Characterization of *In Vivo* and *In Vitro* Drug Screening Models for Gaucher Disease Based on GBA-D409V-KI MICE

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BACKGROUND

It is well-described that mutations in the human GBA (glucosylceramidase-β) gene and associated lowered glucosylceramidase-β (GCase) activity, can cause Gaucher disease (GD). Next to the significance of GCase for GD, the enzyme is highly discussed as therapeutic target in Parkinson`s disease (PD) research. To study both diseases and test possible therapeutic agents *in vivo*, specific mouse models were generated. Here we characterize GBA-D409V-KI mice, that express the mutant D427V GBA protein which corresponds to the D409V mutation in the mature human GBA protein, for expression of typical GD and PD biomarkers. Additionally, a corresponding *in vitro* model, embryonic fibroblasts generated from GBA-D409V-KI mice, is validated by evaluation of GCase activity after treatment with the β –glucosidase inhibitor isofagomine.

MATERIALS and METHODS

Liver samples and brains hemispheres of 4 to 12 months old homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI mice as well as age-matched wild type littermates (wt/wt) were assessed for GCase activity using the 4-MUG assay. Each sample was analyzed in duplicates and a third replicate including 1 mM CBE was used to subtract the GBA1 unspecific signal.

An aliquot of the hemisphere homogenate from 12 months old animals was used for extraction of soluble and Triton X-100 insoluble proteins and subsequently analyzed for murine α-synuclein level with a self-established immunosorbent assay based on the Mesoscale Discovery (MSD) platform.

The corresponding *in vitro* model, mouse embryonic fibroblasts (MEFs) generated from homo- or heterozygous GBA-D409V-KI E14 embryos or the wild type littermates, were validated by testing the effects of isofagomine on GCase activity normalized to viability.

GCase activity was determined with an on-cell 4-MUG assay, viability was assessed using the crystal violet assay. Data are given as relative fluorescent units (RFU) of 4-MUG assay normalized to optical density (OD) values derived from the crystal violet assay.

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RESULTS

GBA1 Activity

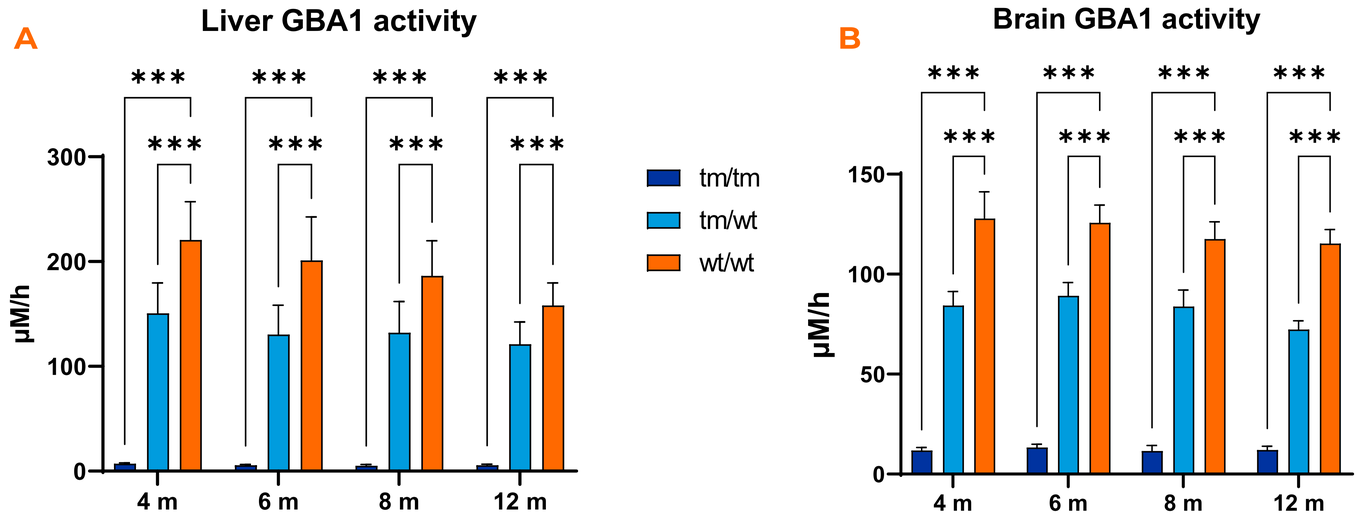

Figure 1. GBA1 activity in liver and brain samples of GBA-D409V-KI mice with age. GBA1 activity as µM/h in liver (A) and brain samples (B) of homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI mice as well as age-matched wild type littermates (wt/wt) at 4, 6, 8 and 12 months of age; n=12 per group. Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; *p<0.05; **p<0.01; ***p<0.001.

