# Innervation of Skin Samples of α-Synuclein Transgenic Mice: Line 61, D-Line, A53T

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## BACKGROUND

Biomarkers for early detection of Parkinson's disease (PD) are in urgent demand. The occurrence of α-synuclein deposits within neurons and neurites of the skin has recently attracted attention. We therefore started to establish techniques for routine multichannel immunofluorescent detection of different α-synuclein types and for investigation of innervation density in three transgenic mouse lines that are established models for Parkinson's disease research.

## MATERIALS and METHODS

Antibody binding on paraffin sections and cryosections of 6 month old transgenic and non-transgenic mice has been investigated.

- Line 61 (TNWT#61): human α-synuclein under murine Thy-1 promoter.
- D-Line (DxJ9M): human α-synuclein under human PDGF-β promoter.
- A53T: human α-synuclein with A53T mutation under human platelet-derived growth factor-β (PDGF-β) promoter.

A total of 16 antibodies against α-synuclein and different histological markers have been tested so far. Routine protocols were established featuring high concentration of Triton X and long incubation times. High level autofluorescence was efficiently reduced by TrueBlack staining.

## RESULTS

Immunoreactivity of α-synuclein was located in fibers around hair follicles and in the epidermis. This pattern was consistently detected using a variety of antibodies targeting different epitopes of α-synuclein. The anti-human α-synuclein antibodies detected no signal in wild type mice, supporting the specificity of the labeling in transgenic mice. Neuronal fibers were successfully identified using different markers (PGP9.5, neurofilament, tyrosine hydroxylase). Labeling was similar in paraffin sections and cryosections. The highest immunoreactivity was detected in Line 61, then D-Line, whereas signal in A53T mice was very low, similar to the finding in the brain.

## RESULTS

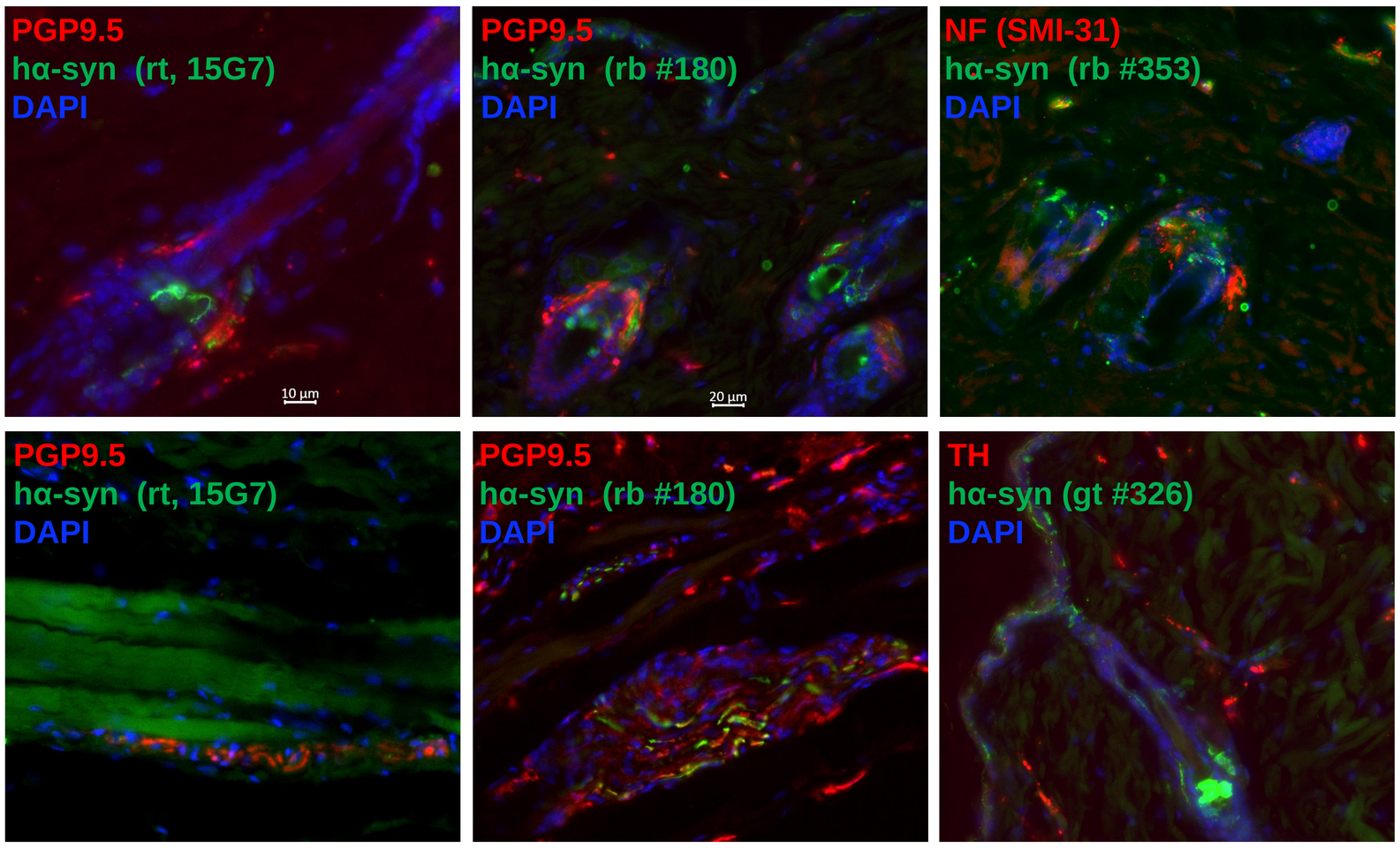


**Figure 2: Consistent labeling pattern in skin samples using different α-synuclein antibodies.** Identical patterns of innervation of hair follicles in D-Line sections were obtained using four different α-synuclein antibodies. Neuronal fibers were labeled by antibodies against PGP9.5, neurofilament (NF), and tyrosine hydroxylase (TH). Note that there is only partial overlay between the neuronal markers and α-synuclein.

