Characterization of Humanized A53T Alpha-synuclein (aSyn A53T KI) and Alpha-synuclein KO (aSyn KO) Rat Models

Teija Parkkari1, Jukka Puoliväli1, Leena Raunala1, Taina-Kaisa Stenius1, Laura Koistinen1, Timo Bragge1, Annina Oksman1, Kaisa M.A. Paldanius1, Yi Chen2, Omar S. Mabrouk2, Kelly E. Glajch2, Warren D. Hirs2, Michael Perkinton2, Nicole K. Polinski2,
1Charles River Discovery, Kuopio, Finland; 2Biogen, Cambridge, MA, USA; 1AstraZeneca, Cambridge, UK; 4The Michael J. Fox Foundation for Parkinson’s Research

1 BACKGROUND
Mutations and multiplications in the SNCA gene encoding the alpha-synuclein protein are linked to an autosomal dominant form of Parkinson’s disease (PD). One such mutation is the alanine-to-threonine substitution at amino acid 53, leading to early onset PD, possibly through an increased propensity for the A53T alpha-synuclein to aggregate. To better understand the biology of this mutation and develop a preclinical model for PD, we have generated a humanized alpha-synuclein rat model with a non-functional rat SNCA gene (aSyn A53T KI). The model was created by applying CRISPR/Cas9 genome targeting strategy where humanized amino acid 53 was inserted for the region spanning amino acids 53-122 in the rat SNCA KI. The model was created by applying CRISPR/Cas9 genome targeting strategy where humanized amino acid 53 was inserted for the region spanning amino acids 53-122 in the rat SNCA KI. In addition, a rat SNCA KO model was developed and preserved with RNAlater, and stored at -80°C. Phenotype of the aSyn A53T KI rat having a partial human cDNA insertion. The wildtype animals have a robust native rat SNCA expression, whereas the KI animals give a positive signal for the humanized transcript. Representative data are shown for substantia nigra (aSyn KO model) or the native rat SNCA gene (aSyn A53T KI model) with custom-prepared QuantGene Plex sets. The SNCA mRNA expression levels were normalized to the geometric mean of 3 different housekeeping genes. Representative data are shown for substantia nigra (aSyn KO) and cerebral cortex (aSyn A53T KI), respectively.

2 METHODS

2.1 Fine Motor Kinematic Analysis
Rats were analyzed in the MotoRater test (TSE) using walking mode. The movement data was captured using a high-speed camera. Different gait parameters and movements were analyzed using a custom-made automated analysis system. The analyzed parameters included: 1) general gait pattern parameters (circle time and speed, step width, stance and swing time during a stride, interlimb coordination), 2) body posture and balance (toe clearance, base width, height, hind limb projection and retraction, tail position and movement), and 3) fine motor skills (swing speed during a stride, jerk metric during swing phase, angle ranges and deviations of different joints, vertical and horizontal head movement).

2.2 Open Field
Exploratory activity was studied in an open field test. Activity chambers (Med Associates Inc, VT, USA; 84 x 90 x 34 cm) were equipped with infrared (IR) beams. Rats were placed in the center of the chamber and their behavior was recorded for 30 minutes.

2.3 Beam Walk
Sensorimotor functions of forelimbs and hindlimbs were tested using tapered/ledged beam. The beam-walking apparatus consisted of a horizontal 160 cm tapered (square) beam with underhanging ledges on each side to permit foot toots without falling. The rats' performance was videotaped and analyzed by calculating the slip ratio (the number of slips/number of total steps).

2.4 Home Cage Motor Activity
Rats were first subjected RF chip implants. Implanted chips provide an identifier of an animal in a cage in home cage setting where receiver plate (Analytical Units, UK) is placed underneath the plastic home cage. Transmitting RF chips and receiver plates allow analysis of home cage behavior of rats. Recordings for rats was performed over 72 h period.

3 RESULTS

3.1 Body Weight and Behavior
During the course of this project, aSyn A53T KI and their wildtype littermates were analyzed. The body weight data was presented as boxplot (A) or mean +/- SEM (B) and is shown in Figure 1. The data is presented in Table 1. The body weight data was analyzed using ANOVA followed by Bonferroni post hoc test. There were no differences in the number of TH-positive cells analyzed in the aSyn A53T KI and WT rats either in the 4 or 8-month cohort. The testing and data analysis for the 12 and 18-month cohorts is ongoing. Tissue sample collection of 6 and 12-month old aSyn KO rats has been completed and results from this will be presented. This will be presented in the SNCA gene expression assay are being presented.

3.2 Fine Motor Kinematic Gait Analysis

Fig. 2. Fine motor kinematics of aSyn KO and WT rats. The overall gait score (A) is based on discontinuous direction vector (B) which emphasizes gait changes associated to aSyn KO rats in all timepoints. The aSyn A53T rats express slower overall speed, slight changes in paw trajectories and head movements (seen in the plot, acceleration, projection, trajectory parameters, and in more head positions). Separate parameters for mean speed, hind limb stance time, and head rotation range are shown in (C). Data presented as mean +/- SEM (C) (n = 10-22).

4 CONCLUSIONS

4.1 Overall, the behavioral phenotypes and body weight of the aSyn A53T KI rats seem to be very similar to their WT littermates through the timepoints tested. However, according to the highly sensitive fine motor kinematic analysis, the aSyn A53T KO rats have significantly altered overall gait score compared to the WT rats at 12-month timepoint. In addition, aSyn A53T KO rats spent more time close to cage walls at the 8-month time point which could potentially be an indicator of an increased anxiety. aSyn A53T KO rats gave a clear signal for the intacted human partial SNCA at the m57 hallmark, whereas they essentially lacked the native rat signal. Conversely, the WT rats had a robust expression of the native rat SNCA, although they also produced some residual signal with the human protein, most likely due to partial sequence homology. These data indicate that genotypes is reflected in the phenotypes as expected.

4.2 There were no differences in the number of TH-positive cells analyzed in the aSyn A53T KI and WT rats either in the 4 or 8-month cohorts. No aSyn immunohistochemistry was observed in the 4-month cohort (data not shown), and therefore, GFAP, Tau, proteine K, and protein A are shown as putative markers for substantia nigra (n = 10-20 in each age cohort).

4.3 The aSyn KO rats exhibited a clear phenotype with significant downregulation of the native rat SNCA gene expression at the m57 hallmark, indicating that transcription and processing of the gene products are altered.