and revealed 12 different genotypes, with the common type 1 N370S allele identified in 14 patients (82%), including five N370S homozygotes. Autopsies were done on four patients and showed Lewy body inclusions, especially in the cerebral cortex and hippocampus.

Parkinson's disease in GBA mutation carriers

Relatives of several Gaucher probands with parkinsonism have been reported to have Parkinson's disease and to be either obligate or confirmed *GBA* mutation carriers.¹⁹ This finding prompted a prospective survey of patients assessed at the Gaucher clinics at the National Institutes of Health, which confirmed the initial finding, with 25% of patients reporting a first-degree or second-degree relative with parkinsonism.²⁰ A survey in a Gaucher clinic in Jerusalem yielded similar results.²¹

The unexpected finding of decreased glucocerebrosidase activity in neuropathological specimens from patients with sporadic Parkinson's disease, confirmed by the finding of heterozygous *GBA* mutations in these cases, launched the investigation in a new direction.²² Brain bank samples from 57 patients with Parkinson's disease were genotyped, and heterozygous *GBA* mutations were identified in 14% of patients.²² Subsequently, investigators screened DNA samples from 99 patients with idiopathic Parkinson's disease from northern Israel who were of Ashkenazi Jewish ancestry and 1543 Ashkenazi Jewish control individuals for six common *GBA* mutations, and reported that 31.3% of the patients with Parkinson's disease carried a *GBA* mutation compared with 6.2% of the control individuals (p<0.0001; table).²³

Since these initial studies, the frequency of mutations in GBA has been assessed in different Parkinson's disease centres worldwide. Some centres screened for specific commonly encountered GBA mutations, such as N370S and L444P, while others fully sequenced all GBA exons (panel 2). The methods used for detection of mutations and the ethnic origins of the people studied differed among centres, which contributed to the variability in the number of mutations identified. However, the frequency of GBA mutations was consistently higher in patients with Parkinson's disease than in control individuals matched for ethnic origin, age, and sex (table).22-50 Heterozygous GBA mutations were reported in 10.7-31.3% of Ashkenazi Jewish patients with Parkinson's disease, whereas the frequency ranged from 2.3% to 9.4% in patients of other ethnic origins.

GBA mutations in familial Parkinson's disease were assessed in a large cohort in the USA, in which the

Panel 1: The three types of Gaucher's disease

Type 1: non-neuronopathic

- Panethnic disorder, although more common in Ashkenazi Jews
- Associated with clinical heterogeneity
- Wide range of symptom severity
- Hepatosplenomegaly, anaemia, and thrombocytopenia are common
- Bone disease is a frequent cause of morbidity
- Treated with enzyme replacement therapy

Type 2: acute neuronopathic

- Rare, panethnic disorder
- Can present prenatally, at birth, or in the first year of life
- Rapidly progressive neurological deterioration
- Enzyme replacement therapy does not reverse or halt neurological progression
- Early death within days to years

Type 3: chronic neuronopathic

- Includes several different phenotypes with variable longevity
- Accompanied by a specific disorder of horizontal saccadic eye movements
- Some patients develop myoclonic epilepsy
- A subgroup of patients develop cardiac calcification, hydrocephalus, and other abnormalities
- Associated with distinct learning disabilities in some patients

mutation rate was 4.1% in cases compared with 1.1% in control individuals.³⁸ A study from Japan identified *GBA* mutations in eight of 34 families with more than one affected individual and in 14.7% of probands.⁴⁰ All affected family members had concordant variants. Two *GBA* variants, E326K and T369M, were frequently encountered.

Because most single-centre studies had limitations in sample size, GBA screening strategies, inclusion of appropriate control individuals, or the extent of data on ethnic origin, a large multicentre collaborative study was done that incorporated 16 research centres from four continents, with 5691 patients with Parkinson's disease (780 Ashkenazi Jews) and 4898 control individuals without Parkinson's disease (387 Ashkenazi Jews; figure 1).⁵¹ All of the patients included were screened for at least two mutations, N370S and L444P, which were identified in 15% of Ashkenazi Jewish patients and 3% of Ashkenazi Jewish control individuals (for N370S odds ratio [OR] 5.62, 95% CI 3.04-10.39; for L444P 4.95, 0.62-39.38) and in 3% of patients with Parkinson's disease with other ethnic ancestries (6.52, 3.62-11.74). In a subset of 1700 participants in whom GBA was fully sequenced, 7% of patients with Parkinson's disease who were not of Ashkenazi Jewish origin were mutation carriers (for N370S 3.3, 1.79-6.10; for L444P 9.68,