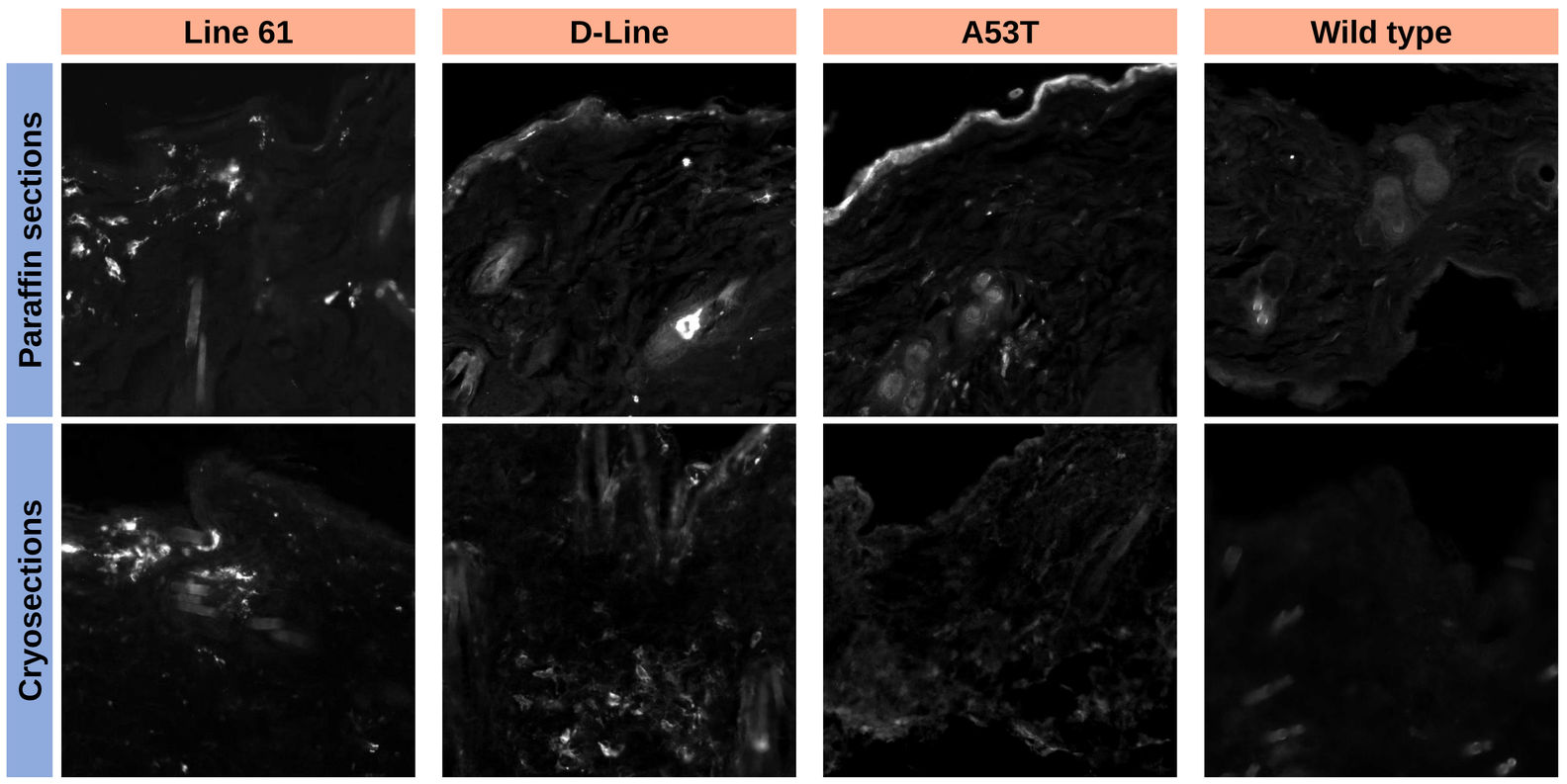


**Figure 2: Level of human α-synuclein immunofluorescence in skin samples of four genotypes.** The highest fiber density is evident in Line 61, followed by D-Line. Positive fibers are rather sparse in A53T samples. Only autofluorescence is visible in wild type mice. Human α-synuclein was detected using rat monoclonal antibody 15G7.

## SUMMARY and CONCLUSION

We detected only rare overlay of α-synuclein and neuronal markers, suggesting there may be pathologic accumulation of transgenic human α-synuclein outside of neuronal fibers. Ongoing experiments aim at elucidating this pattern. We conclude that skin biopsies in mouse models are a promising technique with translational value for establishing peripheral biomarkers to detect PD before the onset of any motor features.

**Contact for more information about the models:**

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