B6-hSNCA E46KA53T

Strain Name： C57BL/6JGpt-Tg(hSNCA-A53T/E46K)99/Gpt
Strain Type： Transgene
Strain Number： T054322
Background： C57BL/6JGpt

Description

Parkinson's disease (PD) is a progressive degenerative disease of the central nervous system, which mainly manifests as motor system disorders such as resting tremors, muscle stiffness, and postural changes. Additionally, some patients may experience cognitive impairment, mental retardation, or dementia, etc. This is the second most common neurodegenerative disease in the world [1]. The pathological features of Parkinson's disease are mainly characterized by the progressive loss of dopaminergic neurons (DA) in the substantia nigra pars compacta (SNpc) and the inclusion of Lewy bodies in the cytoplasm. Studies have shown that PD is related to a series of mechanisms, including oxidative stress, mitochondrial dysfunction, ubiquitin-proteasome system dysfunction, and excitotoxicity [2].

α-Synuclein (α-syn) is ubiquitous in the brain and is the main filamentous component of the characteristic pathological changes of PD, Lewy bodies, and the cause of some family forms of PD. At the same time, it has potential effects on synaptic plasticity, vesicle dynamics, and dopamine synthesis. Therefore, α-syn is considered a molecular marker for some neurodegenerative diseases. In PD, these inclusion bodies are distributed in the medulla oblongata, olfactory bulb, locus coeruleus, substantia nigra, and to a lesser extent in various areas of the cortex. SNCA, the gene that encodes α-synuclein, has three main gene mutation sites: A53T (Ala-Thr), A30P (Ala-Pro), and E46K (Glu-Lys). These mutations can destroy the original molecular spatial structure of the protein, causing α-synuclein to fail to be degraded normally, and abnormally aggregate to form amyloid structures, thereby causing neuronal degeneration [3].

The E46K mutation was first identified in a Spanish family with Parkinson's disease, and the clinical phenotypes were characterized by the early onset of Dementia with Lewy bodies (DLB), which progressed rapidly and severely. It has been reported that E46K mutation can induce α-syn to form a more stable and pathogenic fibril structure, which is more prone to fragmentation and enhances the transmissible virulence of the "toxic seed" [4]. The aggregation level of the E46K mutation in cells was higher than that of

A53T and A30P, suggesting that α-syn formed by different mutants may represent different pathologic forms and play different pathologic roles [5].

At present, therapeutic drugs targeting the clearance of α-synuclein have entered clinical trials one after another, and the development of effective drugs for the treatment of PD continues to receive widespread attention. Gempharmatech developed the B6-hSNCA E46KA53T mice model including the E46K and A53T mutation sites of SNCA. Compared with the B6-hSNCA A53T model, the α-syn aggregation appeared earlier and at higher levels in the B6-hSNCA E46KA53T model, and the motor function of mice decreased significantly before 3 months of age. The median survival time of B6-hSNCA E46KA53T was about 1-2 months longer than that of B6-hSNCA A53T, showing a longer window for efficacy evaluation.

Strategy

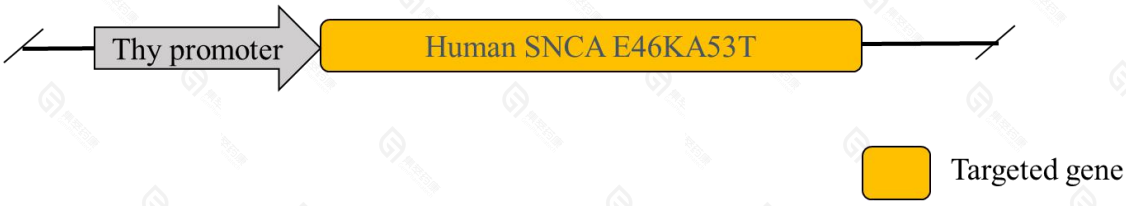

Fig.1 Schematic diagram of B6-hSNCA E46KA53T model strategy.