	Population	Screened mutations	Number of participants		Carrier frequency (%)		p value	Most common variants
			Cases	Controls	Cases	Controls		
Lwin et al 2004 ²²	Mixed	GBA exons	57	44	21.0%	0.0%		N370S
Aharon-Peretz et al 2004 ²³	Ashkenazi	N370S, L444P, c.84dupG, IVS2+A>G, V394L, R496H	99	1543	31.3%	6.2%	<0.0001	N370S
Clark et al 2005 ²⁴	Ashkenazi	N370S	160	92	10.7%	4.3%	0.20	N370S
Sato et al 2005 ²⁵	Caucasians (Canadian origin)	N370S, L444P, IVS2+A>G, K198T, R329C, c.84dupG, RecNci1	88	122	5.7%	0.8%	0.48	RecNci1
Toft et al 2006 ²⁶	Norwegian	N370S, L444P	311	474	2.3%	1.7%	0.58	N3705
Eblan et al 200627	Mixed (non-Jewish)	GBA exons	33	31	12.0%	3.2%		RecNci1, L444P
Tan et al 2007 ²⁸	Chinese	L444P, N370S	331	347	2.4%	0.0%	0.06	L444P
Ziegler et al 2007 ²⁹	Chinese	GBA exons	92	92	4.3%	1.1%		L444P
Wu et al 2007 ³⁰	Taiwanese	L444P, RecNci1, R120W	518	339	3.1%	1.2%	0.07	L444P, RecNci1
Clark et al 2007 ³¹	Mixed (64% Jewish)*	GBA exons	278 (178)	179 (85)	13.7%	4.5%		N370S, c.84dupG
De Marco et al 2008 ³²	Italian	N370S, L444P	395	483	2.8%	0.2%	0.0018	L444P
Spitz et al 2008 ³³	Brazilian	N370S, L444P, G377S	65	267	3.0%	0.0%	0.037	L444P
Mata et al 2008 ³⁴	Mixed	N370S, L444P	721	554	2.9%	0.4%	0.001	N370S, L444P
Gan-Or et al 200835	Ashkenazi	N370S, R496H, L444P, c.84dupG, IVS2+1, V394L, D409H, RecTL	420	333	17.9%	4.2%	<0.0001	N370S
Bras et al 2009 ³⁶	Portuguese	GBA exons	230	430	6.1%	0.7%		N370S, N396T
Kalinderi et al 2009 ³⁷	Greek	GBA exons	172	132	4.7%	0.8%	0.048	H255Q, L444P
Nichols et al 2009 ³⁸	Mixed (<10% Jewish)	N370S, T369M, L444P, IVS6, IVS10, E326K, K303K, R262H, RecNci1	1325	359	12.6%	5.3%		E326K, T369M, N370S, L444P
Neumann et al 2009 ³⁹	British	GBA exons	790	257	4.2%	1.2%	0.01	L444P, N370S
Mitsui et al 2009⁴⁰	Japanese	GBA exons	534	544	9.4%	<0.1%	<0.0001	R120W, RecNci1
Emelyanov et al 201041	Russian	N370S, L444P	330	240	2.7%	0.4%	0.038	N3705
Saunders-Pullman et al 201042	Ashkenazi	N370S, L444P, 84GG, IVS2+1G>A, V394L, del55bp, D409H, R496H	250		12.8%			N370S
Mao et al 201043	Chinese	L444P	616	411	3.2%	0.2%	0.001	
Sun et al 201044	Chinese	L444P, F213I, R353W, N370S	402	413	2.7%	0.0%	0.0007	L444P
Hu et al 201045	Chinese	N3705	328	300	1.8%	0.7%	0.29	
Lesage et al 2011 ⁴⁶	North African (Algeria, Morocco, Tunisia, Libya)	GBA exons, LRRK2 (G2019S)	194	177	4.6%	0.5%	0.01	N370S, L444P, RecNci1
Noreau et al 201147	French-Canadian	GBA exons	212	189	3.8%	0.5%	0.10	L444P, p.L236F, p.S378L, p.W417G
Lesage et al 201148	European	GBA exons	1130	391	6.7%	1.0%	<0.0001	N370S
Huang et al 201149	Chinese	GBA exons	967	780	3.7%	0.3%	0.0001	L444P
Choi et al 2012 ⁵⁰	Korean	GBA exons	277	291	3.2%	0.0%	0.01	N188S, P201H, R257Q, S271G, L444
*Number of Jewish participant	s shown in parentheses.							

4.98-18.83). The study concluded that in patients with Parkinson's disease, the OR for carrying a *GBA* mutation was 5.43 (95% CI 3.89-7.57), confirming that mutations in this gene are a common risk factor for Parkinson's disease.

However, findings from large concurrent genome-wide association studies undertaken on thousands of patients with Parkinson's disease worldwide initially failed to identify *GBA* as a susceptibility gene for parkinsonism, probably because of the presence of several rare variants with incomplete penetrance.⁵²⁻⁵⁴ Moreover, these variants occur on different haplotypes, and thus might not be detected in a study of common variants.⁷ The glucocerebrosidase story was described as "an illustration of how an important risk factor for a complex disease can evade detection by systematic analysis; it only came into the radar because of astute clinical observation".⁵⁵ Subsequent genome-wide association studies specifically looking at *GBA* variants confirmed the glucocerebrosidase locus as a risk factor for Parkinson's disease by focusing on specific single nucleotide polymorphisms in the gene.⁵⁶⁻⁵⁸

Clinical features of GBA-associated parkinsonism Age at onset

Parkinsonian phenotypes in *GBA* mutation carriers and *GBA* homozygotes with parkinsonism seem to be similar. Overall, the onset of motor impairment among carriers occurred 1.7-6.0 years earlier than in those

without mutations.^{23,28,30,31,38,39} Screening showed that in patients with an early onset of Parkinson's disease (<50 years) *GBA* mutations were twice as common as in late-onset cases.^{30,38} Among 951 patients screened for N370S and L444P with an onset of Parkinson's disease before age 51 years, $6 \cdot 7\%$ carried either *GBA* mutation, which is equivalent to the frequency of patients with homozygous or heterozygous *PARK2* mutations.⁵⁹ Among the patients who developed Parkinson's disease before age 50 years, *GBA* mutation carriers developed clinical symptoms at an earlier age than non-mutation carriers.^{31,35}