Soluble and Insoluble Cerebral α-synuclein

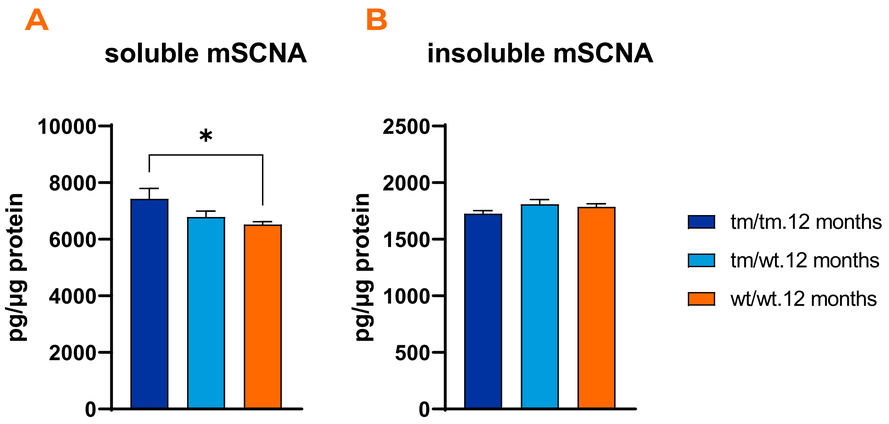

Figure 2. Soluble and insoluble α-synuclein in brain samples of 12 months old animals. Murine α-synuclein (mSCNA) in soluble (A) and insoluble (B) brain fractions of homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI mice as well as age-matched wild type littermates (wt/wt) at 12 months of age; n=12 per group. Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; *p<0.05.

Mouse Embryonic Fibroblasts

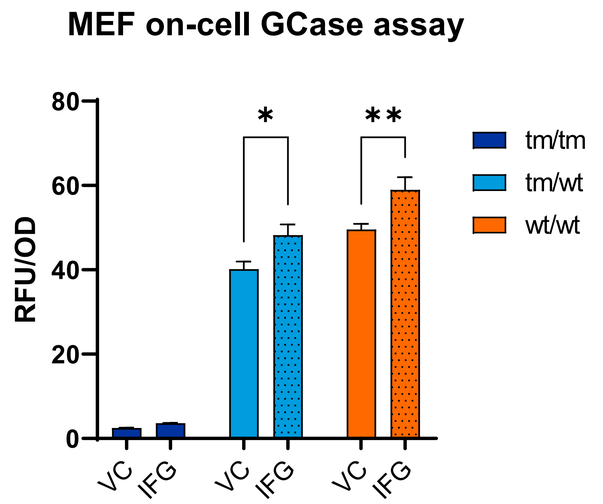

Figure 3. GCase activity in mouse embryonic fibroblasts (MEFs) of different genotypes treated with vehicle (VC) or Isofagomine (IFG). Mouse embryonic fibroblasts were isolated from homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI E14 embryos as well as age-matched wild type littermates (wt/wt). Cells were cultivated in 96-well plates and treated with either vehicle (0.1 % DMSO) or 20 µM isofagomine for 7 days. Thereafter, cells were subjected to either an adapted on-cell 4-MUG or crystal violet assay. Data are given as relative fluorescent units (RFU) of 4-MUG assay normalized to optical density (OD) values derived from crystal violet assay; n=5 per group. Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; *p<0.05; **p<0.01.

SUMMARY and CONCLUSION

In summary, we provide a baseline characterization of GD and PD biomarkers in GBA-D409V-KI mice and corresponding MEFs. Genotype-specific reduction of GCase activity is reliably present in both models. MEFs are a suitable *in vitro* screening tool before selected compounds are tested in the corresponding *in vivo* mouse model.