α-synuclein Pre-Formed Fibril-Induced Aggregation in SNCA and GBA Mutant iPSC-Derived Neurons and Rescue by Antibody Therapy

bit.bio
THE CELL CODING COMPANY

scantox

Loeffler T.[1], Oosterveen T.[2], Turner A.[2], Perez-Ruiz I.[1], Schilcher I.[1], Daurer M.[1], Breznik L.[1], Flunkert S.[1], Prokesch M.[1]

[1]Scantox Neuro GmbH, Grambach, Austria | [2]bit.bio Ltd., Cambridge, United Kingdom

For further information and inquiries contact office-austria@scantox.com

Objectives

To investigate how different Parkinson's disease-associated genotypes influence α-synuclein (α-syn) pathology and neuronal damage, we compared induced pluripotent stem cell (iPSC)-derived glutamatergic neurons carrying mutations in SNCA (A53T/A53T) and GBA (null/null, null/N409S, null/R159W) with their isogenic controls. We aimed to assess glucocerebrosidase (GCase) activity, neurofilament light chain (NF-L) secretion, and α-syn levels at baseline and following exposure to α-syn pre-formed fibrils (PFFs) and therapeutic antibody treatment.

Materials and Methods

Human iPSC-derived ioGlutamatergic Neurons from mutant and isogenic control lines were cultivated and deterministically programmed according to established protocols by bit.bio. Baseline GCase activity, NF-L release, and α-syn expression were quantified across genotypes. Cultures were then treated with recombinant α-syn PFFs to induce aggregation. Soluble and insoluble α-syn fractions were analyzed via an immunosorbent assay. To test therapeutic modulation, fibril-challenged neurons were treated with an α-syn antibody, and effects on α-syn species were quantified with the same methods.

Results

Neurons harboring GBA mutations displayed reduced GCase activity and elevated NF-L secretion compared to isogenic controls. Baseline α-syn levels were only slightly increased in mutant lines. Upon PFF treatment, a marked accumulation of both soluble and insoluble α-syn was observed in all genotypes with higher insoluble level in GBA mutated lines. Antibody treatment effectively reduced both soluble and insoluble α-syn species across genotypes.