Applications

1. Study on the pathogenesis and efficacy of Parkinson's disease
2. Study on pathogenesis of dementia with Lewy bodies
3. Studies on synaptic signal transduction and transport

Data support

1. Detection of a-Synuclein protein expression

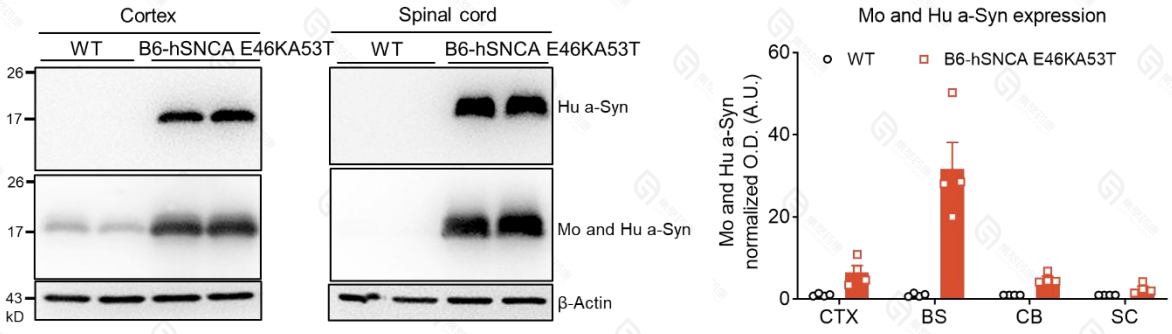

Fig 2. Expression of a-Synuclein protein.

a-Synuclein (mouse and human) and human a-Synuclein protein was detected in brain, spinal cord, cerebellum and brainstem from 2-month-old wild type and B6-hSNCA E46KA53T mice by Western Blot.
All data represent as MEAN ± SEM, Unpaired two-tailed Student's t test.

2. Dopaminergic neuron loss

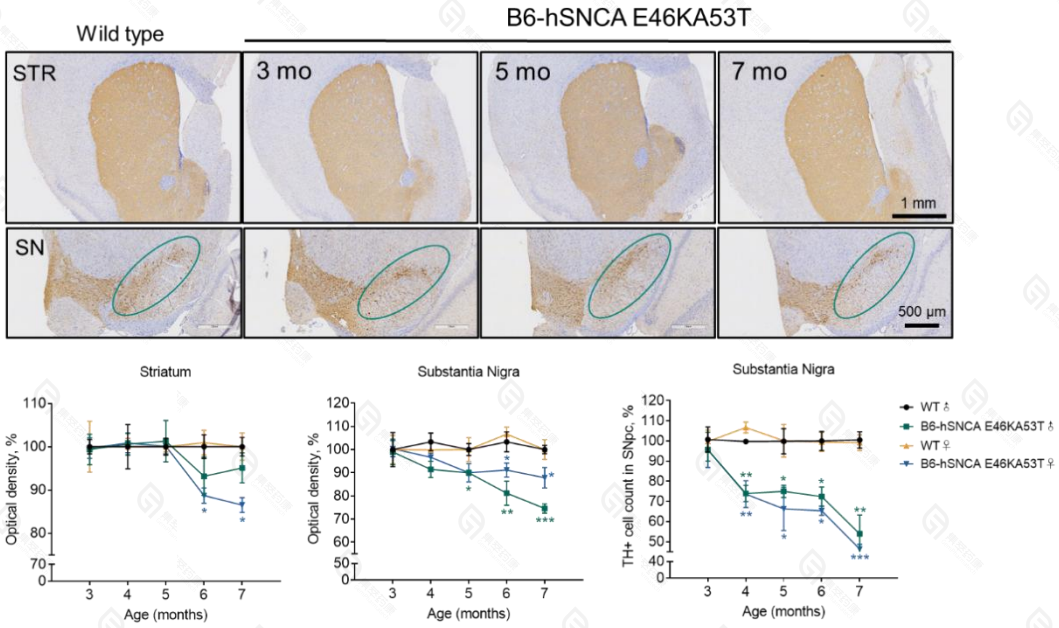

Fig 3. Dopaminergic neuron loss.

