Augmenting CNS glucocerebrosidase activity as a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies

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Mutations of *GBA1*, the gene encoding glucocerebrosidase, represent a common genetic risk factor for developing the synucleinopathies Parkinson disease (PD) and dementia with Lewy bodies. PD patients with or without *GBA1* mutations also exhibit lower enzymatic levels of glucocerebrosidase in the central nervous system (CNS), suggesting a possible link between the enzyme and the development of the disease. Previously, we have shown that early treatment with glucocerebrosidase can modulate α-synuclein aggregation in a presymptomatic mouse model of Gaucher-related synucleinopathy (*Gba1*$^{D409V/D409V}$) and ameliorate the associated cognitive deficit. To probe this link further, we have now evaluated the efficacy of augmenting glucocerebrosidase activity in the CNS of symptomatic *Gba1*$^{D409V/D409V}$ mice and in a transgenic mouse model overexpressing A53T α-synuclein. Adeno-associated virus-mediated expression of glucocerebrosidase in the CNS of symptomatic *Gba1*$^{D409V/D409V}$ mice completely corrected the aberrant accumulation of the toxic lipid glucosylsphingosine and reduced the levels of ubiquitin, tau, and proteinase K-resistant α-synuclein aggregates. Importantly, hippocampal expression of glucocerebrosidase in *Gba1*$^{D409V/D409V}$ mice (starting at 4 or 12 mo of age) also reversed their cognitive impairment when examined using a novel object recognition test. Correspondingly, overexpression of glucocerebrosidase in the CNS of A53T α-synuclein mice reduced the levels of soluble α-synuclein, suggesting that increasing the glycosidase activity can modulate α-synuclein processing and may modulate the progression of α-synucleinopathies. Hence, increasing glucocerebrosidase activity in the CNS represents a potential therapeutic strategy for *GBA1*-related and non-*GBA1*–associated synucleinopathies, including PD.

lysosomal storage diseases | mouse models | MAPT | memory defect

Mutations in the gene for glucocerebrosidase (*GBA1*) present the highest genetic risk factor for developing synucleinopathies such as Parkinson disease (PD) and dementia with Lewy bodies (DLB) (1–5). The central nervous system (CNS) of Gaucher patients and carriers who present with parkinsonism and dementia harbor deposits of α-synuclein–positive Lewy bodies (LBs) and Lewy neurites (LNs) in hippocampal neurons and their processes resembling those noted in patients with classical PD and DLB (6, 7). Aspects of these characteristics have also been noted in the CNS of several mouse models of neuropathic and nonneuropathic Gaucher disease (8–10). Consequently, a causal relationship has been suggested between the loss of glucocerebrosidase activity or the lysosomal accumulation of undegraded metabolites and the development of PD and DLB. A more direct link between glucocerebrosidase activity and α-synuclein metabolism has been highlighted by studies of Gaucher cells and mice indicating that a reduction in glucocerebrosidase activity by pharmacological or genetic interventions resulted in increased levels of α-synuclein aggregates (9–12). Moreover, a decrease in glucocerebrosidase activity has been noted in cerebrospinal fluid (CSF) and brain samples from patients with PD and DLB (regardless of whether they harbor mutations in *GBA1*), suggesting that a reduction in glucocerebrosidase activity may contribute to the development of synucleinopathies (13–15).

A role for glucocerebrosidase in the development of synucleinopathies is further supported by clinical observations of patients with Gaucher-associated parkinsonism. These individuals present with increased frequencies and severities of nonmotor symptoms (e.g., cognitive impairment) that substantially erode their quality of life (16, 17). Individuals harboring mutations in *GBA1* also have a higher incidence of dementia that is correlated with the presence of neocortical accumulation of aggregates of α-synuclein (18, 19). Indeed, mutations in *GBA1* are now recognized as an independent risk factor for development of cognitive impairment in PD patients (20). Another gene associated with an increased risk for dementia in PD is *MAPT* (21), the gene encoding the microtubule-associated protein tau, which helps maintain cytoskeletal organization and integrity. Tau-associated and α-synuclein–associated pathology frequently occurs in patients with PD and LBD (22–24) although the relative roles of these proteins are not well defined. Tau is more explicitly involved in Alzheimer's disease (25).

We previously described a Gaucher-related synucleinopathy in mice with progressive CNS accumulation of proteinase K-resistant α-synuclein/ubiquitin aggregates reminiscent of LNs (10). These mice also have high CNS levels of the neurotoxin glucosylsphingosine (GlcSph) and a hippocampal memory deficit. We showed these biochemical and behavioral aberrations to be ameliorated by CNS administration into presymptomatic animals of a recombinant adeno-associated viral (AAV) vector encoding human glucocerebrosidase. The present study further characterizes the pathological features in this Gaucher-associated synucleinopathy model, adding increased protein tau and demonstrating cognitive improvement and moderation of CNS pathology when glucocerebrosidase was administered at a clinically relevant postsymptomatic stage. Finally, to further probe the glucocerebrosidase/α-synuclein relationship, the effect of the lysosomal hydrolase on α-synuclein levels in the A53T α-synuclein mouse was evaluated.

