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Glucocerebrosidase haploinsufficiency in A53T α-synuclein mice impacts disease onset and course

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ABSTRACT

Mutations in *GBA1* encountered in Gaucher disease are a leading risk factor for Parkinson disease and associated Lewy body disorders. Many *GBA1* mutation carriers, especially those with severe or null *GBA1* alleles, have earlier and more progressive parkinsonism. To model the effect of partial glucocerebrosidase deficiency on neurological progression *in vivo*, mice with a human A53T α-synuclein ($SNCA^{A53T}$) transgene were crossed with heterozygous null *gba* mice ($gba^{+/-}$). Survival analysis of 84 mice showed that in $gba^{+/-}//SNCA^{A53T}$ hemizygotes and homozygotes, the symptom onset was significantly earlier than in $gba^{+/+}//SNCA^{A53T}$ mice (p-values 0.023–0.0030), with exacerbated disease progression (p-value < 0.0001). Over-expression of $SNCA^{A53T}$ had no effect on glucocerebrosidase levels or activity. Immunoblotting demonstrated that *gba* haploinsufficiency did not lead to increased levels of either monomeric SNCA or insoluble high molecular weight SNCA in this model. Immunohistochemical analyses demonstrated that the abundance and distribution of SNCA pathology was also unaltered by *gba* haploinsufficiency. Thus, while the underlying mechanism is not clear, this model shows that *gba* deficiency impacts the age of onset and disease duration in aged $SNCA^{A53T}$ mice, providing a valuable resource to identify modifiers, pathways and possible moonlighting roles of glucocerebrosidase in Parkinson pathogenesis.

1. Introduction

Parkinson disease (PD), a common neurodegenerative disorder associated with aging, is characterized pathologically by intraneuronal Lewy bodies and the loss of dopaminergic neurons in the substantia nigra [1]. Clinically, patients manifest with resting tremors, bradykinesia, rigidity and postural instability [2], as well as non-motor symptoms including cognitive impairment [3]. Identifying genetic contributions to Parkinson disease has illuminated molecular mechanisms underlying disease pathogenesis [4,5], particularly the gene encoding alpha-synuclein (*SNCA*), a major component of Lewy bodies. The Ala53Thr (A53T) mutation in SNCA, initially identified in rare families with Parkinson disease [6] increases its propensity to aggregate [7,8].

The genetic landscape of Parkinson disease now includes nearly thirty genes [9]. Heterozygous mutations in *GBA1,* the gene mutated in Gaucher disease, encoding the lysosomal enzyme glucocerebrosidase (GCase, EC 3.2.1.45) are the most common genetic risk factor for

Abbreviations: CAT, cathepsin; CBE, conduritol-β-epoxide; ER, endoplasmic reticulum; $gba^{+/-}$, heterozygous null mouse *gba* allele; GCase, glucocerebrosidase; GD, Gaucher disease; HMW, high molecular weight; LAMP2, lysosomal membrane protein 1; LIMP2, lysosomal integral membrane protein 2; PD, Parkinson disease; SNCA, α-synuclein; $SNCA^{A53T}$, mutant human A53T α-synuclein; TH, tyrosine hydroxylase

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Parkinson disease as well as dementia with Lewy bodies, often associated with an earlier onset and more severe cognitive and non-motor symptoms [10,11]. Severe *GBA1* mutations, such as loss of function mutations c.84insG and IVS2 + 1G > A, confer a higher risk for Parkinson disease and a more progressive course compared to milder mutations like N409S (N370S) [12,13]. Many groups have investigated the relationship between SNCA levels and GCase deficiency or inhibition, but only a few have utilized animal models [14–17], including mice treated with the GCase inhibitor conduritol-β-epoxide (CBE) [16,18]. To further investigate the link between *GBA1* mutations and Parkinson disease, we sought to develop an *in vivo* mouse model recapitulating the accelerated Parkinson disease course observed in loss-of-function *GBA1* mutation carriers, by crossing a $SNCA^{A53T}$ transgenic mouse that develops severe motor impairment and neuronal SNCA inclusions [19], with heterozygotes for a functionally null *gba* allele [20]. While homozygous null *gba* mice die perinatally, heterozygotes, with 40–50% of wildtype GCase enzyme activity, develop normally [20], and mimic patients with Parkinsonism carrying loss-of-function *GBA1* mutations. Unlike the CBE treated models, these mice age naturally, enabling the analysis of factors associated with aging.

2. Materials and methods

2.1. The generation of $gba^{+/-}//SNCA^{A53T}$ mice

In this study, $gba^{+/-}$ mice [20] were crossed with mice with a human $SNCA^{A53T}$ transgene [19]. $gba^{+/-}//SNCA^{A53T}$ homozygotes and hemizygotes, along with their $gba^{+/-}$ and $gba^{+/+}//SNCA^{A53T}$ controls, were followed for two years. All housing and breeding of mice were performed under NHGRI Animal Care and Use Committee-approved protocols.