Representative images of TH^+ neruons in the striatum and substantia nigra of 3 to 7-month-old wild type and B6-hSNCA E46KA53T mice. Dopaminergic neurons were detected by the immunohistochemistry staining of the sections using Tyrosine Hydroxylase Antibody. Scale, 1 mm/500 μm.
All data represent as MEAN ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001; unpaired two-tailed Student's t test.

3. a-Synuclein aggregation

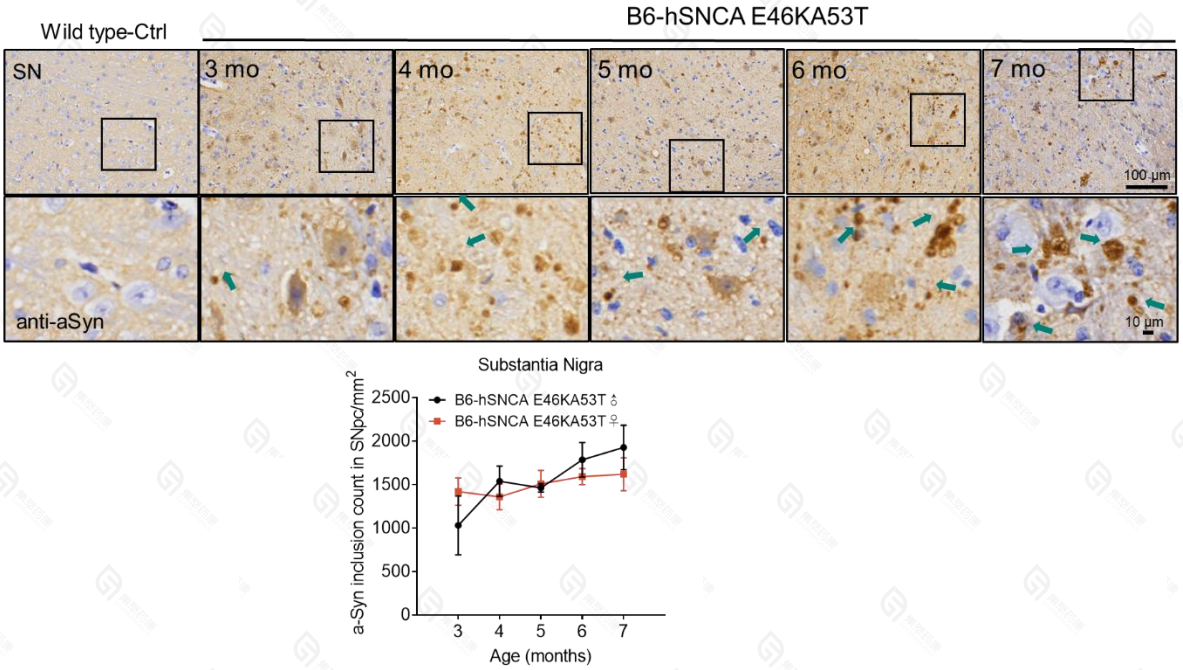

Fig 4. a-Synuclein aggregation.

Representative images of a-Synuclein inclusion in the substantia nigra of 3 to 7-month-old wild type and B6-hSNCA E46KA53T mice. a-Synuclein aggregation were detected by the immunohistochemistry staining of the sections using alpha Synuclein Monoclonal Antibody. Scale, 100 μm/10 μm.
All data represent as MEAN ± SEM.