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Results

CNS of a Mouse Model of Gaucher Disease Exhibits Accumulation of Tau Aggregates. Accumulation of α-synuclein and tau inclusions with resultant dementia are the hallmarks of a number of neurodegenerative diseases, including PD and DLB (22, 25, 26). We have reported that a mouse model of Gaucher disease harboring a single point mutation in the murine *Gba1* locus ($Gba1^{D409V/D409V}$) exhibits progressive, marked accumulation of α-synuclein/ubiquitin aggregates in the CNS and a measurable deficit in hippocampal memory (10). To determine whether mutations in *Gba1* with resultant loss of glucocerebrosidase activity also promote the accumulation of tau in the CNS, brain sections of 12-mo-old $Gba1^{D409V/D409V}$ mice were examined immunohistochemically with a specific anti-tau antibody. Marked punctate staining was noted primarily in the hippocampal regions (Fig. 1*A*), although immunoreactivity was also observed in other areas, including cerebral cortex and cerebellum. The onset and rate of accumulation of the tau aggregates in the brains of $Gba1^{D409V/D409V}$ mice were determined as well. At 2 mo of age, tau immunoreactivity in $Gba1^{D409V/D409V}$ mice was not different from that in wild-type controls (Fig. 1 *A* and *B*). However, the level of tau staining in 6-mo-old $Gba1^{D409V/D409V}$ mice was significantly higher than in the age-matched controls. Accumulation was progressive, with 12-mo-old $Gba1^{D409V/D409V}$ mice displaying higher amounts of tau aggregates (Fig. 1 *A* and *B*).

A common finding in tauopathies and related neurodegenerative diseases is an increase in hyperphosphorylated tau comprising the neurofibrillary tangles (27, 28). These phosphorylated species can be detected with specific antibodies, such as AT270 (Thr-181), AT8 (Ser-202/Thr-205), and AT180 (Thr-231). To probe the phosphorylation status of the tau aggregates in the CNS of $Gba1^{D409V/D409V}$ mice, Western blot analysis was performed on hippocampal lysates from 18-mo-old mice. Staining the blots with the Tau-5 antibody that recognizes all tau species revealed that the overall levels of the protein were not different between $Gba1^{D409V/D409V}$ and wild-type mice (Fig. 1*C*), nor were differences in the extent of staining observed between controls and age-matched $Gba1^{D409V/D409V}$ mice when the blots were probed with AT180 or AT270 antibodies (Fig. 1*C*). However, AT8 staining, which detects phosphorylation on Ser-202 and Thr-205, was modestly but significantly increased in the lysates of $Gba1^{D409V/D409V}$ mice (1.3 ± 0.1 compared with wild-type, $n = 6$, $P < 0.05$; Fig. 1*C*). This increased phosphorylation on Ser-202 and Thr-205, coupled with the progressive accumulation of the tau aggregates (in addition to α-synuclein), indicates that the CNS of $Gba1^{D409V/D409V}$ mice recapitulates pathological features in PD and DLB patients.

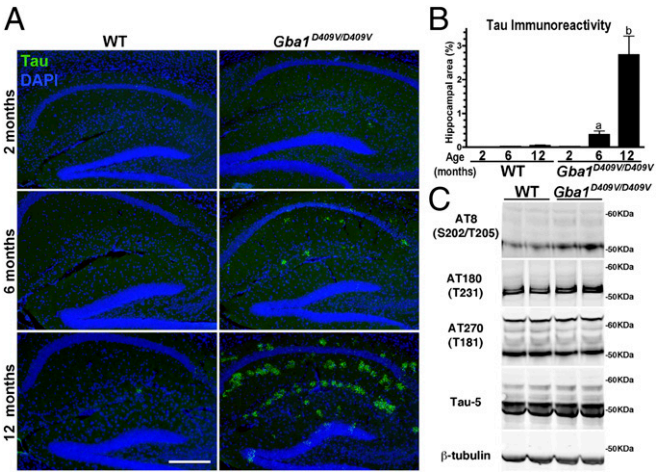

Fig. 1. Progressive accumulation of tau aggregates in the brains of $Gba1^{D409V/D409V}$ mice. (*A*) Images show immunostaining with an anti-tau serum (green) and nuclear staining (DAPI; blue) in the hippocampi of 2-, 6-, and 12-mo-old $Gba1^{D409V/D409V}$ and age-matched wild-type (WT) mice. (Scale bar, 500 μm.) (*B*) Quantification of Tau-5 immunoreactivity in WT and $Gba1^{D409V/D409V}$ hippocampi at 2, 6, and 12 mo shows progressive accumulation of aggregates with age ($n \geq 5$ per group). (*C*) Shown are representative immunoblots of hippocampal lysates from 18-mo-old $Gba1^{D409V/D409V}$ mice and age-matched controls for AT8, AT180, AT270, Tau-5, and β-tubulin. Each lane represents an independent mouse brain. Clone AT8 antibody shows increased tau phosphorylation (S202/T205) in aged $Gba1^{D409V/D409V}$ mice. No differences between mutant and wild-type mice were observed in total tau levels (Tau-5) or other phosphorylated species (AT180 or AT270). The results are represented as the means ± SEM. Bars marked with different letters are significantly different from each other ($P < 0.05$).