Clinical characteristics of patients with Gaucher's disease who develop parkinsonism

Generally, patients with Gaucher's disease and Parkinson's disease have mild Gaucher's symptoms. No specific Gaucher's genotype is associated with parkinsonian features, although the mutation most frequently identified in most American, European, and Ashkenazi Jewish cohorts is N370S.^{19,60}

A review of the International Collaborative Gaucher Group (ICGG) Gaucher Registry,60 which included data on 4051 patients with Gaucher's disease, revealed that 68 patients had been diagnosed with Parkinson's disease. Those with Parkinson's disease had no specific pattern of associated Gaucher manifestations, and their mean age at symptom onset was 57 years. 54 of the 68 patients had received enzyme replacement therapy for their Gaucher manifestations, which suggests that this treatment did not prevent Parkinson's disease. In another study of 444 patients with Gaucher's disease undertaken in New York (NY, USA) and New Haven (CT, USA), the age at onset of Gaucher-related symptoms, age at Gaucher diagnosis, and sex distribution of patients with parkinsonism were similar to those in patients with Gaucher's disease without Parkinson's disease.61

Although most patients with Gaucher's disease and Parkinson's disease present with Gaucher symptoms many years before the development of parkinsonian manifestations, some develop Parkinson's disease before Gaucher's disease is diagnosed.16,42,60 Screening of 250 Ashkenazi Jewish patients with Parkinson's disease in New York identified four GBA homozygotes who were unaware of their Gaucher's disease.⁴² In the initial series of 17 patients with Gaucher's disease and Parkinson's disease,19 most patients had typical Parkinson's disease features, including asymmetric onset, bradykinesia, rigidity, and resting tremor. Characterisation of Parkinson's disease manifestations associated with GBA mutations in ten patients revealed nine with an asymmetric onset.62 Tremor and bradykinesia were the most common initial symptoms, and motor fluctuations were frequent. In a review of 49 patients with Gaucher's disease and Parkinson's disease, including ten new patients from France, resting tremor was noted in 69% of patients.63 Atypical features such as perceptive deafness18

Panel 2: Screening patients for GBA mutations

GBA is located on chromosome 1q21 and there is a highly homologous pseudogene sequence located only 16 kb downstream. There are approximately 300 described mutations in GBA, some of which originate from pseudogene sequences. The N370S mutation accounts for about 70% of mutant alleles in the Ashkenazi Jewish population. In Caucasian populations, N370S and L444P mutations are common. Sequencing of all exons, using primers specific for the GBA gene, and not the pseudogene, is the most reliable means to screen for GBA mutations. However, many centres continue to screen only for several specific mutant alleles.

and supranuclear oculomotor signs¹⁹ have been reported, but whether these manifestations are attributable to the parkinsonian phenotype as opposed to Gaucher symptoms is difficult to establish. Alonso-Canovas and colleagues⁶⁴ described a 66-year-old Ashkenazi Jewish patient with Gaucher's disease and atypical parkinsonism who developed apraxia and supranuclear gaze abnormalities. One non-Jewish patient with Gaucher's disease from Poland who had a 24-year history of parkinsonism was assessed 12 years after the initial report. This patient ultimately developed cognitive decline, akinetic mutism, and cortical atrophy.⁶⁵

Reports regarding the efficacy of levodopa treatment in *GBA* mutation carriers have been inconsistent. Although parkinsonian symptoms in patients with Gaucher's disease were initially thought to be poorly responsive to levodopa treatment,¹⁹ most subsequent studies have reported good or excellent responses, similar to those in patients with sporadic Parkinson's disease.^{17,30,34,39,41,62}

A group of 30 adult patients with Gaucher's disease from the Lysosomal Disorders Unit at the Royal Free Hospital (London, UK) were screened for potential prodromal symptoms of early neurodegeneration.⁶⁶ Although an increased occurrence of hyposmia and cognitive impairment was reported, larger prospective studies are needed to identify the specific cognitive areas most affected in these patients.

The effect of treatment for Gaucher's disease on parkinsonian manifestations

Two types of treatments are approved for Gaucher's disease. Enzyme replacement therapy using recombinant glucocerebrosidase can be used to control visceral and haematological complications of Gaucher's disease, but does not cross the blood–brain barrier and does not affect neurological manifestations of Gaucher's disease. The treatment does not seem to have any effect on the progression of parkinsonian symptoms.^{18,60,62,63} Thus, using the development of parkinsonian manifestations as a criterion to start this costly treatment is not recommended. An alternative treatment for Gaucher's disease that is less widely used is designed to reduce



Figure 1: The 16 centres that participated in the international collaborative study on the frequency of GBA mutations in Parkinson's disease The numbers shown are the number of patients from each centre. Numbers in parentheses are the number of GBA mutation carriers identified at each site. Numbers in red show centres that sequenced the GBA gene for genotyping.

glucosylceramide synthesis with an iminosugar inhibitor. However, substrate reduction therapy with the iminosugar miglustat was used in two patients with Gaucher's disease and Parkinson's disease without any effect on the progression of their parkinsonism.⁶³

