# GBA1-Linked Parkinson's Disease AAV Gene Therapy

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## INTRODUCTION AND BACKGROUND

Parkinson's disease (PD) is a chronic, progressive, neurodegenerative disorder characterised by α-synuclein accumulation, Lewy Body formation and loss of dopaminergic neurones within the substantia nigra pars compacta (SNpc) which controls movement and coordination. Non-motor symptoms include cognitive decline, sleep disturbance and psychiatric symptoms. Mutations in *GBA1* which encodes the lysosomal enzyme glucocerebrosidase (GCase) are linked to Gaucher disease (GD), one of the most common lysosomal storage diseases. In this population, approximately 9.1% of patients are likely to develop PD before the age of 80, compared to 3-4% for the wider population[1]. Heterozygous *GBA1* GD mutation carriers and a number of non-Gaucher PD associated *GBA1* mutations share a similar risk of developing PD. 5-15% of people with PD carry mutations in *GBA1*, resulting in dysfunctional enzyme and lysosomes[1].

AAV-mediated gene therapy providing sustained functional GCase may reduce the early and accelerated rate of disease progression observed for *GBA1*-linked PD. An engineered GCase called variant 85 (GCase85), with two amino acid substitutions to the wild type GCase (GCase WT), has been established as a more stable enzyme[2] while retaining the same specific activity as wild type, resulting in net improved activity and substrate clearance in multiple organs when assessed in mouse models of GD (Fig 1 shows bone marrow as example). It also shows clinical benefit in GD (GALILEO-1 clinical trial NCT05324943, Goker-Alpan et al, Late-Breaking Abstracts I[3]). *GBA1-85*, the gene encoding GCase85, offers a potential for improved GCase delivery and distribution in the brain for *GBA1*-linked PD. In this study, AAV9-GBA1-85 in brain cells exhibit approximately an order of magnitude more measured activity than AAV9-GBA1-WT *in vitro* and *in vivo*, reflecting the improved stability. Direct brain injection of AAV9-GFP, AAV9-GBA1-WT or AAV9-GBA1-85 in mice show effective retrograde transport from the site of injection in the caudate putamen to the substantia nigra, with GCase85 exhibiting a broader distribution across the brain.

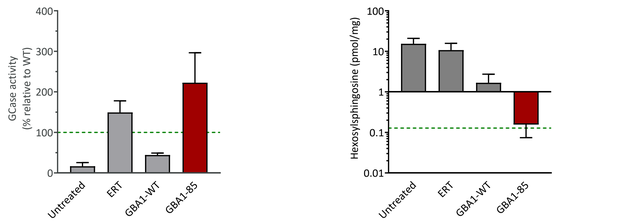



**Fig 1 *GBA1-85* results in net higher activity and better substrate clearance in bone marrow of GCase-deficient mice.** Treatments in Gba$^{9v/-}$ Gaucher disease mouse model, comparing systemic ERT (velaglucerase alfa, 60 U/kg every other week, seven cycles), AAV8-GBA1-WT or AAV8-GBA1-85 with enhanced stability (AAV dose 2x10$^{12}$ vg/kg). Bone marrow activity and substrate levels 12 weeks after injection (2h post-ERT), dotted line shows levels in healthy wild type (WT) animals. Data denoted as mean ± SD; n = 9 to 12 per treatment group. ERT: enzyme replacement therapy; GBA1-WT: wild type GBA1; GBA1-85: GBA1 variant 85.

## METHODS AND RESULTS

### *GBA1-85* delivers higher measured GCase activity in brain and neuronal cell lines

To investigate the potential of GCase85 in GBA1-linked Parkinson's disease, we assessed its activity 72h post-transduction in brain and neuronal cells.

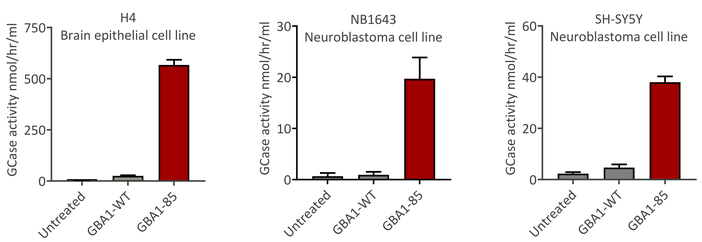
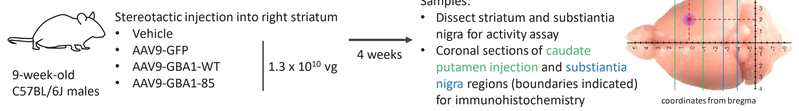



**Fig 2 GCase activity in supernatant of AAV9-GBA-WT or AAV9-GBA-85 treated cells** in different cell lines as indicated; n=3, data denoted as mean ± SEM.

### Design of *GBA1-85 in vivo* expression study




### GCase85 shows higher measured GCase activity *in vivo* compared to WT

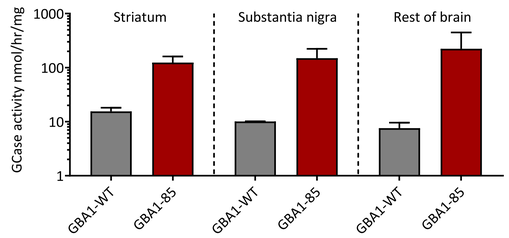



**Fig 3 GCase activity in brain regions** injected with indicated AAVs, samples dissected from striatum, substantia nigra or the rest of the brain. The GCase activity is normalised for VG, n=3, data denoted as mean ± SD.