Administration of Glucocerebrosidase into the Hippocampus Reverses the Biochemical and Memory Aberrations of Postsymptomatic $Gba1^{D409V/D409V}$ Mice. To determine whether reconstitution of the CNS with recombinant glucocerebrosidase can correct the biochemical aberrations and memory deficits of symptomatic $Gba1^{D409V/D409V}$ mice, a recombinant self-complementary AAV vector (serotype 1) encoding human glucocerebrosidase (AAV–GBA1) was administered bilaterally into the hippocampi of early and late symptomatic mice (4- and 12-mo-old, respectively). Immunohistochemical examination of the CNS of $Gba1^{D409V/D409V}$ mice that had been administered AAV–GBA1 at 12 mo of age and were analyzed 6 mo later revealed abundant and widespread hippocampal expression of glucocerebrosidase (Fig. 2*A*). Mice treated with a control virus that did not encode a transgene (AAV-EV) showed no staining (Fig. 2*A Inset*). The enzymatic activity in AAV–GBA1-treated (Fig. 2*B*, red bar) mice was ~10-fold higher than the baseline value (Fig. 2*B*, black bar) and the activity in $Gba1^{D409V/D409V}$ mice administered AAV-EV (Fig. 2*B*, blue bar). A similar distribution of the enzyme was noted in the CNS of $Gba1^{D409V/D409V}$ mice treated at 4 mo of age and analyzed 6 mo posttreatment. Expression of glucocerebrosidase in the 12-mo-old mice was associated with normalization of the hyperelevated levels of brain GlcSph after 6 mo (Fig. 2*C*, red bar). In contrast, $Gba1^{D409V/D409V}$ mice treated with the control virus exhibited continued accumulation of the proinflammatory lipid over the same time interval (Fig. 2*C*, blue bar). Glucosylceramide (GlcCer), another glucocerebrosidase substrate, was not affected by any of the treatments or genotypes (Fig. S1).

Hippocampal memory was evaluated with the novel object recognition test. Testing of 4-mo-old $Gba1^{D409V/D409V}$ mice before treatment confirmed that they exhibited impairments in novel object recollection (Fig. 2*D*). Treatment of these mice with AAV–GBA1 reversed memory deficits when the mice were tested 2 mo later (at 6 mo of age; Fig. 2*E*, red bars; $n = 10$, $P < 0.05$), whereas $Gba1^{D409V/D409V}$ mice treated with the control viral vector showed no discernible improvement (Fig. 2*E*, blue bars; $n = 9$). A similar result was obtained in a separate cohort of $Gba1^{D409V/D409V}$ mice treated with AAV–GBA1 at 12 mo of age (i.e., with higher levels of preexisting pathology) and tested 2 mo later (Fig. 2*F*, red bars, $n = 12$, $P < 0.05$; AAV-EV, blue bars, $n = 12$). Hence, augmenting CNS glucocerebrosidase activity in postsymptomatic $Gba1^{D409V/D409V}$ mice corrected the pathological accumulation of GlcSph and, importantly, their memory impairments.

Administration of Glucocerebrosidase into the Hippocampus of Symptomatic $Gba1^{D409V/D409V}$ Mice Reduces the Levels of Aggregated Proteins in the Brain. Because $Gba1^{D409V/D409V}$ mice exhibit reduced glucocerebrosidase activity and progressive accumulation of ubiquitin, α-synuclein, and tau aggregates in the hippocampus, we sought to test whether augmenting glucocerebrosidase levels in the brain would decrease the levels of these aberrant proteinaceous materials in symptomatic animals. The hippocampi of 4- and 12-mo-old $Gba1^{D409V/D409V}$ mice (the latter presented with