Parkinsonian features in GBA mutation carriers

Findings from several studies of patients with Parkinson's disease who were being managed at movement disorders clinics have confirmed the earlier age at onset of Parkinson's disease symptoms in patients heterozygous for GBA mutations compared with those without mutations. Neumann and colleagues39 described clinical features of patients with Parkinson's disease in a large non-Jewish cohort. They screened 790 patients with Parkinson's disease for GBA mutations and examined the age at disease onset, sex, levodopa response, and motor and non-motor symptoms (cognition and visual hallucinations). GBA carriers were predominantly men, were more likely to have cognitive dysfunction or dementia, and had more frequent hallucinations (not associated with drug treatment) compared with patients without GBA mutations. Another study from Spain reported that dementia was more frequent among GBA mutation carriers with Parkinson's disease than noncarriers.⁶⁷ Scales including the Unified Parkinson's disease rating scale, mini-mental state examination, and Hoehn

and Yahr staging have not detected significant differences in either the extent of Parkinson's disease manifestations or the rate of progression of disease between *GBA* carriers and non-carriers.⁵¹ However, some studies have reported a higher frequency of cognitive decline,^{39,62} bradykinesia,⁶⁸ and olfactory dysfunction,⁶² and less rigidity³¹ associated with *GBA* mutations.

Non-motor symptoms have also been described in *GBA* mutation carriers with Parkinson's disease. In one prospective study, the most consistent non-motor feature was olfactory dysfunction. Other associated features were variable, including depression, anxiety, and cognitive decline.⁶² A German study of Parkinson's disease that compared 20 patients with *GBA* mutations with 20 patients without concluded that mutation carriers were more likely to have dementia, neuropsychological disturbances, and autonomic dysfunction.⁶⁹

Neuroimaging

PET scanning has been used to look for possible dopaminergic dysfunction in patients with Parkinson's disease and *GBA* mutations. Two sisters with Parkinson's disease from South Korea, one with Gaucher's disease and one a carrier, were assessed with ¹⁸F-N-fluoropropyl-2βcarbomethoxy-3β-(4-iodophenyl)nortropane PET at ages 44 years and 55 years, respectively.⁷⁰ Both showed decreased reuptake of dopamine in the posterior putamen, in a pattern similar to that noted in sporadic Parkinson's disease. Four additional patients assessed with ¹⁸F-fluorodopa PET also showed decreased striatal uptake.⁴² Findings from a larger study showed that although the pattern of dopamine loss in patients with Gaucher's disease and Parkinson's disease was similar to that in patients with sporadic Parkinson's disease, H₂¹⁵O PET showed decreased resting regional cerebral blood flow activity in a pattern characteristic of diffuse Lewy body disease.⁷¹

Findings from preliminary studies with transcranial sonography suggest increased echogenicity in the substantia nigra of patients with *GBA*-associated Parkinson's disease.⁶⁹ Three other patients with Gaucher's disease and Parkinson's disease exhibited increased nigral echogenicity compared with control individuals.⁴²

Studies of CSF

CSF concentrations of 13 fatty acids were assessed by gas chromatography in eight patients with Parkinson's disease carrying a *GBA* mutation and were compared with concentrations from 41 patients with idiopathic Parkinson's disease.⁷² Findings from this preliminary study suggested specific abnormalities of fatty acid metabolism in *GBA*-associated Parkinson's disease, which merits further assessment in larger cohorts.

In one patient with Gaucher's disease with a long history of Parkinson's disease, reverse-phase highperformance liquid chromatography of CSF revealed low concentrations of monoamine metabolites, suggesting the involvement of norepinephrinergic and serotonergic neurons. Concentrations of tau and amyloid- β , markers of dementia, were normal.⁶⁵

Genetic counselling

Most patients with GBA mutations never develop Parkinson's disease. Care should be taken when counselling patients with Gaucher's disease and carrier relatives about risk factors for neurodegenerative disorders. In particular, the reported increased incidence of dementia in GBA-associated Parkinson's disease66,67 can be a source of great concern for patients with Gaucher's disease and their families. Careful clinical assessments should be done and detailed histories taken in patients with Gaucher's disease and their relatives, and any evidence of parkinsonian features or cognitive difficulties should be investigated further. As more is known about the pathophysiological mechanisms underlying GBA-associated parkinsonism and as potential neuroprotective drugs become available, early identification of those at highest risk will be important.

Based on published data from the ICGG Gaucher Registry,⁶⁰ among patients with Gaucher's disease the probability of developing Parkinson's disease before age 70 years was 5–7% and before age 80 years was 9–12%. However, of 203 patients in the registry who were born before 1929, only ten had developed Parkinson's disease. Thus, the enzymatic deficiency in most cases was not predictive of Parkinson's disease. Bultron and colleagues⁶¹ attempted to estimate the risk of Parkinson's disease in a series of 444 consecutively assessed patients from the USA who had type 1 Gaucher's disease. 11 patients developed Parkinson's disease over a 12-year follow-up period. Despite this limited sample size, the authors attempted to compare the lifetime risk ratio for developing Parkinson's disease in patients with Gaucher's disease to that in the general population, and estimated it to be increased by 20 times. However, this finding is not substantiated by the ICGG Gaucher Registry,⁶⁰ and larger, better-designed studies are needed to address this question accurately.