2.1.1. Mouse background

gba knock-out (gba^{tm1Nsb}; maintained as heterozygotes) and transgenic $SNCA^{A53T}$ (Tg(Prnp-SNCA*A53T)83Vle) mice were originally on mixed backgrounds (C57BL/6 X FVB/N and C57BL/C3H, respectively). Both lines were backcrossed against the C57Bl/6 J (B6) background, with matings being informed by genetic markers. The Mouse Medium Density (MD) Linkage panel (1449 SNPs; Illumina) was used to define blocks of heterozygosity. At each subsequent generation, pups were genotyped for the presence of the mutation (*gba*-) or transgene ($SNCA^{A53T}$) and were assessed using progressively more focused panels of microsatellite markers within these heterozygous blocks. Markers, first chosen at intervals of < 5 Mb within each heterozygous block, were selected at decreased intervals as heterozygosity diminished with each generation. The two male pups with the highest levels of homozygosity were mated to B6 females at each generation. This process was repeated in excess of ten generations for gba^{tm1Nsb}, and five generations for Tg(Prnp-SNCA*A53T) mice, at which time the Y chromosome was fixed by mating a female mutant/transgenic pup with a B6 male mouse.

2.1.2. Mouse genotyping

Genomic DNA was prepared from tail biopsies as previously described [21]. The *SNCA* primers used for PCR amplification to confirm the presence of human *SNCA*, and neo primer set used to screen for the *gba* null allele are shown below.

SNCA-F 5′-TGC CTG TGG ATC CTG ACA AT-3′.
SNCA-R 5′-GGG GAG GGG CAA ACA ACA GA -3′.
NEO-1F 5′-ACA GAC AAT CGG CTG CTC TGA TGC -3′.
NEO-2R 5′-CTC GTC AAG AAG GCG ATA GAA GGC-3′.

2.1.3. Evaluation of SNCA copy number

To distinguish hemizygous from homozygous $SNCA^{A53T}$ mice, transgene copy-number was evaluated using TaqMan (applied Biosystems, Foster City, CA) Copy Number real-time PCR assays. Two FAM-labeled probes were used to measure the human *SNCA* in genomic DNA: Hs05969230_cn in exon 2 and Hs02236645_cn in exon 7. The mouse *Tfrc* gene assay (VIC-labeled; 4,458,366) was used as an endogenous control. Assays were performed according to the manufacturer's directions, using 20 ng of genomic DNA. Reactions were run on a StepOnePlus Real-Time PCR system and data were analyzed using the StepOne Software v2.3. Relative quantitation was calculated using the $2^{-\Delta\Delta CT}$ method. Heterozygote and homozygote controls were included in each panel of reactions.

2.1.4. Phenotyping of mice

Six groups of mice were followed: wildtype controls ($gba^{+/+}$), *gba* null heterozygotes ($gba^{+/-}$), $SNCA^{A53T}$ hemizygotes with and without the null *gba* allele (($gba^{+/-}//SNCA^{A53T}$) and ($gba^{+/+}//SNCA^{A53T}$)) and $SNCA^{A53T}$ homozygotes with and without the null *gba* allele (($gba^{+/-}//SNCA^{A53T/A53T}$) ($gba^{+/+}//SNCA^{A53T/A53T}$)). An equal number of male and female mice were included in this study. Weight was recorded weekly from age 6 months. Once a 13% drop in weight was observed, measurements continued at least biweekly. Mice were monitored for gait abnormalities, hunching or arching of the back, abnormal grooming, and urinary retention, and symptoms were recorded or filmed. Mice were euthanized when total limb paralysis and/or bladder dysfunction developed, together with the analogous aged-matched controls.

2.2. Tissue collection and processing

2.2.1. Murine tissues

Mice were deeply anesthetized and perfused with PBS, followed by 70% ethanol/150 mM NaCl. Brains and spinal cords were dissected out and further fixed in 70% ethanol/150 mM NaCl followed by paraffin infiltration [19] for imaging. For RNA and protein studies, brain and other tissues were collected and immediately snap-frozen in liquid N_2.

2.2.2. Human brain samples

Total protein from five cortical brain samples collected from autopsies performed at the NIH Clinical Center or from the Massachusetts General Hospital Brain Bank was extracted in RIPA buffer. Samples included a normal control, two patients with type 1 Gaucher disease (genotype N370S/N370S), one with and one without Parkinson disease, a c.84insG heterozygote with parkinsonism, and a patient with Parkinson disease without *GBA1* mutations. Each subject was male and above age 55 years. Samples were processed as described for mouse brain samples.

2.3. Protein extraction and evaluation

2.3.1. Protein extraction

Total protein was extracted from forebrain, midbrain and total mouse brain samples using two different extraction buffers: 1:10 (w/v) citrate-phosphate buffer (pH 5.4, 0.25%) Triton X-100 and protease inhibitor cocktail) and RIPA buffer (Thermo Scientific, Waltham, MA). Samples were homogenized on ice, sonicated for 10 s, and centrifuged at 5000 rpm for 10 min.

2.3.2. Glucocerebrosidase levels

The lysates in citrate-phosphate buffer were used for GCase activity and immunoblotting using a fluorescent activity-based probe specific for GCase (MDW933) [22]. 15 μg of midbrain and forebrain lysate and 1.2 μM imiglucerase (Genzyme, Cambridge, MA), used as a control, were incubated with 100 nM of the GCase-specific MDW933 fluorescent probe at 37° C for 90 min and run on a 4–20% Criterion: TGX™ gel (Bio-Rad laboratories). The results were analyzed using an excitation wavelight of 488 nm and emission of 520 nm to measure the fluorescent signal in the gel [23].