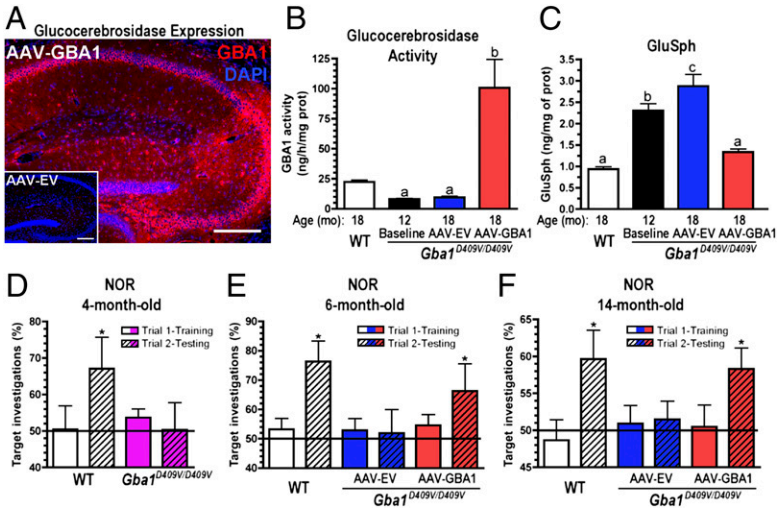

Fig. 2. CNS administration of AAV–GBA1 reduces GlcSph levels and reverses memory deficits. Four-mo-old and 12-mo-old *Gba1*$^{D409V/D409V}$ mice were given bilateral hippocampal injections of either AAV–EV or AAV–GBA1. Uninjected *Gba1*$^{D409V/D409V}$ littermates were euthanized at the time of surgeries as baselines for biochemical and histological endpoints ($n = 8$). Age-matched, uninjected wild-type (WT; $n = 9$) mice were used as a positive control. In both cohorts, tissues were collected for biochemical and pathological analysis at 6 mo postinjection. (*A*) Hippocampal expression of the recombinant enzyme 6 mo after stereotaxic injections. Image shows glucocerebrosidase immunoreactivity (red) and nuclear (DAPI; blue) stains in an AAV–GBA1-injected *Gba1*$^{D409V/D409V}$ mouse. (Scale bar, 400 μm.) *Inset* depicts glucocerebrosidase and nuclear staining in an AAV–EV-injected mouse. (*B* and *C*) Hippocampal administration of AAV–GBA1 into *Gba1*$^{D409V/D409V}$ mice increased glucocerebrosidase activity (*B*, red; $n = 11$, $P < 0.05$) and promoted clearance of GlcSph to WT levels (*C*, red; $n = 11$, $P < 0.05$), whereas AAV–EV-treated *Gba1*$^{D409V/D409V}$ mice showed no change in glucocerebrosidase activity (*B*, blue; $n = 12$, $P > 0.05$) and continued to accumulate GluSph compared with baseline levels (*C*, black; $n = 8$, $P < 0.05$). (*D*) Presurgical evaluation of 4-mo-old wild-type (WT) and *Gba1*$^{D409V/D409V}$ mice revealed no object preference when exposed to two identical objects. The results from trial 1 (training) are shown as white (WT) and purple (*Gba1*$^{D409V/D409V}$ mice) filled bars. After a 24-h retention period, mice were presented with a novel object. In trial 2 (testing, hatched bars), WT mice investigated the novel object significantly more frequently ($n = 9$, $P < 0.05$). In contrast, *Gba1*$^{D409V/D409V}$ mice ($n = 11$; purple hatched bar) showed no preference for the novel object, indicating a cognitive impairment. (*E*) At 2 mo postinjection, mice were subjected to the novel object recognition (NOR) test. AAV–GBA1-treated *Gba1*$^{D409V/D409V}$ mice ($n = 10$; blue hatched bar), but not AAV–EV-treated animals ($n = 9$; red hatched bar), exhibited a reversal of their memory deficit when presented with the novel object during the testing trial. (*F*) A separate cohort of 12-mo-old *Gba1*$^{D409V/D409V}$ mice were injected with AAV–EV ($n = 12$) or AAV–GBA1 ($n = 12$). Similar to the 4-mo-old cohort, reversal of the memory dysfunction was observed when these animals were tested at 2 mo postinjection (14 mo of age). The results are represented as means ± SEM. (*D–F*) The horizontal line demarcates 50% target investigations, which represents no preference for either object (*, significantly different from 50%, $P < 0.05$). (*B* and *C*) Bars with different letters are significantly different from each other ($P < 0.05$).

greater accumulation of aggregates and pathology) were stereotaxically injected bilaterally with 2E11 DNase-resistant particles (drp) of AAV–GBA1 or –EV. Analysis of brain tissues of *Gba1*$^{D409V/D409V}$ mice at the start of the study (at 4 and 12 mo of age) and at 6 mo postinjection with the control AAV–EV vector showed accumulation of ubiquitin, α-synuclein, and tau aggregates over this period (Fig. 3). In contrast, gene delivery of AAV-GBA1 into the 4-mo-old *Gba1*$^{D409V/D409V}$ mice led to reductions of hippocampal ubiquitin, proteinase K-resistant α-synuclein, and tau aggregates (Fig. 3). However, the reduction of ubiquitin, but not the reductions in α-synuclein or tau, reached statistical significance. CNS expression of glucocerebrosidase in the older (12-mo-old) mice produced a similar, but more modest, effect than that noted in the younger cohort when assayed 6 mo later (Fig. 3). Delivery of glucocerebrosidase appeared to have slowed the rates of accumulation of tau and α-synuclein but had no effect on ubiquitin levels, suggesting that the mechanisms for accumulation of these proteins may be different. It is possible that the higher levels of aggregates present in the older animals require a longer period or more glucocerebrosidase to be efficiently reduced. Nevertheless, the data suggest that augmenting glucocerebrosidase activity in the CNS can retard the extent of accumulation of pathologically misfolded protein aggregates in symptomatic *Gba1*$^{D409V/D409V}$ mice.