A study from France attempted to estimate the Parkinson's disease penetrance in families of *GBA* mutation carriers.⁷³ Focusing on 24 *GBA* mutation carriers with Parkinson's disease, the authors found that 26 of 32 relatives with Parkinson's disease carried *GBA* mutations, whereas 31 of 71 tested relatives who did not have Parkinson's disease had *GBA* mutations.⁷³ The authors concluded that this high penetrance estimate suggested that *GBA* is a dominant causal Parkinson's disease gene with reduced penetrance. This interpretation is of concern, because it could lead to inappropriate genetic counselling in settings where the counsellor is not fully aware of the overlap between Mendelian inheritance with reduced penetrance and complex multigenic inheritance.

Thus, at present, genetic counselling about the risk of Parkinson's disease in patients with a *GBA* mutation is complicated. Most patients with Gaucher's disease and *GBA* mutation carriers never develop Parkinson's disease. Taking a careful family history to investigate other possible risk factors for Parkinson's disease is essential. Although *GBA* mutation carriers do seem to be at an increased risk for Parkinson's disease, further studies might reveal other genetic or non-genetic factors that modify Parkinson's disease risk in such patients.

Neuropathological findings in GBA-associated parkinsonism

Typically, patients with type 1 Gaucher's disease have few neuropathological findings. However, findings from autopsies of brains from patients with GBA-associated parkinsonism show a-synuclein-immunoreactive cortical-type and brain-stem-type Lewy bodies and Lewy neurites. Additionally, Lewy bodies have been found in more atypical locations, such as in hippocampal regions CA2-4, which are areas of vulnerability in neuronopathic Gaucher's disease and dementia with Lewy bodies.19,74 In one study, patients with GBA mutations had a more diffuse distribution of Lewy bodies than the brainstem and limbic distribution noted in patients with typical Parkinson's disease.⁷⁵ Moreover, α-synuclein inclusions were found in the cortical areas, which corresponded to Braak stages 5-6, resembling findings reported in patients with dementia with Lewy bodies.76 However, in



Figure 2: Immunofluorescence assessment of glucocerebrosidase in Lewy bodies in patients with parkinsonism without and with GBA mutations (A, B, and C) The substantia nigra area from a patient without GBA mutations stained for glucocerebrosidase (A, red) and α -synuclein (B, green). The merged image with the nuclear stain (blue) shows a paucity of glucocerebrosidase staining in the Lewy body structure (C). (D, E, and F) The substantia nigra area from a GBA mutation carrier stained for glucocerebrosidase (D, red) and α -synuclein (E, green) showing colocalisation and accumulation in the Lewy body structure (F).

another study of brain samples from 33 patients with Parkinson's disease, including 17 with heterozygous GBA mutations, no association was identified between GBA status and the density of cortical Lewy bodies when corrected for age, sex, duration of Parkinson's disease, and the presence of dementia.77 Seven brain samples from patients carrying GBA mutations who had pathological diagnoses of Parkinson's disease or dementia with Lewy bodies were used to assess the contribution of glucocerebrosidase to the development of parkinsonian pathological abnormalities with immunofluorescence and confocal microscopy.78 Glucocerebrosidase was present in 32-90% of both ubiquitinated and non-ubiquitinated Lewy bodies, whereas in samples from patients without mutations, less than 10% of Lewy bodies were glucocerebrosidase positive (figure 2). Whether or how the presence of the enzyme in Lewy inclusions contributes to disease pathogenesis is still not known.

GBA mutations in other Lewy body disorders

Studies of *GBA* have been extended to other Lewy body disorders, including dementia with Lewy bodies and multiple systems atrophy. Deposition of fibrillated α -synuclein, either in the brainstem or in cortical inclusion bodies, is characteristic of such disorders.⁷⁹⁻⁸¹ *GBA* mutations were first identified in 23% of 35 cases with pathologically confirmed dementia with Lewy bodies.⁸² In another study, screening for N370S and L444P detected *GBA* alterations in 3 · 5% of 57 patients with dementia with Lewy bodies compared with 0 · 4% of 554 control individuals (p=0 · 045).³⁴ Farrer and colleagues⁸³ studied 50 brain samples from individuals with pathologically confirmed dementia with Lewy bodies and identified *GBA* mutations in 6%; whereas Clark and colleagues⁷⁶ reported the presence of *GBA* mutations in 28% of 95 patients with dementia with Lewy bodies, 10% of 60 patients with Alzheimer's disease, and 3% of 35 control individuals (p<0.001). In these studies, *GBA* carriers were significantly more likely than non-carriers to have Lewy bodies at autopsy. Findings from a large multicentre analysis of *GBA* mutations in patients with dementia with Lewy bodies,⁸⁴ which was similar to the international study in Parkinson's disease,⁵¹ suggested that the frequency of *GBA* mutations in patients with dementia with Lewy bodies is even higher than in patients with Parkinson's disease, with an OR of 8.28 (95% CI 4.78–14.88)