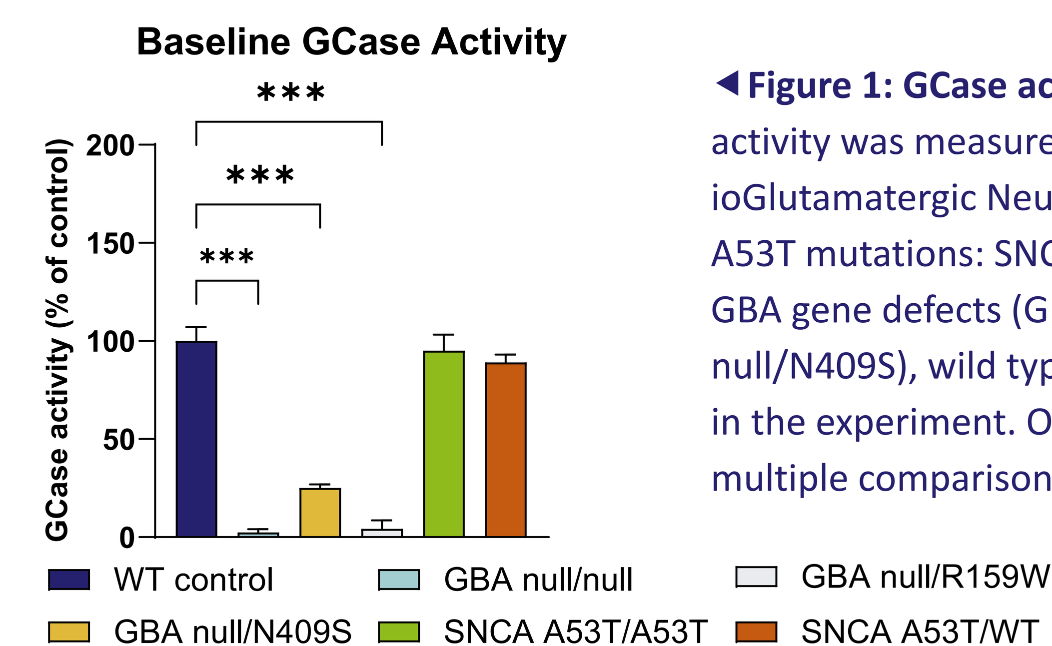

Figure 1: GCase activity ioGlutamatergic Neurons. GCase activity was measured on DIV9 on different types of ioGlutamatergic Neurons (25,000 cells/well, 96wp) with SNCA A53T mutations: SNCA A53T/A53T and SNCA A53T/WT as well as GBA gene defects (GBA null/null, GBA null/R159W and GBA null/N409S), wild type (WT) control neurons were also included in the experiment. One-way ANOVA followed by Dunnett's multiple comparison test. ***p <0.001.

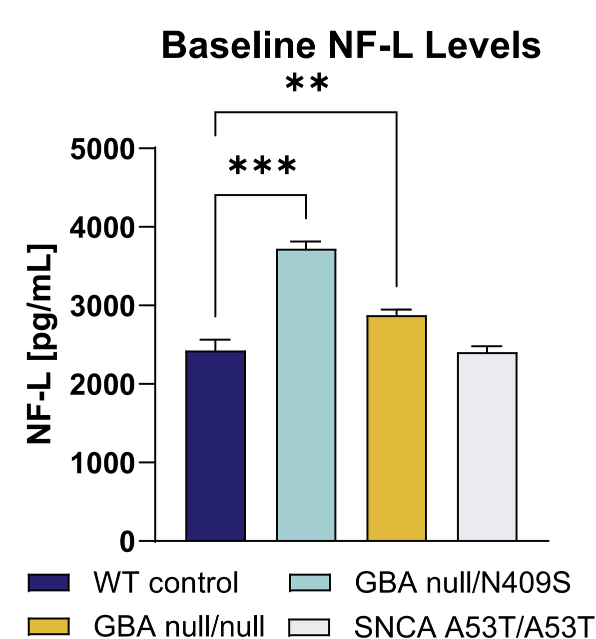

Figure 2: NF-L levels in supernatant of ioGlutamatergic Neurons. NF-L baseline levels were measured at DIV10 in supernatants of different types of ioGlutamatergic Neurons (25,000 cells/well, 96wp) with SNCA A53T mutations: SNCA A53T/A53T as well as GBA gene defects (GBA null/null and GBA null/N409S), wild type (WT) control neurons were also included in the experiment. NF-light® ELISA 10-7001 CE from UmanDiagnostics was used. One-way ANOVA followed by Dunnett's multiple comparison test.**p <0.01; ***p <0.001.