CNS of Transgenic A53T α-Synuclein Mice Is Associated with Lower Glucocerebrosidase Activities. Analyses of CSF and brain samples of patients with PD or DLB have shown that glucocerebrosidase activity is lower in affected than in unaffected individuals, suggesting a causal role of the lysosomal enzyme in the development of these synucleinopathies (13–15). Recent data have also suggested that α-synuclein has the capacity to inhibit lysosomal glucocerebrosidase activity (12, 29). To determine whether overexpression of α-synuclein negatively affects the activity of glucocerebrosidase, brain lysates from transgenic A53T α-synuclein mice (expressing mutant human α-synuclein bearing the A53T mutation) were studied (30). Similar to findings in PD patients without mutations in *GBA1* (15), A53T α-synuclein mice exhibited significantly lower lysosomal glucocerebrosidase activity than did wild-type animals (Fig. 4*A*). This effect was dependent on the levels of α-synuclein, because the CNS of homozygous A53T α-synuclein mice showed greater reductions in enzymatic activity than their (Het) littermates who expressed lower levels of α-synuclein (Fig. 4*A*, hatched bars). This decrease was selectively associated with glucocerebrosidase, because the activities of other lysosomal enzymes (i.e., hexosaminidase and β-galactosidase) were unaffected (Fig. 4*A*). These results support the contention that high levels of α-synuclein can inhibit lysosomal glucocerebrosidase activity, because greater inhibition was correlated with higher levels of α-synuclein.

AAV-Mediated Expression of Glucocerebrosidase in the CNS of Transgenic A53T α-Synuclein Mice Lowers α-Synuclein Levels. Earlier, we noted that overexpression of glucocerebrosidase reduced the accumulation of α-synuclein aggregates in the CNS of symptomatic *Gba1*$^{D409V/D409V}$ mice (Fig. 3*B*). To confirm the therapeutic potential of glucocerebrosidase in moderating the accumulation of α-synuclein, we next tested whether this reduction could also be realized in A53T α-synuclein mice (30). The striatum of 4-mo-old heterozygous A53T α-synuclein mice was unilaterally injected with either AAV–GBA1 or a control virus encoding GFP (AAV–GFP). As expected, glucocerebrosidase activity was significantly increased (approximately sevenfold) in the ipsilateral striatum of AAV–GBA1-injected mice compared with the contralateral side or to AAV–GFP-injected controls (Fig. 4*B*). Striatal tissue homogenates were also subjected to serial fractionation (9) to separate the cytosolic-soluble, membrane-associated, and cytosolic-insoluble forms of α-synuclein. Quantitation by ELISA revealed that the levels of cytosolic soluble α-synuclein were significantly reduced ($86 \pm 3\%$ of control, $n = 5$, $P < 0.01$) by striatal expression of glucocerebrosidase (Fig. 4*B*). The levels of membrane-associated α-synuclein also exhibited a modest reduction ($81 \pm 9\%$ of control, $n = 5$, $P = 0.07$) upon expression of glucocerebrosidase (Fig. 4*B*). However, the amount of the insoluble fraction was unchanged by treatment.

