Phenotypic Characterization of hA53Ttg Mice as Parkinson's Disease Model

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BACKGROUND

Aggregation of α-synuclein (α-syn) plays a crucial role in Parkinson's disease (PD) and other synucleinopathies. Point mutations in α-syn have been identified in rare forms of familial PD and are reported to accelerate α-syn oligomerization and aggregation as well as age of symptom onset. Here, we characterized human α-syn transgenic mice with A53T mutation (hA53Ttg) developed by Sudhof and colleagues for brain pathology and motor deficits.

MATERIALS and METHODS

hA53Ttg mice at an age of 2, 4 and 6 months were tested for motor deficits in the beam walk test. Afterwards, animals were euthanized, and brain tissue evaluated for human α-syn, pSer129 α-syn, as well as GFAP as marker for neuroinflammation. Plasma of older animals was further evaluated for neurofilament light chain levels as marker for neurodegeneration using a commercially available assay. Tissues were analyzed by immunofluorescent labeling and biochemical methods. All experiments were performed in animals of both sexes and compared to age-matched non-transgenic littermates.

RESULTS

Already at the age of 2 months, hA53Ttg mice present severe motor deficits in the wire hanging and beam walk test. At 4 months of age also differences in the pasta gnawing test were observed. Highly increased human α-syn levels are present already in young hA53Ttg animals, but no progression could be detected.
Significant progression of disease-related markers was observed for pSer129 α-syn, GFAP and Iba1, most evident in the brainstem of 10 months old hA53Ttg mice, suggesting that severe pathology is a regional event in this mouse model. A similar increase was found for plasma neurofilament light chain (NF-L), highly correlating with pSer129 α-syn in the brainstem.

RESULTS

Severe and Progressing Motor Deficits

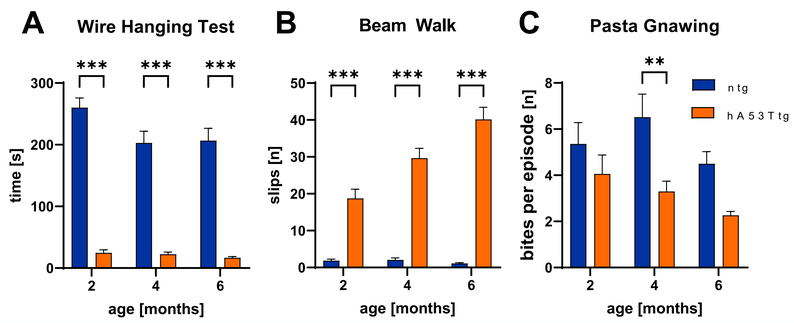

Figure 1. Assessment of motor deficits. (A) Mean latency to fall in the wire hanging test as well as (B) number of slips in the beam walk test are significantly affected already in 2 months old hA53Ttg animals compared to age-matched ntg controls. (C) Bites per episode in the pasta gnawing test are reduced in hA53Ttg mice, reaching significance at 4 months of age. Kruskal-Wallis test followed by Dunn's *post hoc* test; **p<0.01, ***p<0.001. Mean + SEM (n=12-16 per group).

Neurofilament Light Chain in Plasma

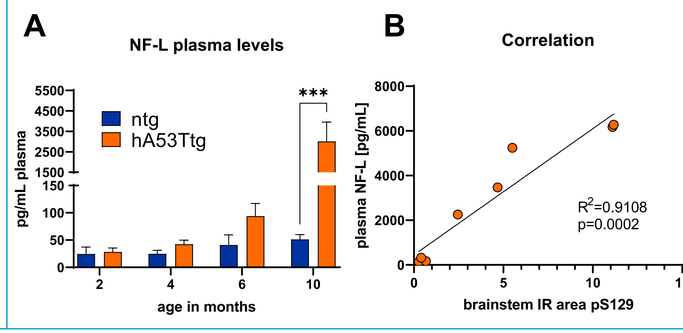

Figure 4. Quantification of neurofilament light chain (NF-L) in the plasma. (A) NF-L levels in pg/mL in the plasma of non-transgenic (ntg) and hA53Ttg animals at 2 to 10 months of age. One-way ANOVA and Sidak's *post hoc* test; ***p<0.001 Mean + SEM. (n=8 per group). (B) High correlation of plasma NF-L levels with pS129 α-syn immunoreactive area in the brainstem of 10 months old hA53Ttg animals.