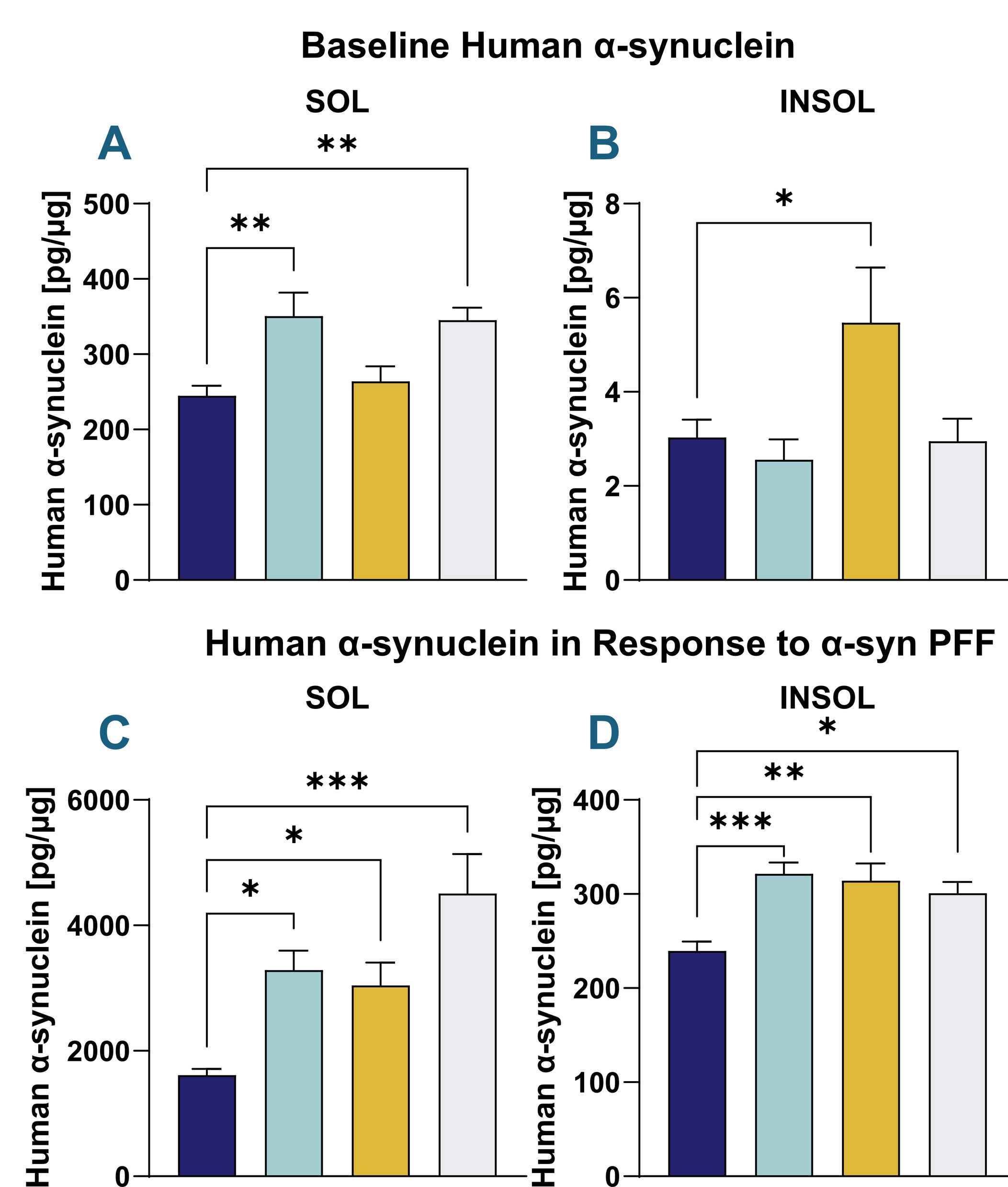

Figure 3: Soluble (SOL) and insoluble (INSOL) human α-syn levels in the cell extract from ioGlutamatergic Neurons of different genotypes. Cells were cultured for 12 days and Triton X-100 soluble and insoluble human α-syn levels were assessed by MSD assay. Baselines measurement (**A, B**) and response to α-syn PFF (**C, D**) are shown. Data are given as pg α-syn per µg total protein. Mean + SEM; n = 5-6; One-Way ANOVA followed by Dunnett's multiple comparison test. *p <0.05; **p <0.01; ***p <0.001.