### Intrastriatal injection of AAV9-*GFP* shows transduction in both caudate putamen and substantia nigra

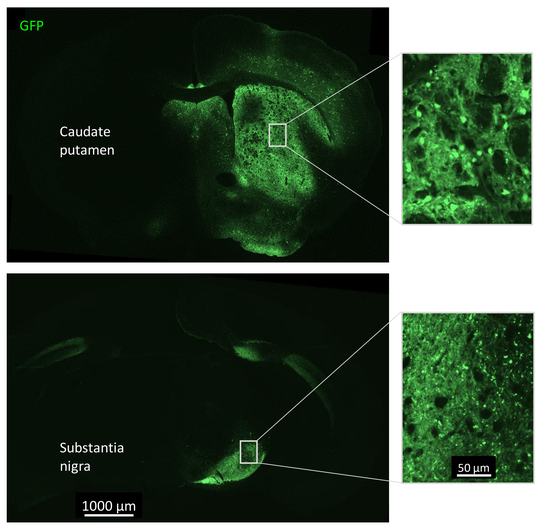



**Fig 4 AAV9 direct brain delivery.** Representative coronal sections from 4 animals injected with AAV9-GFP. GFP (which is not secreted) is present in spiny neurons and dopaminergic fibres; and distributed from the caudate putamen to the substantia nigra by retrograde transport (boxed insets).

### AAV9-GBA1-85 results in higher enzyme levels and broader distribution of GCase in the brain compared to AAV9-GBA1-WT

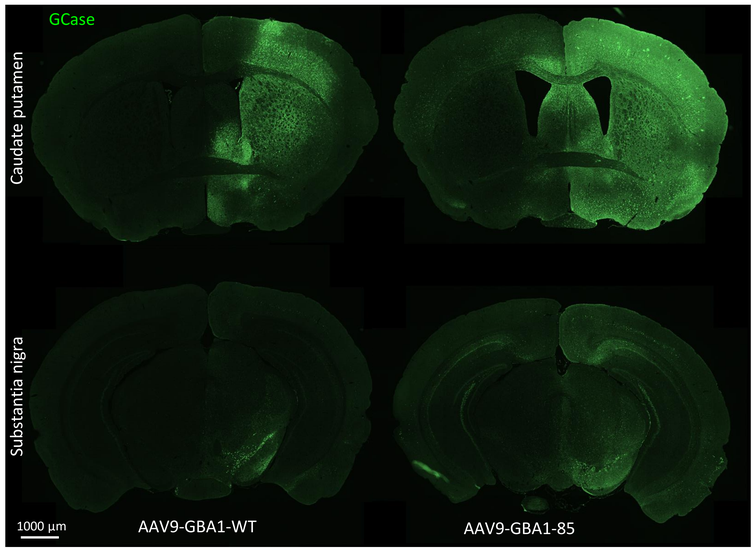



**Fig 5 GCase distribution in the brain**. Representative coronal sections of animals injected with either AAV9-GBA1-WT or AAV9-GBA1-85 labelled for GCase, n=4. GCase is a secreted protein and GBA1 variant 85 is engineered for stability. In animals with AAV9-GBA1-85 administration, GCase signal is more intense in both somata and neuropil of the injected right hemisphere and also in the left hemisphere compared to AAV9-GBA1-WT. In sections of the substantia nigra region, a clear GCase signal was observed in tyrosine hydrolase-positive somata within the substantia nigra pars compacta of animals from both groups.

## CONCLUSIONS

This study shows the potential for using the engineered GCase in a AAV delivered gene therapy for GBA1-linked Parkinson's disease

1. AAV9-GBA1-85 results in an order of magnitude higher GCase activity compared to AAV9-GBA1-WT *in vitro* and *in vivo*
2. Direct brain injection of AAV9 constructs to the caudate putamen is effectively distributed to the target cells of the substantia nigra
3. AAV9-GBA1-85 results in broader GCase distribution than AAV9-GBA1-WT when delivered by direct brain injection into mice

**References**

1. Smith, L.; Schapira, A.H.V. GBA Variants and Parkinson Disease: Mechanisms and Treatments. Cells 2022, 11, 1261. https://doi.org/10.3390/cells11081261

2. Comper, F. et al. Poster #41 Generation of β-Glucocerebrosidase variants with increased half-life in human plasma for liver directed AAV gene therapy aimed at the treatment of Gaucher disease type 1. WORLDSymposium 8-12 February, 2021

3. Goker-Alpan O. et al. Results from GALILEO-1, a first-in-human clinical trial of FLT201, an AAV-gene therapy, in adults with Gaucher disease Type 1. Late-Breaking Abstracts I, Abstract #2. Ballroom 3, Thursday May 9, 2024 8:00 am, ASGCT 27th Annual Meeting

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