The association between *GBA* mutations and multiple systems atrophy seems to be different from that in dementia with Lewy bodies. A study of 12 cases of multiple systems atrophy did not identify *GBA* mutations.⁸² In a study of 108 autopsy-verified cases of multiple systems atrophy and 257 control individuals from the UK,⁸⁵ all *GBA* exons were screened and no significant differences were noted in cases compared with control individuals (p=0.66). A Polish study that examined 66 patients diagnosed with multiple systems atrophy also failed to identify *GBA* mutations.⁸⁶ Since in multiple systems atrophy α-synuclein is deposited mostly in oligodendroglial cytoplasmic inclusions, rather than in neurons, this distinction might be relevant to disease pathogenesis.⁸¹

Proposed mechanisms for GBA-associated parkinsonism

The mechanisms underlying the relation between GBA mutations and the development of Parkinson's disease and associated disorders remain elusive. However, several recent studies provide some new perspectives. Generally, in autosomal recessive forms of Parkinson's disease, such as those involving PARK2, DJ-1, and PINK1, loss-of-function mutations are implicated, and these patients have an early onset of disease manifestations. By contrast, gain-of-function mutations are usually associated with autosomal dominant forms of Parkinson's disease, such as with SCNA and LRRK2.87 Although Gaucher's disease is an autosomal recessive disorder, the inheritance of parkinsonism associated with GBA mutations does not follow a strict Mendelian pattern, leading investigators to propose both gain-offunction and loss-of-function theories (panel 3).

Support for gain-of-function and loss-of function hypotheses

The finding that most mutant alleles identified result in a misfolded protein supports a gain-of-function role for mutations in *GBA*. This abnormal conformation could contribute to the development of parkinsonism by increasing α -synuclein aggregation. Alternatively, the misfolded protein could lead to lysosomal dysfunction, overwhelming the ubiquitin-proteasome pathway or causing impairment of autophagy.

Additionally, parkinsonism could arise as a consequence of glucocerebrosidase deficiency, due to the loss of function of the enzyme. A misfolded protein can be degraded, leading to enzymatic deficiency and substrate accumulation. The potential role of lipid homoeostasis and lysosomal function in the cause of Parkinson's disease is a topic of great interest, and other ceramide-related genes have been implicated in Parkinson's disease.⁸⁸ Furthermore, α -synuclein can bind to the lipid-raft-associated ganglioside GM1.⁸⁹ Lipid rafts have an essential role in the proper trafficking of α -synuclein to presynaptic membranes; hence, lipid storage could cause changes in lipid homoeostasis, with subsequent alterations in α -synuclein processing.⁹⁰

Although both theories have some support, there are serious deficiencies with each. First, some *GBA* mutations encountered in patients with parkinsonism are null mutations—a finding in conflict with the gainof-function hypothesis. In fact, carriers of null alleles might have an even higher risk of development of parkinsonism.⁶⁸ Second, the clinical finding that most patients with Gaucher's disease never develop Parkinson's disease, despite having a deficiency of glucocerebrosidase exceeding that of heterozygotes, argues against a solely loss-of-function hypothesis. This theory is supported by the low frequency of Parkinson's disease reported in the large Gaucher cohort in the ICGG Gaucher Registry.⁶⁰ Thus, in most cases, the enzymatic deficiency is not predictive of Parkinson's disease.

In another study, Cullen and colleagues⁹¹ concluded that both loss-of-function and gain-of-function mechanisms were consistent with their data. In culture, neuronal cell lines stably overexpressing α -synuclein and different GBA mutations showed a gain-of-function effect; however, findings from the sensitive sandwich ELISA assay, developed by the authors to measure α -synuclein, showed that raised concentrations of α -synuclein were dependent on the amount of expressed mutant GBA, but not its activity. Using a point mutation knock-in mouse model of Gaucher's disease with genotype D409V/D409V, the authors noted that both mutant GBA and downregulation of glucocerebrosidase led to upregulation of membrane-associated a-synuclein in the hippocampus; in heterozygote mice (D409V/+) this was not identified.

Using this same mouse model, other investigators reported that α -synuclein accumulates in hippocampal neurons and that this accumulation increases with age.⁹² Moreover, these mice developed a corresponding memory deficit that was reversed when recombinant glucocerebrosidase was administered directly into the brain. The α -synuclein accumulation was associated more with increased concentrations of glucosylsphingosine than glucosylceramide. However, although this mouse model is used because it has decreased enzymatic activity, it is not ideal because the D409V mutation is not reported in Gaucher's disease,

Panel 3: Arguments for a gain-of-function versus a loss-offunction mechanism for GBA-associated parkinsonism

Gain of function

- Parkinson's disease occurs in GBA heterozygotes
- Most GBA mutations are missense, leading to a misfolded protein
- Mutant glucocerebrosidase is found in Lewy bodies, suggesting a role in α-synuclein oligomerisation or impaired degradation
- Most patients with Gaucher's disease do not develop Parkinson's disease, despite low glucocerebrosidase activity
- Mutant glucocerebrosidase can accumulate in the endoplasmic reticulum and burden the proteasomal and lysosomal systems responsible for degrading proteins

Loss of function

- Null GBA alleles (eg, c.84dupG and IVS2+1G>A) have been reported in patients with Parkinson's disease
- Mouse models of Gaucher's disease show increased levels of $\alpha\mbox{-synuclein}$
- Lipid rafts and ceramides play a part in Parkinson's disease pathogenesis
- Chemical inhibition of glucocerebrosidase in mice and in cells increases concentrations of $\alpha\mbox{-synuclein}$
- The lipids glucocerebroside and glucosylsphingosine can accumulate in the lysosome, affecting its function

and this mouse model does not manifest typical Gaucher features. Although the authors concluded that the reported pathological abnormalities are a combination of loss of activity and toxicity related to the mutant enzyme, neuropathological changes in both D409V homozygote and heterozygote (but not heterozygous knockout) mice suggest that α -synuclein aggregation is not associated with glucocerebrosidase activity or substrate accumulation.