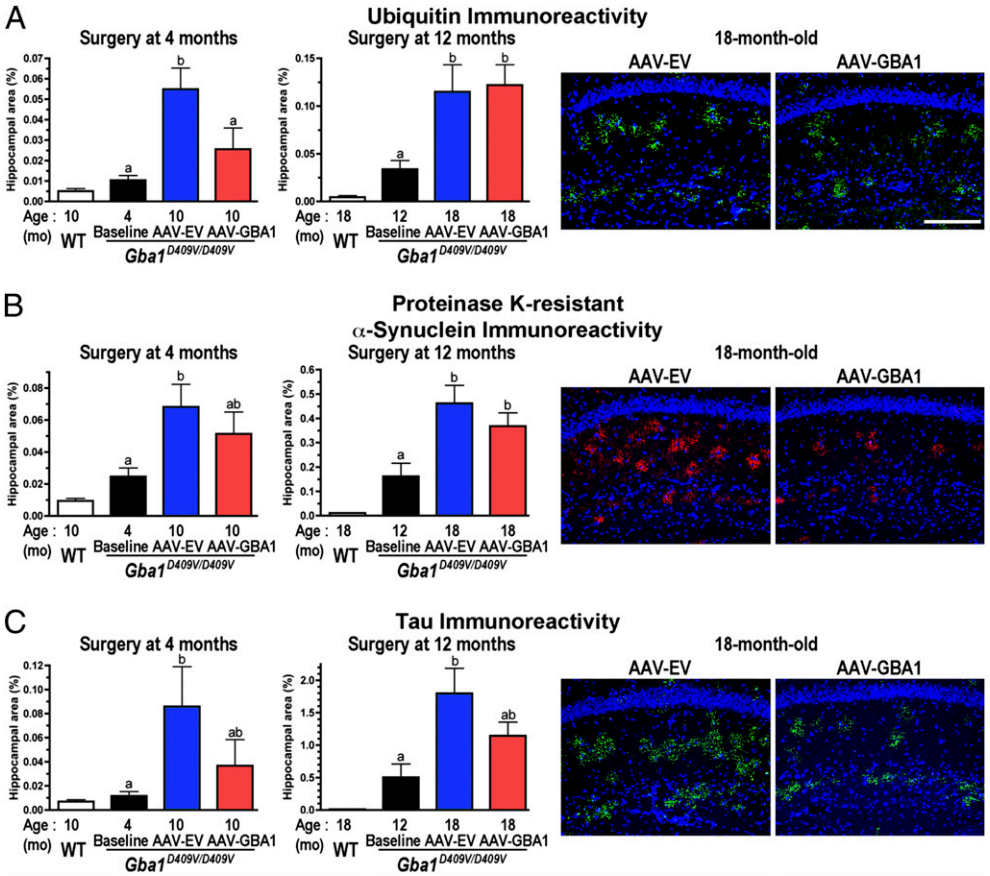

Fig. 3. Expression of glucocerebrosidase in symptomatic *Gba1*$^{D409V/D409V}$ mouse hippocampi slows accumulation of aggregated α-synuclein and tau. Two cohorts of *Gba1*$^{D409V/D409V}$ mice were injected with either AAV–EV or –GBA1 bilaterally into the hippocampus at 4 or 12 mo of age. Age-matched, uninjected WT mice were left untreated as positive controls. *Gba1*$^{D409V/D409V}$ littermates were harvested at the time of the injections as a baseline group. Injected animals were killed 6 mo after surgery. Graphs represent hippocampal quantifications of ubiquitin (*A*), proteinase K-resistant α-synuclein (*B*), and tau immunoreactivity (*C*) for the cohorts injected at 4 (*Left*) or 12 (*Center*) mo of age. Glucocerebrosidase augmentation in the CNS of symptomatic *Gba1*$^{D409V/D409V}$ mice reduced the levels of aggregated proteins, although this treatment was less effective in older animals. Images (*Right*) show ubiquitin (*A*, green), proteinase K-resistant α-synuclein (*B*, red) and tau (*C*, green) immunoreactivity in the hippocampi of 18-mo-old *Gba1*$^{D409V/D409V}$ mice treated with AAV–EV or –GBA1. DAPI nuclear staining is shown in blue. (Scale bar, 100 μm.) The results are represented as means ± SEM, with $n \geq 8$ per group. Bars with different letters are significantly different from each other ($P < 0.05$).

The efficacy of glucocerebrosidase in reducing α-synuclein levels in the spinal cord of A53T α-synuclein mice was also determined. Newborn A53T α-synuclein mice were injected with AAV–GBA1 or –GFP into both cerebral lateral ventricles and the upper lumbar spinal cord for a total dose of 3E11 drp per pup (31). As expected, robust expression of glucocerebrosidase (approximately threefold higher than controls) in the spinal cord was achieved following administration of AAV–GBA1 but not the control vector (Fig. 4*C*). Human α-synuclein mRNA levels were similar in control and treated mouse brains (control, 100 ± 5%; AAV-GBA1, 95 ± 5%). Analogous to the striatal injections, administration of AAV–GBA1 lowered α-synuclein levels in the soluble fraction to 67 ± 7% of control ($P < 0.01$; Fig. 4*C*). However, despite these reductions in α-synuclein, we did not observe a significant survival benefit [the median survival of AAV–GFP-treated mice was 290 d ($n = 13$) vs. 313 for AAV–GBA1-treated mice ($n = 18$)]. Nevertheless, these results indicate that augmenting the activity of glucocerebrosidase can lower α-synuclein levels in the CNS of A53T α-synuclein mice.

Discussion

Following the initial description of *GBA1* mutations as a risk factor for PD and DLB, findings from several independent studies have supported a role for glucocerebrosidase in the pathogenesis of these devastating diseases (4). A decrease in glucocerebrosidase activity and presence of mutant enzyme can purportedly induce increases in CNS α-synuclein/ubiquitin aggregates (8–12). Analyses of mouse Gaucher models harboring mutations in *Gba1* suggest that a decrease in enzymatic activity promotes neuronal protein misprocessing and cognitive deficits, two characteristics of PD and DLB (8–10, 32). However, the extent to which *Gba1* deficiency contributes to the pathogenesis of these ailments has not been established. This study provides further support for a role of glucocerebrosidase in α-synuclein processing, confirms a potential feedback loop between glucocerebrosidase activity and α-synuclein levels, and validates glucocerebrosidase augmentation in the CNS as a therapeutic approach for diseases associated with α-synuclein misprocessing, such as PD and DLB.

Although the precise pathologies of PD and LBD remain unclear, the findings of progressive accumulation of α-synuclein and other proteins in LBs have implicated protein misfolding as a potential causative mechanism (25, 33). This proteinopathy is replicated in the *Gba1*$^{D409V/D409V}$ mouse model of Gaucher disease, which shows a progressive accumulation of tau in addition to the described increases in α-synuclein and ubiquitin aggregates (10). Tau and α-synuclein likely play pivotal roles in disparate sets of neurodegenerative diseases (25). Mutations in their genes, *MAPT* and *SNCA*, that lead to formation of tau and α-synuclein, respectively, result in Alzheimer's disease, PD, DLB, and frontotemporal dementia (21, 25, 34). Although the mechanisms by which these proteins aggregate appear to be different, α-synuclein, for example, is spontaneously self-polymerizing (35), whereas tau requires the presence of an inducing agent (36, 37). However, the disease mechanisms need further clarification, because α-synuclein fibrils may be able to promote the polymerization of tau (24, 38), so the observed tau aggregation in the CNS of *Gba1*$^{D409V/D409V}$ mice may be occurring secondary to α-synuclein fibrillation.

