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Accumulation and distribution of α-synuclein and ubiquitin in the CNS of Gaucher disease mouse models

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ABSTRACT

Gaucher disease, a prevalent lysosomal storage disease, is caused by insufficient activity of acid β-glucosidase (GCase) and resultant glucosylceramide accumulation. Recently in Parkinson disease (PD) patients, heterozygous mutations in GCase have been associated with earlier onset and more progressive PD. To understand the pathogenic relationships between GCase variants and Parkinsonism, α-synuclein and ubiquitin distributions and levels in the brains of several mouse models containing GCase variants were evaluated by immunohistochemistry. Progressive α-synuclein and ubiquitin aggregate accumulations were observed in the cortex, hippocampus, basal ganglia, brainstem, and some cerebellar regions between 4 and 24 weeks in mice that were homozygous for GCase [D409H (9H) or V394L (4L)] variants and also had a prosaposin hypomorphic (PS-NA) transgene. In 4L/PS-NA and 9H/PS-NA mice, this was coincident with progressive neurological manifestations and brain glucosylceramide accumulation. Ultrastructural studies showed electron dense inclusion bodies in neurons and axons of 9H/PS-NA brains. α-synuclein aggregates were also observed in ventricular, brainstem, and cerebellar regions of older mice (>42-weeks) with the GCase variant (D409H/D409H) without overt neurological disease. In a chemically induced GCase deficiency, α-synuclein aggregates and glucosylceramide accumulation also occurred. These studies demonstrate a relationship between glucosylceramide accumulation and α-synuclein aggregates, and implicate glucosylceramide accumulation as risk factor for the α-synucleinopathies.

1. Introduction

Gaucher disease, an autosomal recessive disorder, is a common lysosomal storage disease [1,2] that results from insufficient activity of acid β-glucosidase (GCase, encoded by the *GBA1* gene) and resultant accumulation of its substrates glucosylceramide and glucosylsphingosine. Accumulations of glucosylceramide and glucosylsphingosine produce the visceral and CNS manifestations by as yet ill-defined mechanisms. Classically, the three clinical phenotypes include the "non-neuronopathic" (type 1), and "neuronopathic" (type 2 and 3) variants [1,3,4]. In all variants, glucosylceramide engorged visceral macrophages or "Gaucher cells" are hallmark of the disease. In the "neuronopathic" variants, the CNS pathology includes neuronal cell death.

The distinction between the "nonneuronopathic" and "neuronopathic" variants has become somewhat blurred. Recent studies showed that Parkinson disease patients have a 3- to 7-fold increased risk of being heterozygous for *GBA1* variants [5–13]. Such associations with Parkinsonism are not related to specific *GBA1* mutations [14]. Parkinsonism also occurs in Gaucher disease type 1 or 3 patients and in heterozygotes for *GBA1* mutations [6–8,11], but the risk of *GBA1* heterozygotes of developing Parkinsonism is unknown. Parkinson signs and symptoms include memory loss, resting tremor, uncontrolled movements, kinetic rigidity syndrome, asymmetric onset, horizontal myoclonus, supranuclear gaze palsy, typical progression rigidity, difficulty ambulating, and bradykinesia [5–13,15]. This spectrum of manifestations is similar in persons with or without *GBA1* mutations, but can be more severe in their presence. These observations indicate that mutant *GBA1* even in heterozygotes, is a significant risk factor for potentiating the effects of Parkinsonism. Neither the basis for these effects or the general pathology and their relationships to glucosylceramide accumulation are known.

These findings contrast with the neuropathology of Gaucher disease types 2 and 3 in which neuronal loss and degeneration are the most consistent findings, particularly in the basal ganglia, nuclei of the midbrain, pons and medulla, cerebellum, dentate nucleus and hypothalamus [16–19]. Cerebral cortical laminar necrosis [16,19] and neuronal loss with astrogliosis [20,21] also have been reported, but only in some type 2 patients. Importantly, α-synuclein inclusion-

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associated neurodegenerative lesions (α-synucleinopathies) were reported in similar brain regions from some PD patients who also had Gaucher disease type 1 [7,15,22].

α-synuclein is a small presynaptic cytosolic protein that is abundant in nerve terminals of dopaminergic system. Its normal function is incompletely defined, but it has been implicated in dopamine metabolism and synaptic vesicle homeostasis [23–25]. Mutations in the gene for α-synuclein (e.g., Ala30Pro or Ala53Thr) have been implicated directly in the pathogenesis of Parkinson disease [26,27], as has the over-expression of a human wild-type α-synuclein [28–30]. The presence of α-synuclein insoluble intracellular aggregates (Lewy bodies) is a feature of Parkinson disease and other neurodegenerative disorders [31,32]. Although α-synuclein aggregates have been observed in several lysosomal diseases and their animal analogues, only *GBA1* mutations show a clear and, potentially direct risk association with α-synucleinopathies and Parkinson disease for this reason these shared clinical and neuropathologic findings suggested that *GBA1* mutations or glucosylceramide excess act as contributory risk factors that interfere with the clearance of or promote the aggregation of α-synuclein in some patients.

Here, *Gba1* point-mutated mice bearing a prosaposin hypomorph (4L/PS-NA and 9H/PS-NA) [33] or having CBE induced-GCase deficiency [34] were used as models of Gaucher disease. Prosaposin is a precursor of the four saposins A, B, C, and D that are essential proteins for the optimal activity of selected glycosphingolipid hydrolyases [35]. Saposin C optimizes GCase hydrolysis of glucosylceramide and other substrates, as well as protecting GCase from proteolytic digestion [35,36]. Saposin C's protective function accounts for the decreased GCase protein and activity with excess glucosylceramide in the CNS and visceral organs of 4L/PS-NA and 9H/PS-NA mice [33]. Extensive histological and immunohistological analyses of such mice showed a particular pattern of α-synuclein accumulation that implicates mutant GCase and/or excess glucosylceramide in the development of α-synuclein accumulation.