4. Serine 129 phosphorylated a-synuclein expression

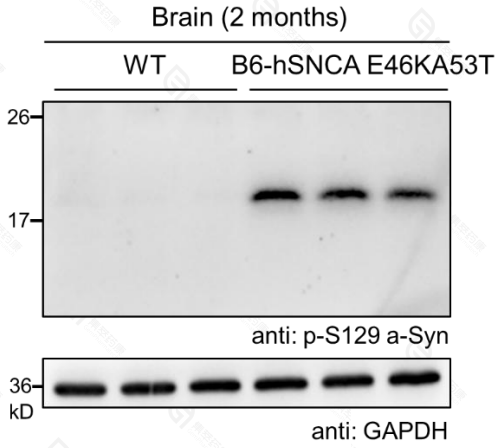

Fig 5. Expression of Serine 129 phosphorylated a-synuclein.

Serine 129 phosphorylated a-synuclein protein was detected in brain from 2-month-old wild type and B6-hSNCA E46KA53T mice by Western Blot.

5. Body weight change and survival data of B6-hSNCA E46KA53T mice

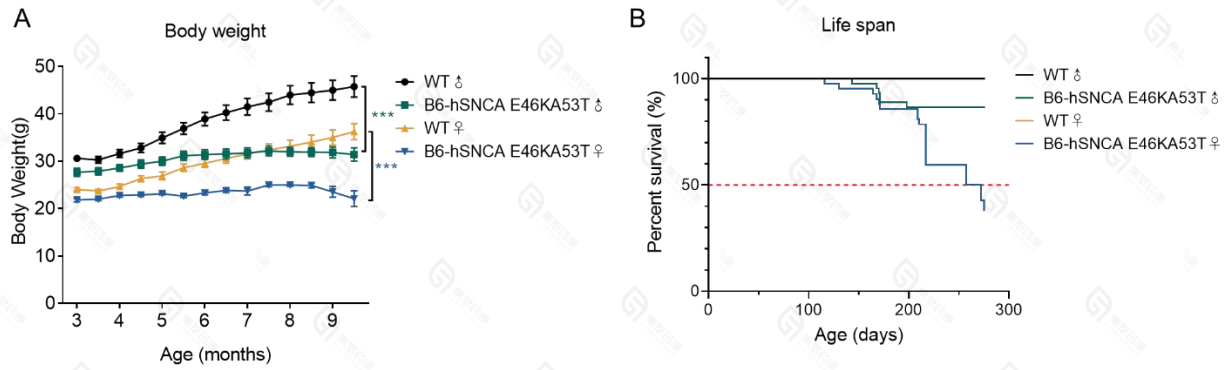

Fig 6. Body weight change and survival data of B6-hSNCA E46KA53T mice.

Body weight change and survival data in B6-hSNCA E46KA53T mice aged 3 to 8 months. (A)N=10 each group, (B) N=30 each group. All data represent as MEAN ± SEM. ***p < 0.001, unpaired two-tailed Student's t test.