Although PD typically presents as a movement disorder, patients may present various degrees of cognitive impairment, including dementia. PD patients harboring mutations in *GBA1* typically have lower cognitive scores than their non-*GBA1*-mutation–bearing counterparts, suggesting that altered *GBA1* increases susceptibility to development of cognitive deficits (20). The *Gba1*$^{D409V/D409V}$ Gaucher mouse model recapitulates many of the aberrant biochemical characteristics noted in brains from PD and DLB patients and also features measurable deficits in memory. We

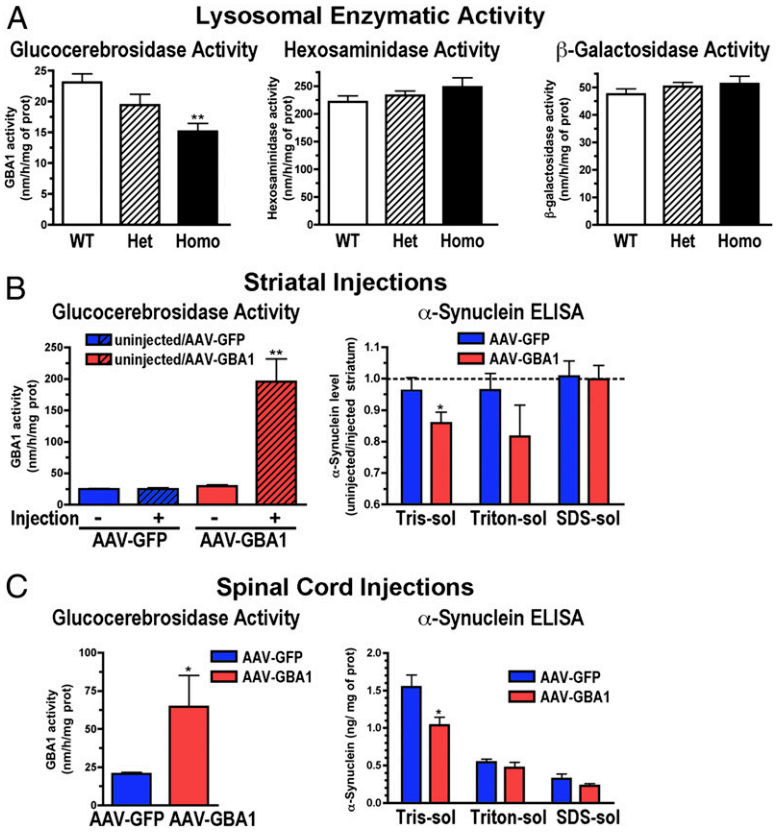

Fig. 4. Glucocerebrosidase augmentation in A53T α-synuclein mouse brain decreases α-synuclein levels. A53T α-synuclein transgenic mice exhibit decreased brain glucocerebrosidase activity. (*A*) The activity of various lysosomal enzymes was determined in cortical homogenates from homozygous ($n = 9$) and heterozygous ($n = 8$) α-synuclein transgenics and wild-type littermates ($n = 9$). Glucocerebrosidase activity was inversely correlated with α-synuclein levels, with homozygous mice showing a greater reduction of hydrolase activity. The enzymatic activities of two other lysosomal hydrolases, hexosaminidase and β-galactosidase, remained unchanged by the expression of A53T α-synuclein. (*B*) Four-mo-old A53T α-synuclein mice were each injected with either AAV–GFP ($n = 6$) or AAV–GBA1 ($n = 5$) unilaterally into the right striatum. The left striatum was used as an uninjected control for each animal to reduce the variability in α-synuclein levels between subjects. Four mo later, mice were euthanized, and both striata were collected. Robust glucocerebrosidase activity was observed in the AAV–GBA1-injected striatum (sevenfold over the uninjected contralateral side). Expression of glucocerebrosidase promoted decreased α-synuclein levels in the cytosolic fraction (Tris-soluble, non-membrane–associated; $P < 0.05$). (*C*) Newborn (P0) A53T α-synuclein mice were injected with either AAV–GFP or –GBA1 into the lumbar spinal cord. As expected, robust glucocerebrosidase activity was noted in AAV–GBA1-injected mice (threefold over controls). As in the striatum, expression of glucocerebrosidase reduced α-synuclein levels in the cytosolic fraction (Tris-soluble, nonmembrane associated; $n = 7$ per group, $P < 0.05$). Data are represented as means ± SEM. *$P < 0.05$.

have shown that these CNS manifestations can be ameliorated in presymptomatic animals by supplementation with exogenous enzyme (10). Because very few patients carrying *GBA1* mutations will develop cognitive impairment, it was pertinent to test whether the same salutary effects can also be realized in animals with overt disease. We showed that AAV-mediated expression of glucocerebrosidase in both early and late symptomatic $Gba1^{D409V/D409V}$ mice was also effective in reversing cognitive impairment. This recovery in cognition was associated with complete clearance of GlcSph and measurable reductions in the accumulation of the pathological aggregates. Augmenting glucocerebrosidase activity in the CNS of $Gba1^{D409V/D409V}$ mice may possibly reduce the levels of "toxic" metabolites and thereby improve lysosomal function, a requirement for correct synaptic activity (39, 40) and proper functioning of pathways that degrade aggregated proteins (41, 42). Importantly, these results strongly suggest that augmenting CNS glucocerebrosidase activity may impede progression of (and perhaps even reverse) some aspects of Gaucher-related parkinsonism and associated synucleinopathies.