Histological Assessment of pS129 α-syn, Astroglia and Microglia Reveals Development of Regional Pathology

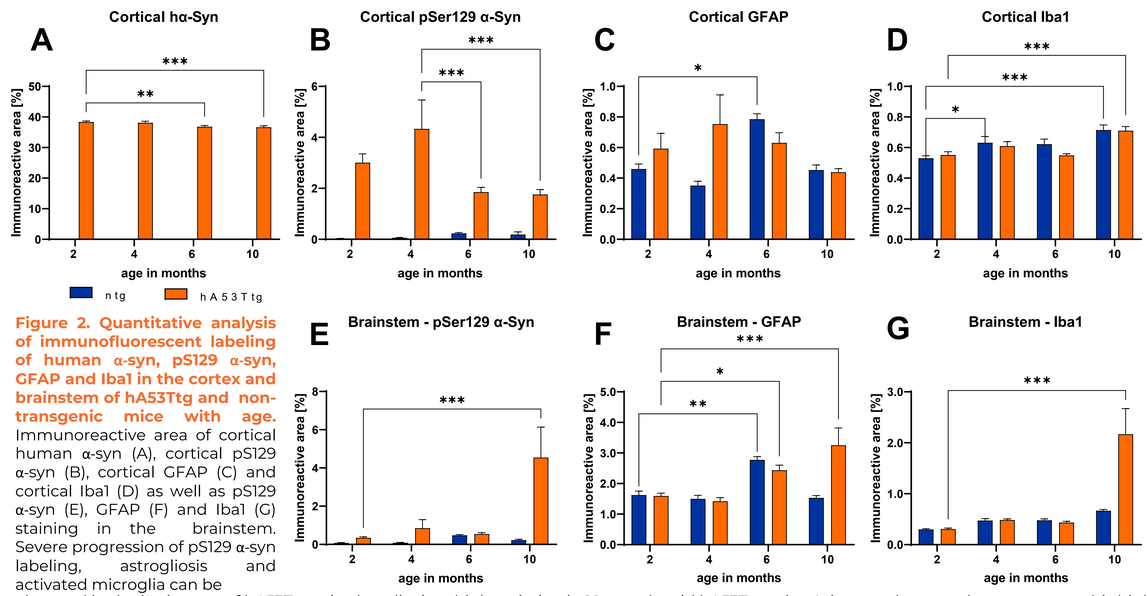

Figure 2. Quantitative analysis of immunofluorescent labeling of human α-syn, pS129 α-syn, GFAP and Iba1 in the cortex and brainstem of hA53Ttg and non-transgenic mice with age. Immunoreactive area of cortical human α-syn (A), cortical pS129 α-syn (B), cortical GFAP (C) and cortical Iba1 (D) as well as pS129 α-syn (E), GFAP (F) and Iba1 (G) staining in the brainstem. Severe progression of pS129 α-syn labeling, astrogliosis and activated microglia can be observed in the brainstem of hA53Ttg mice but displays high variation in 10 months old hA53Ttg mice. It is yet unknown what may cause this high variability and whether there is any heritable element that may allow to separate low and high pathology mice. Two-way ANOVA with Bonferroni's *post hoc* test, progression with age vs. 2 months; *p<0.05, **p<0.01, ***p<0.001. Mean + SEM (n=8 per group).

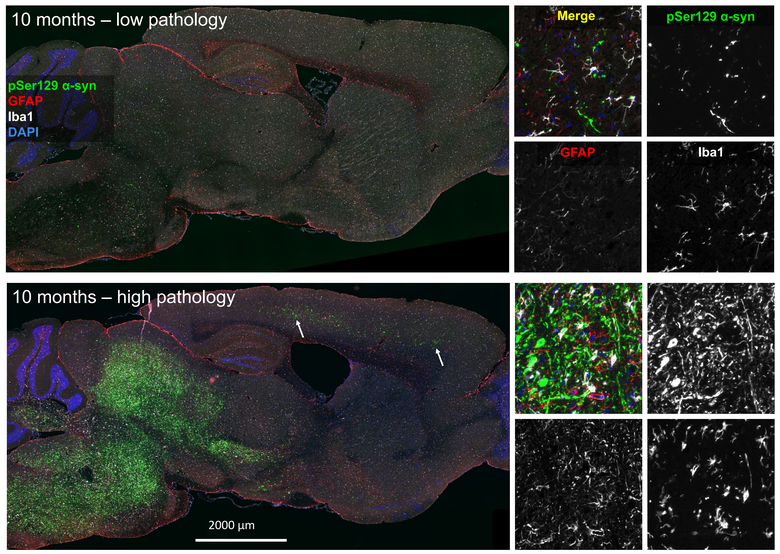

Figure 3. Immunofluorescence in hA53Ttg mice at the age of 10 months. Labeling of pSer129 α-syn, GFAP, and Iba1 shows striking differences between individuals of the same age. "Low pathology" mice display little labeling for all three markers, whereas a large amount of pSer129 α-syn and associated gliosis is evident in the brainstem of "high pathology" mice. Images thus support a high within-group variability of A53Ttg mice at the age of 10 months.