6. Deficiency in balance and coordination

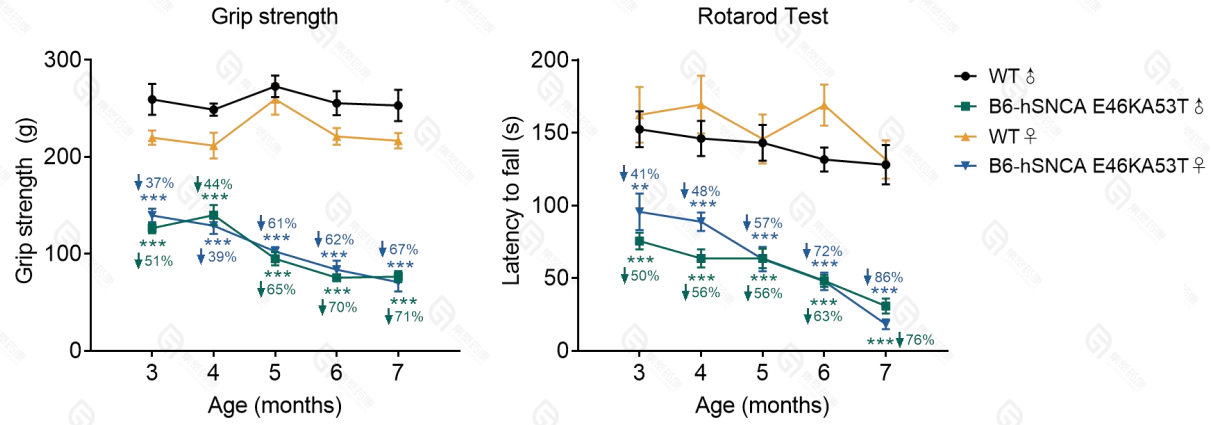

Fig 7. Deficiency in balance and coordination.

(A)Grip strength in B6-hSNCA E46KA53T mice. The limbs grip strength at 3 to 7-month-old of wild type and B6-hSNCA E46KA53T mice in grip strength test. (B) Rotarod test in B6-hSNCA E46KA53T mice. The latency (seconds fall in the rotarod) of 3 to 7-month-old of wild type and B6-hSNCA E46KA53T mice in the rotarod test.

All data represent as MEAN ± SEM. **p < 0.01, ***p < 0.001, unpaired two-tailed Student's t test.

7. Gait abnormality

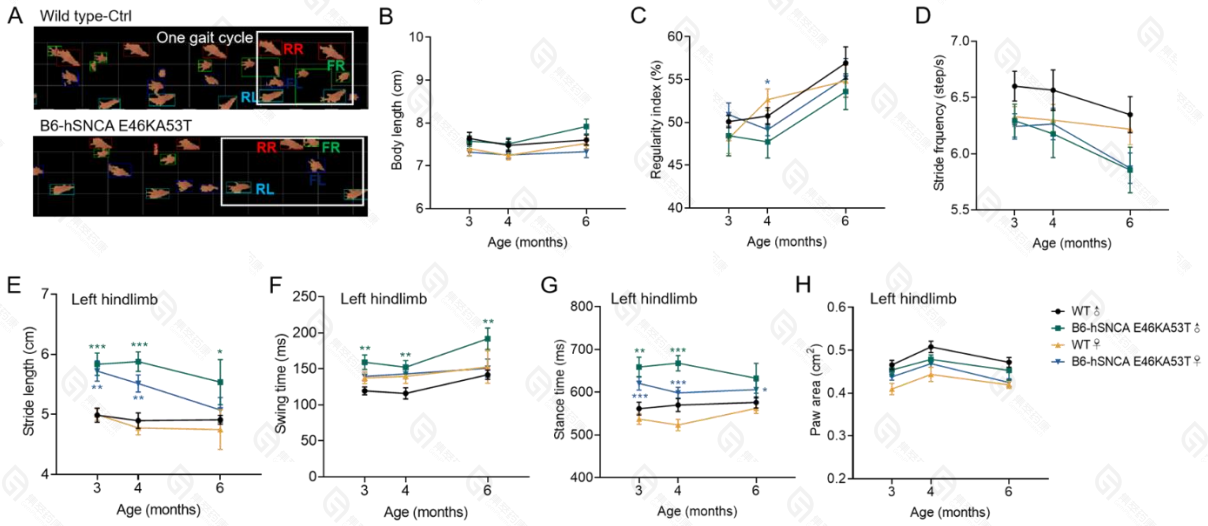

Fig 8. Abnormal gait in B6-hSNCA E46KA53T mice.

(A) Representative images of wild-type (up) and B6-hSNCA E46KA53T strides (down) in the gait test. Left forelimb (FL, blue), right forelimb (FR, green), left hindlimb (RL, cyan) and right hindlimb (RR, red). (B-D) Body length, Regularity index and Stride frequency in B6-hSNCA E46KA53T mice aged 3, 4 and 6 months. (E-H) Stride length, Swing time, Stance time and Paw area of left hindlimb in B6-hSNCA E46KA53T mice aged 3, 4 and 6 months.