Other attempts to model the association between Gaucher's disease and parkinsonism in animals have yielded similarly conflicting results. Treatment of wild-type mice with the glucocerebrosidase inhibitor conduritol β epoxide moderately increased concentrations of α -synuclein.⁹³ Gaucher mice homozygous for V394L or D409H, which have a normal lifespan and do not show storage of glucosylceramide in the CNS, were crossed with mice deficient in prosaposin, including the peptide saposin C, which is necessary for glucocerebrosidase activation, yielding mice that developed α -synuclein aggregation in cortical neurons. However, this aggregation was not related to glucosylceramide accumulation.⁹⁴

The prion hypothesis

Another hypothesis that has been proposed to explain this association is that α -synuclein aggregates might have a prion-like mechanism of cell-to-cell transmission,

and that macrophages, the cells most affected in Gaucher's disease, might serve as a carrier of such aggregates.⁹⁵ The author suggested that both endogenous and exogenous α -synuclein might accumulate and spread in the lipid-rich environment found in Gaucher's cells. However, this suggestion does not explain why carriers would be at risk, because they generally do not have storage in macrophages. One possible explanation is that a second hit—a new somatic *GBA* mutation—could occur in isolated macrophages, which would initiate α -synuclein accumulation and spread.

The role of α-synuclein

Findings from recent studies have further implicated α -synuclein in the pathogenesis of *GBA*-associated parkinsonism. Yap and colleagues⁹⁶ reported a physical interaction between α -synuclein and purified recombinant glucocerebrosidase, occurring only at the acidic pH present in the lysosomal compartment, using four different methodologies. First, when incubated with glucocerebrosidase at pH 5.5, labelled Dns-136- α -



Figure 3: The bidirectional loop theory

Decreased glucocerebrosidase increases the lysosomal concentrations of glucosylceramide, which increases the formation of soluble α -synuclein oligomers. These oligomers also disrupt transport of newly synthesised glucocerebrosidase between the endoplasmic reticulum and Golgi apparatus, further compounding the problem.

synuclein caused a marked spectral shift with increased intensity, suggesting a physical interaction between the two molecules in lysosomes. However, this shift did not occur when the experiment was done at pH 7.4 or when the label was placed on the N-terminal of α-synuclein. Residue level mapping by nuclear MRI at pH 5.5 was done to examine the relative spectra intensity ratio of α-synuclein-glucocerebrosidase to α-synuclein alone as a function of α -synuclein residues and showed that the interaction occurred at residues 118–137 of α -synuclein. Again, no significant interaction was noted at pH 7.4. In brain samples from patients with Parkinson's disease, glucocerebrosidase co-immunoprecipitated with native α -synuclein at pH 5.5 but not at pH 7.4. Lastly, confocal microscopy of M17 neuroblastoma cells stably overexpressing glucocerebrosidase and a-synuclein showed colocalisation of the enzyme with α -synuclein in cathepsin-D-positive cellular structures. The C-terminal region of a-synuclein seems to interact with glucocerebrosidase, whereas the N-terminal helix is bound to a glycolipid-rich vesicle inside the lysosome. This binding at lysosomal pH might facilitate α-synuclein degradation or prevent aggregation. Because much of the processing of a-synuclein occurs in the cytoplasm, this interaction was unexpected, but it supports a role for lysosomal pathways in α-synuclein degradation. That glucocerebrosidase might have a beneficial role in a-synuclein processing that is disrupted when GBA is mutated or absent is intriguing.

Studies of brain samples have shed some light on the association between α -synuclein pathology and glucocerebrosidase. Proteins extracted from cerebral cortex samples from patients with synucleinopathies with and without *GBA* mutations, as well as samples from control individuals and patients with Gaucher's disease, were studied by western blotting. Patients with *GBA*-associated parkinsonism showed aggregated oligomeric forms of α -synuclein in the insoluble fraction, whereas only monomeric α -synuclein was noted in patients with *GBA* mutations without parkinsonism, including samples from patients with neuronopathic Gaucher's disease. Thus, aggregation of α -synuclein is not related to glucocerebrosidase deficiency alone.⁹⁷

The association between *a*-synuclein pathology and glucocerebrosidase was recently assessed from a different perspective in a complex study by Mazzulli and colleagues.⁹⁸ Using cultured primary neurons treated with short hairpin RNA by lentiviral infection to knock down the expression of glucocerebrosidase, a 50% reduction in glucocerebrosidase and reduced concentrations of endoglycosidase H-resistant glucocerebrosidase were reported and were ascribed to the depletion of the lysosomal form of the enzyme. This glucocerebrosidase knock-down also increased α -synuclein concentrations by 1.8 times. The authors then showed that human induced-pluripotent-stem-cellderived neurons made from Gaucher fibroblasts also



Figure 4: How mutant glucocerebrosidase might result in enhanced α-synuclein aggregation