2. Materials and methods

2.1. Materials

The following were from commercial sources: Conduritol B epoxide (CBE, Calbiochem, San Diego, La Jolla, CA). 4-methylumbelliferyl-β-D-glucopyranoside (4MU-Glc; Biosynth AG, Switzerland). Sodium taurocholate and Protease Inhibitor Cocktail (Calbiochem, La Jolla, CA). Triton X-100 (Sigma, St. Louis, MO). Antibody sources are as follows: mouse monoclonal anti-α-synuclein, rabbit polyclonal anti-mouse α-synuclein, rabbit polyclonal anti-ubiquitin and tyrosine hydroxylase (Abcam, Inc. Cambridge, MA). Rat monoclonal to CD68 (Serotec, Raleigh, NC). Mouse monoclonal anti-GFAP (Sigma, St. Louis, MO). Mouse monoclonal anti-β-actin antibody (Sigma, St. Louis, MO). Goat anti-rabbit/rat (Alexa-488, FITC), or Goat anti-mouse-biotinylated antibody with streptavidin-Alexa-610 (Molecular Probes, Irvine, CA). Hybond ECL Nitrocellulose Membrane (Amersham, Piscataway, NJ). 10% Bis-Tris Gel (Invitrogen, Carlsbad, CA). BCA Protein Assay Kit, Peroxidase-Conjugated Goat anti-mouse IgG, M-Per Mammalian Protein Extraction Reagent and ECL Kit (PIERCE, Rockford, IL).

2.2. Gaucher disease and other lysosomal disease mouse models

Several GCase, prosaposin, and other lysosomal disease mouse models were analyzed (Table 1). The homozygous point-mutated *Gba1* mice, V394L (4L), D409H (9H), and D409V (9V), have normal life spans and no overt CNS disease [37]. Hypomorphic prosaposin mice (PS-NA) and prosaposin knockout mice (PS-/-) had glucosylceramide accumulation in the CNS with CNS degenerative disease [33,38,39]. PS-NA mice that were also homozygous for V394L or D409H (4L/PS-NA or 9H/PS-NA, respectively), exhibit greater excesses of glucosylceramide accumulation in the CNS than PS-NA mice [33] at least in part due to additive deficiencies of GCase activity. These mice were used here as models for chronic effects of GCase deficiency and glucosylceramide accumulation since no other viable models for the CNS variants of GCase deficiency available. Other mice with lysosomal storage diseases were used for comparative purposes and included those with individual saposin deficiencies (saposin A, B, C or D), Niemann-Pick type C1 (NPC1), mucopolysaccharidosis type I (MPS I), and lysosomal acid lipase (LAL) deficiency. NPC1 is characterized by intracellular accumulations of free cholesterol and glycosphingolipids, including glucosylceramide, in the endosomal/lysosomal compartment [40]. MPS I, α-L-iduronidase deficiency, leads to defective catabolism of the glycosaminoglycans heparan and dermatan, sulfate, and have secondary accumulations of GM2 in neurons [41]. Lysosomal acid lipase is essential for the hydrolysis of the triglycerides and cholesteryl esters in lysosomes and deficient mice do not exhibit overt CNS disease during their 8–10 month lifespan [42]. Age-matched wild-type mice in the FVB background were from Jackson Laboratory (FVB/NJ, Stock No 001800). The mice were maintained in microisolators in accordance with institutional guidelines under IACUC approval at the animal facility of Cincinnati Children's Hospital Research Foundation. Brains were collected from each genotype at the designated times for histological and biochemical analyses.

Table 1
Gaucher and LSD mouse models.

Genotypes	Life span (weeks)	GCase activity[a] (%WT)	Brain GC level[b]	CNS phenotype	References
Variant gaucher disease mice					
9H/PS-NA	22	34.2	2-fold[c]	Wobble/ataxia	[33]
4L/PS-NA	22	11.0	4-fold[c]	Wobble/ataxia	
GCase point mutant					
V394L/V394L	>100	27.4	–	Normal	[37]
D409H/D409H	>100	25.6	–	Normal	[34]
D409V/D409V	>100	22.5	–	Normal	
CBE induced Gaucher disease mice					
V394L/V394L	>16	12.9	+	Wobble/ataxia/seizure	[34]
D409H/D409H	>16	13.1	+	Wobble/ataxia/seizure	
D409V/D409V	>16	7.2	+	Wobble/ataxia/seizure	
WT	>16	10.2	+	Wobble/ataxia/seizure	
LSD mouse models					
PS-NA	28	88	+	Wobble/ataxia	[39]
PS-/-	4	80	+	Ataxia/seizure	[36,38]
LAL	20	N/A[d]	N/A[d]	Normal	[42]
NPC1	13	N/A[d]	+	Ataxia/seizure	[72]
MPS1	52	N/A[d]	N/A[d]	Degeneration	[41,73]

[a] GCase activity in the brain.
[b] Glucosylceramide levels in the brain determined by TLC: (−) undetectable, (+) low level.
[c] Glucosylceramide level compared with that in WT by LC/MS.
[d] N/A, data not available.

2.3. Histological studies

For histological studies, 3 to 4 mice from each genotype at the indicated ages, a minimum of 2 sections from each tissue were examined. Brains were dissected after perfusion with 1×PBS, pH 7.4 and 4% paraformaldehyde in 1×PBS, pH 7.4. The brains were post fixed overnight in 10% formalin or 4% paraformaldehyde, and then