Induction of α-syn Aggregation with α-syn PFF

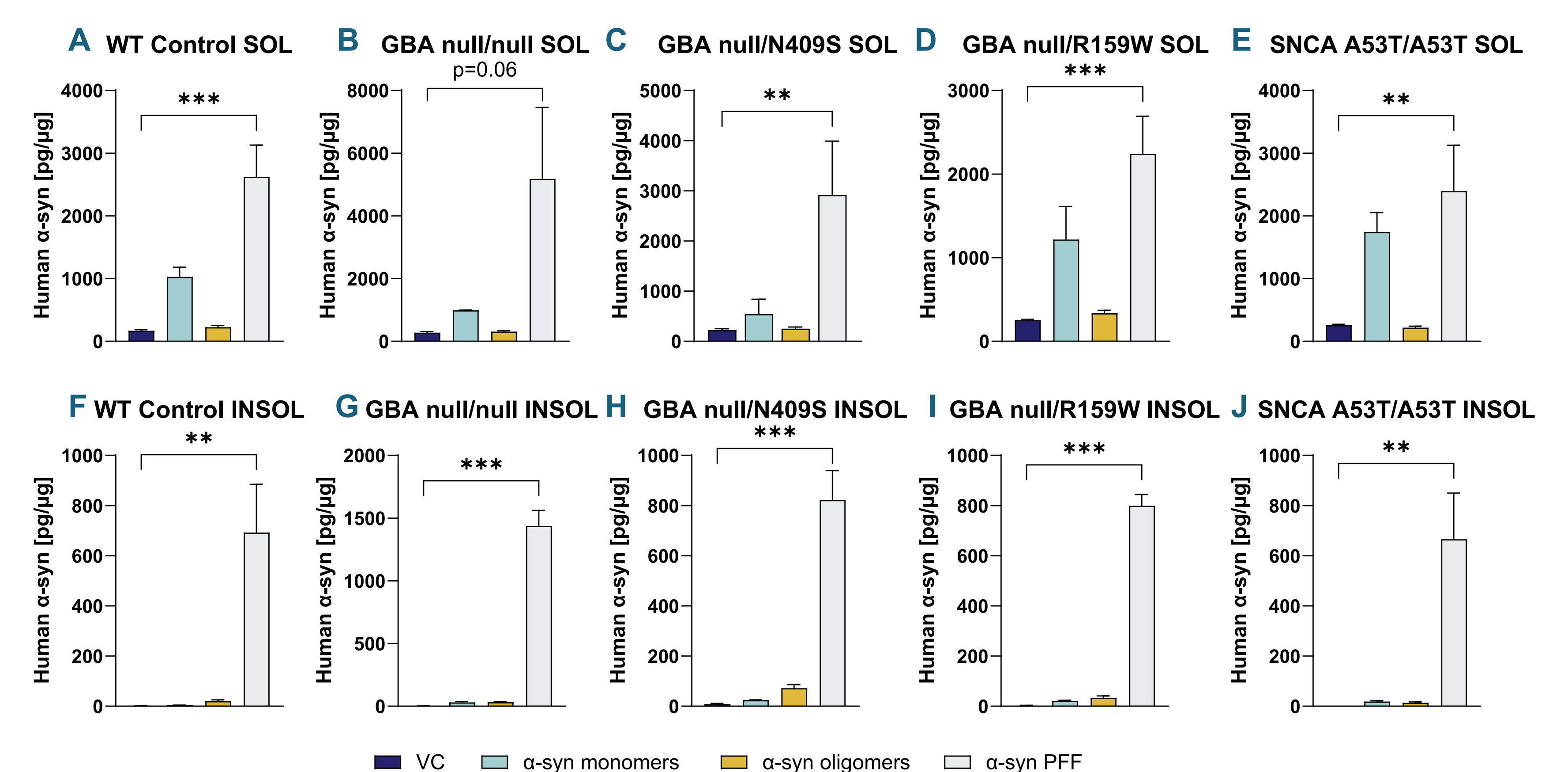

Figure 4: Soluble and insoluble human α-syn levels in the cell extract from ioGlutamatergic Neurons of different genotypes. Wild type (WT) control (**A, F**), GBA null/null (**B, G**), GBA null/N409S (**C, H**), GBA null/R159W (**D, I**) & SNCA A53T/A53T (**E, J**) neurons were treated with 0.4 µM α-syn monomers, oligomers or pre-formed fibrils for 12 days and Triton X-100 soluble (SOL) and insoluble (INSOL) human α-syn levels were assessed by MSD assay. Data are given as pg α-syn per µg total protein. Mean + SEM; n = 5; One-Way ANOVA followed by Dunnett's multiple comparison test. **p <0.01; ***p <0.001.