(A) In the normally functioning lysosome, wild-type glucocerebrosidase might interact with α -synuclein, facilitating the lysosomal component of α -synuclein degradation. (B) In most cases, when glucocerebrosidase is mutated, α -synuclein remains in the monomeric form and other processes are active in its degradation. (C) In some patients, glucocerebrosidase is mutated and the cell is unable to degrade α -synuclein. Lysosomal function is compromised and increased oligomeric forms of α -synuclein lead to neuronal cell death and the development of parkinsonism. This situation might enhanced by ageing, when the number of lysosomes and lysosome function decrease and cellular α -synuclein concentrations increase.

had an accumulation of α -synuclein that resulted in neurotoxicity attributed to aggregation-dependent mechanisms. They concluded that deficiency of glucocerebrosidase resulted in impaired lysosomal proteolysis that preferentially affected a-synuclein. Working with a mouse model, Caenorhabditis elegans, and human brain samples, an attempt was made to establish that increased concentrations of lysosomal glucosylceramides resulting from diminished enzymatic activity promoted the formation of soluble α -synuclein oligomers and fibrils. Furthermore, they noted that increased concentrations of cytoplasmic α-synuclein blocked endoplasmic reticulum-Golgi apparatus trafficking of glucocerebrosidase, thus compounding the problem (figure 3). This further decrease in lysosomal glucocerebrosidase amplified the increase in glucosylceramide and the production of additional stable a-synuclein oligomers. The authors concluded that a bidirectional pathogenic loop had formed, leading to self-propagating disease. The exciting aspect of this hypothesis is that even without mutations, decreased glucocerebrosidase concentrations might have a role in Parkinson's disease pathogenesis. However, how the 50% reduction in glucocerebrosidase reported in their cell-based model resulted in glucosylceramide accumulation and complete lysosomal dysfunction is difficult to understand. This finding does not typically occur in patients with type 1 Gaucher's disease, who have a 70-90% reduction in glucocerebrosidase activity.²

Thus, although this work provides ideas for future research, it does not completely explain what occurs clinically.

Does mutated GBA affect autophagy?

Lysosomes are involved in the degradation of substrates both through enzymatic degradation and through different autophagic pathways that can include chaperonemediated autophagy, macroautophagy, or microautophagy.⁹⁹ In addition to proteasomal pathways, α -synuclein is also degraded via chaperone-mediated autophagy.^{99,100} Alterations in autophagy have been reported in several neurodegenerative lysosomal storage disorders and in a Gaucher mouse model.¹⁰¹ In a murine study, overexpression of D409V glucocerebrosidase resulted in α -synuclein accumulation that could be reversed using rapamycin, an inducer of macroautophagy.⁹¹ Parkin, an E3 ubiquitin ligase, might also play a part in the degradation and accumulation of mutant glucocerebrosidase.¹⁰²

Conclusions

Further studies using techniques in cell biology, neuropathology, and genetics are needed to better piece together the mechanisms that contribute to *GBA*associated parkinsonism and to identify other risk factors working in concert with *GBA* that favour the development of parkinsonism. Since Parkinson's disease is a disorder of ageing, factors that change during the ageing process probably play a part.¹⁰³ As we

Search strategy and selection criteria

References for this Review were identified through searches of PubMed and the Web of Science from 1990 to May, 2012, with the search items "glucocerebrosidase", "GBA", "Gaucher", "Parkinson", and "parkinsonism". The final reference list was selected on the basis of relevance to the subject of this review.

age, cellular concentrations of α -synuclein increase.^{104,105} In parallel, ageing is associated with diminished numbers of lysosomes and poorer lysosomal function.106 Thus, one might speculate that there is a crucial balance between the amount of α -synuclein needing to be degraded and the lysosomal capacity, which becomes compromised as one ages, or as the lysosomes become engorged with storage products. Mutant or absent glucocerebrosidase might somehow contribute to this scenario by further compromising the ability of the lysosome to function normally. Either the absence of this protein, which in this case might have a totally different purpose than its known enzymatic function, or altered lipid metabolism might affect the normal lysosomal breakdown of α-synuclein aggregates, resulting in the increased risk of Parkinson's disease (figure 4).

The identification of glucocerebrosidase as a risk factor for parkinsonism is, and will continue to be, a source of considerable excitement in the specialty. In contrast to most other identified genes associated with Parkinson's disease, a great deal is known about this protein, its processing, and its function. This knowledge provides the opportunity for new hypotheses and research directions for years to come. Moreover, different therapeutic strategies under consideration and development for the treatment of Gaucher's disease could lead to stabilisation of the protein or enhancement of enzymatic activity and affect the course of the neurodegeneration that occurs in parkinsonism.

However, despite the substantial progress that has been made, the basis for *GBA*-associated parkinsonism remains enigmatic. Unravelling the factors underlying this association is imperative to enable improved genetic counselling for people who carry mutations in this gene. Moreover, a better understanding of the role of this lysosomal enzyme in the development of parkinsonism will contribute to new and improved therapeutic strategies for *GBA*-associated parkinsonism, which might affect the treatment of all individuals with Parkinson's disease, a common and complex disease.

Contributors

ES and GL reviewed the published work and wrote the manuscript.

Conflicts of interest

We declare that we have no conflicts of interest.

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