All data represent as MEAN ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, unpaired two-tailed Student's t test.

8. The timeline of disease progression in B6-hSNCA E46KA53T mice

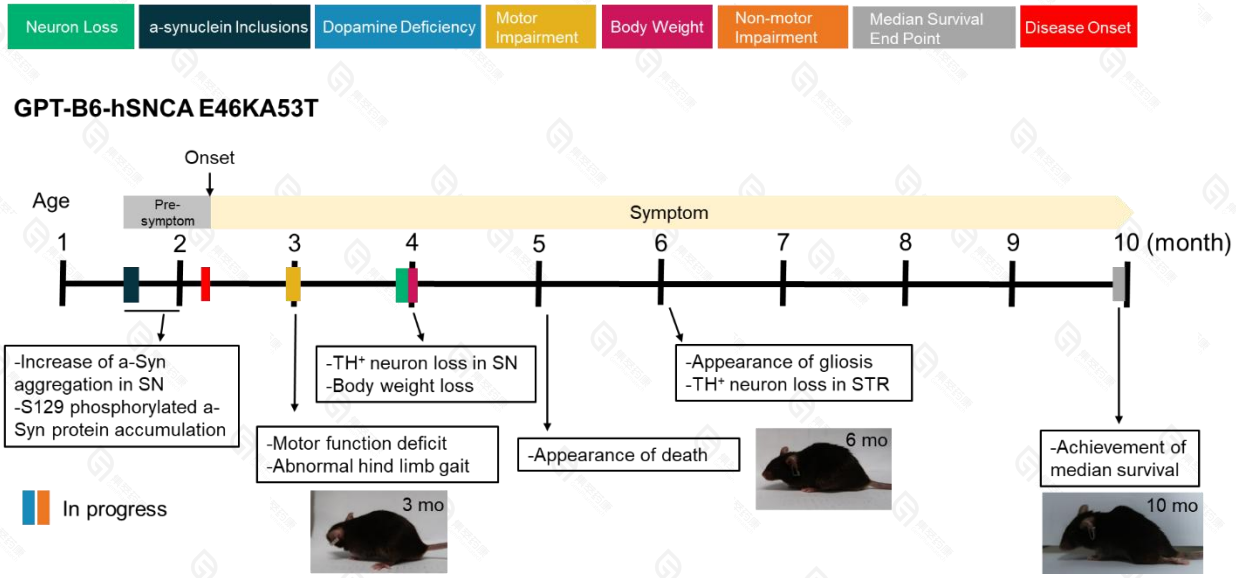

Fig 9. The timeline (month) of disease progression in B6-hSNCA E46KA53T mice.

References

1. Fernagut PO, Chesselet MF. "Alpha-synuclein and transgenic mouse models." Neurobiol Dis. 2004 Nov;17(2):123-30.

2. Yang W, Hamilton JL, Kopil C, Beck JC, Tanner CM, Albin RL, Ray Dorsey E, Dahodwala N, Cintina I, Hogan P, Thompson T. “Current and projected future economic burden of Parkinson's disease in the U.S.” NPJ Parkinsons Dis. 2020 Jul 9;6:15.
3. Conway KA, Rochet JC, Bieganski RM, Lansbury PT Jr. “Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct.” Science. 2001 Nov 9;294(5545):1346-9.
4. Zhao K, Li Y, Liu Z, Long H, Zhao C, Luo F, Sun Y, Tao Y, Su XD, Li D, Li X, Liu C. “Parkinson's disease associated mutation E46K of α-synuclein triggers the formation of a distinct fibril structure.” Nat Commun. 2020 May 26;11(1):2643.
5. Boyer DR, Li B, Sun C, Fan W, Zhou K, Hughes MP, Sawaya MR, Jiang L, Eisenberg DS. “The α-synuclein hereditary mutation E46K unlocks a more stable, pathogenic fibril structure.” Proc Natl Acad Sci U S A. 2020 Feb 18;117(7):3592-3602.