Modulation of α-syn Aggregation with α-syn Antibody

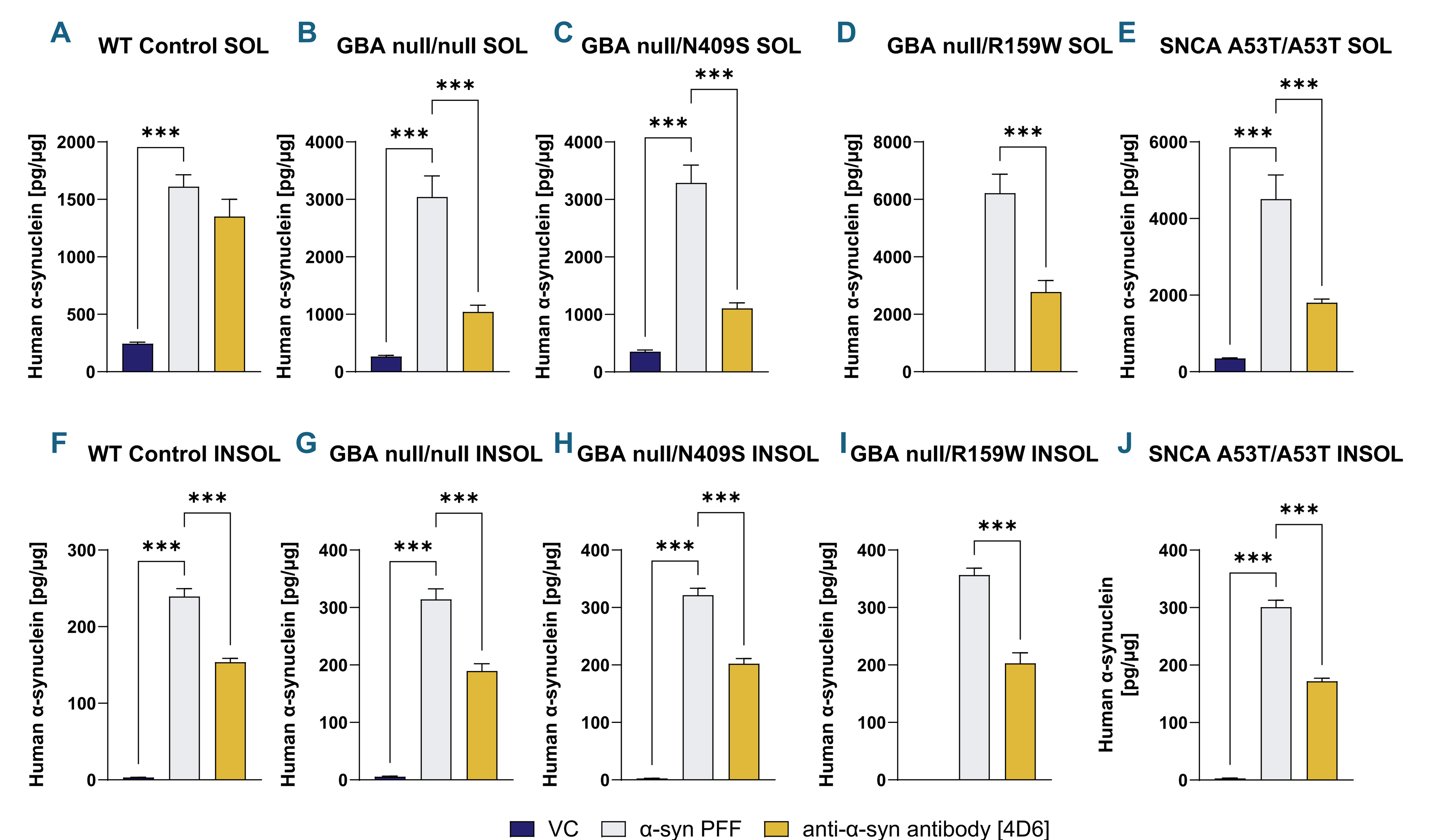

Figure 5: Soluble and insoluble human α-syn levels in the cell extract from treated ioGlutamatergic Neurons of different genotypes. Wild type (WT) control (**A, F**), GBA null/null (**B, G**), GBA null/N409S (**C, H**), GBA null/R159W (**D, I**) & SNCA A53T/A53T (**E, J**) neurons were treated with α-syn pre-formed fibrils (α-syn PFF) at 0.3 µM alone or in combination with anti-α-syn antibody [4D6] at 20 nM for 12 days and Triton X-100 soluble (SOL) and insoluble (INSOL) human α-syn levels were assessed by MSD assay. Data are given as pg α-syn per µg total protein. Mean + SEM; n = 5-6; One-Way ANOVA followed by Dunnett's multiple comparison test. ***p <0.001.

Conclusion

The increased susceptibility of mutant neurons to α-syn aggregation following PFF challenge underscores their disease relevance. Importantly, α-syn antibody treatment mitigated fibril-induced α-syn pathology, supporting therapeutic strategies targeting α-syn in genetically defined subtypes of Parkinson's disease.

Meet Scantox at booth #67

For more information about the models please visit: www.scantox.com

Scantox Neuro GmbH, Parkring 12, 8074 Grambach, Austria