Ongoing investigations continue to provide greater insights into the relationship between glucocerebrosidase and α-synuclein (4, 32). It is evident that a decrease in glucocerebrosidase activity or the presence of mutant glucocerebrosidase can promote the aberrant accumulation of α-synuclein (32). Reportedly, α-synuclein can also interact with glucocerebrosidase to reduce its trafficking to the lysosomes or inhibit its activity, thereby exacerbating the disease state (12, 29). A role for glucocerebrosidase in the disease process is also supported by findings of decreased glucocerebrosidase activity in the brains and CSF of sporadic PD patients, irrespective of whether they harbor *GBA1* mutations (15). To complement these findings, we studied transgenic A53T α-synuclein mice that overexpress A53T α-synuclein in the CNS (30). Measurements of brain lysates from A53T α-synuclein mice showed that mice with higher levels of α-synuclein were correlated with lower amounts of glucocerebrosidase activity. Importantly, increasing glucocerebrosidase activity in the brains of A53T α-synuclein mice reduced α-synuclein levels. These results suggest that augmenting glucocerebrosidase activity in the CNS of A53T α-synuclein mice, through its "synuclease" activity, may interrupt the deleterious feedback of α-synuclein on glucocerebrosidase activity and thereby restore the cell's capacity to degrade α-synuclein. Hence, augmenting glucocerebrosidase activity in the CNS via administration of the recombinant enzyme, gene transfer vectors encoding the lysosomal enzyme, or small-molecule activators of the hydrolase may reduce the extent of accumulation of misfolded proteins and may thereby slow disease progression of PD in patients with or without *GBA1* mutations.

In summary, the efficacy of increasing glucocerebrosidase in modulating the extent of accumulation of aggregates in the CNS was demonstrated in two murine models of synucleinopathy. In a symptomatic mouse model of Gaucher-related parkinsonism and proteinopathy, augmenting glucocerebrosidase activity in the CNS corrected the aberrant storage of lipids, reversed cognitive dysfunction, and reduced the levels of aggregated α-synuclein and tau. Increasing glucocerebrosidase levels in the CNS was also effective in decreasing α-synuclein levels in the A53T α-synuclein mouse model, indicating that the positive feedback between α-synuclein increase and loss of glucocerebrosidase activity (10, 12) can be influenced by increasing enzymatic activity of glucocerebrosidase in lysosomes. Together, these results provide further support for the development of glucocerebrosidase augmentation therapies for PD and related synucleinopathies.

Materials and Methods

Animals. The Institutional Animal Care and Use Committee at Genzyme, a Sanofi Company, approved all procedures. The $Gba1^{D409V/D409V}$ mouse model of Gaucher disease harbors a point mutation at residue 409 in the murine *Gba1* gene (43). Transgenic A53T α-synuclein mice express human

A53T α-synuclein (line M83) under the transcriptional control of the murine PrP promoter (30).

AAV Vectors and Injections. A detailed description of the AAV vectors and the injection procedures used in these studies is available in *SI Materials and Methods*.

Western Blotting. Western blotting procedures are described in *SI Materials and Methods*.

Measurements of Glucocerebrosidase Activity and Glycosphingolipid Levels. Brain and hippocampal glucocerebrosidase activities were determined as described with 4-methylumbelliferyl (4-MU)-β-D-glucoside as the artificial substrate (10). Hexosaminidase and β-galactosidase activities were determined with 4-MU-*N*-acetyl-β-D-glucosaminide and 4-MU-β-D-galactopyranoside, respectively. Tissue GlcCer and GlcSph levels were measured by mass spectrometry as described (10).

Immunohistochemistry. Immunohistochemistry procedures are described in *SI Materials and Methods*.

Mice Behavioral Tests. A detailed description of the behavioral tests is available in *SI Materials and Methods*.

Fractionation and Quantification of α-Synuclein. Striatum and spinal cord from A53T–α-synuclein mice were homogenized as described (9) to obtain three fractions: cytosolic (Tris-soluble), membrane-associated (Triton X-100–soluble), and insoluble (SDS-soluble). The concentration of human α-synuclein in the different fractions was quantified by sandwich ELISA (Invitrogen). Protein concentration was determined by the microBCA assay (Pierce).

Statistical Analysis. Statistical analyses were performed by Student's *t* test or ANOVA followed by Newman–Keuls' post hoc test. Preference for novelty was defined as investigating the novel object >50% of the time by a one-sample *t* test. All statistical analyses were performed with GraphPad Prism v4.0 (GraphPad Software). $P < 0.05$ was considered significant.

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