CROSS COMPARISON OF NEUROLOGICAL DEFICITS IN SOD1 G93A AND PROFILIN 1(PFN1 G118V) MOUSE MODELS OF MOTOR NEURON DISEASE

I. Morganstern[1], N. Roberts[1], E. Sabath[1],L. Theide[1], M. Kiaei[2], T. Hanania[1]

[1]PsychoGenics Inc., Tarrytown, NY, USA

[2]The Center for Translational Neuroscience, The University of Arkansas for Medical Sciences, Little Rock, AR, 72223, USA

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Transgenic mice over-expressing human SOD1-G93A are commonly employed as an animal model of familial ALS. However this model presents several challenges in drug discovery as the disease onset is early and progression occurs rapidly with only a limited window of opportunity for therapeutic intervention. Recent evidence from the University of Arkansas for Medcial Sciences (Dr. Mahmoud Kiaei's Lab) has introduced a new mouse model of motor neuron disease that may display ALS-like phenotypes and pathologies. These animals over-express mutant human profilin1 (hPFN1G118V), a small actin-binding protein critical for monomeric (G)-actin conversion to filamentous (F)-actin that has recently been implicated in familial ALS (FALS)[1,2].

The present study was aimed at identifying behavioral tests that are most sensitive to the emergence of behavioral/neurological deficits in the standard SOD1 G93A mice (SOD1) in a cross comparison with the newly proposed hPFN1G118V (PFN1) mouse model. Specifically, the test battery consisted of commonly used metrics such as survival, rotarod, respiration and more complex proprietary algorithm-based behavioral platforms such as NeuroCube ® and SmartCube ® Systems.

METHODS

ANIMALS

➢Male and female mice transgenic for human *SOD1 G93A [B6SJL-TgN(SOD1-G93A)1 GUR]* (**SOD1,** Jackson Laboratoris, Bar Harbor ME) or profilin1 (hPFN1G118V) (**PFN1,** University of Arkansas Medical Center) were used in this study starting at an age of 7 weeks and 12 weeks, respectively.

BEHAVIOR ANALYSIS

➢**Rotarod:** Motor coordination was assessed by Rotarod. Mice were placed on an accelerating rotarod and the latency to fall was recorded. The mice were given three accelerating trials of 5minutes (0-40 rpm).

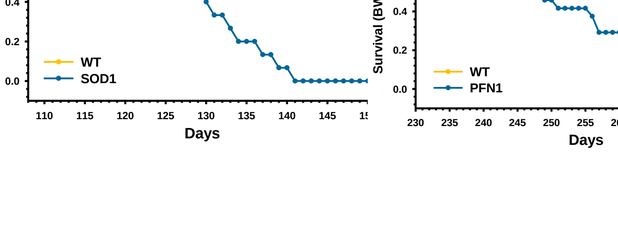
➢**Respiration:** Specialized whole body plethysmographs for the measurement of ventilation in conscious animals was used to measure respiratory function (Data Sciences International).

➢**SmartCube® System:** This proprietary platform uses computer vision to automatically capture and score changes in activity, spatial patterns, spontaneous behavior, reactive behavior, gait, and other measures in mice.

➢**NeuroCube™ System:** This proprietary platform uses computer vision to automatically capture and score changes in gait (geometry and dynamics), paw pressure, paw imaging, body positioning, and other measures in mice or rats.

➢**Feature Analysis:** Data are typically presented as: Control and Disease (SOD-1, PFN-1). We first transform original feature set to the non-redundant de-correlated ranked features space and plot Control and Disease in the coordinate system formed by the two highest-ranked (best-discriminating between the two group's new features). Quality Measure of Disease Model = Overlap between the Control and Disease groups (Discrimination Probability = 100% - Overlap)

➢**Statistical Analyses:** Survival data, where loss of righting reflex was used as a surrogate measure for SOD1 mice and a 30% drop in BW from peak was used for PFN1 mice, were analyzed with a Kaplan-Meier analysis. Data obtained in, rotarod and open field tests were analyzed using multi-factorial analyses of variance (ANOVA). For each group 13-15 mice were used.



Discrimination Probability = 100% - Overlap

Figure 1: Decline in Survival and Body Weight in SOD1 and PFN1 Mice

RESULTS

Figure 2: Decline in Motor Coordination with Disease Progression in SOD1 and PFN1 Mice

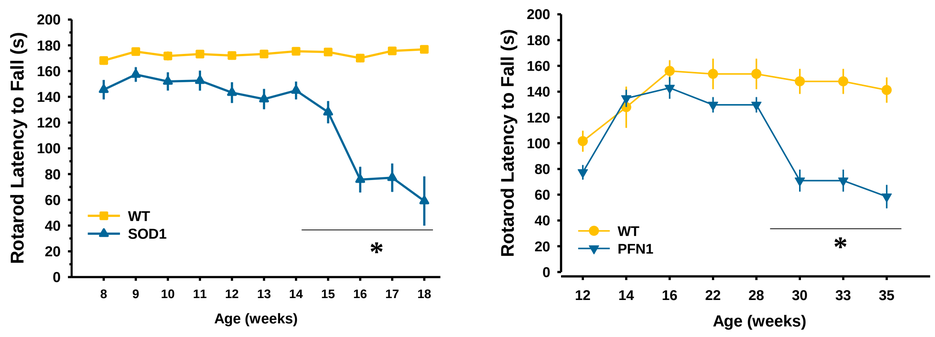


Figure 3: Abnormal Respiration in PFN1 Mice During Late Stage Disease

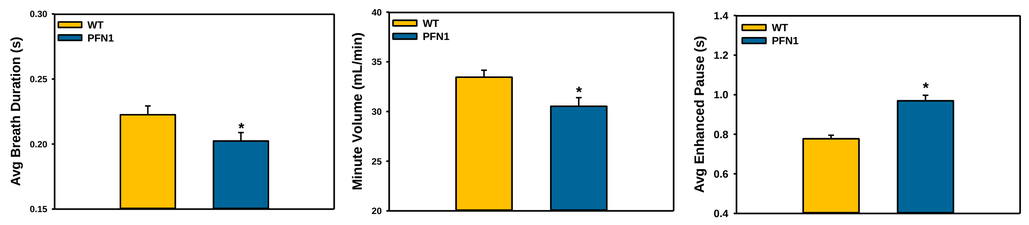
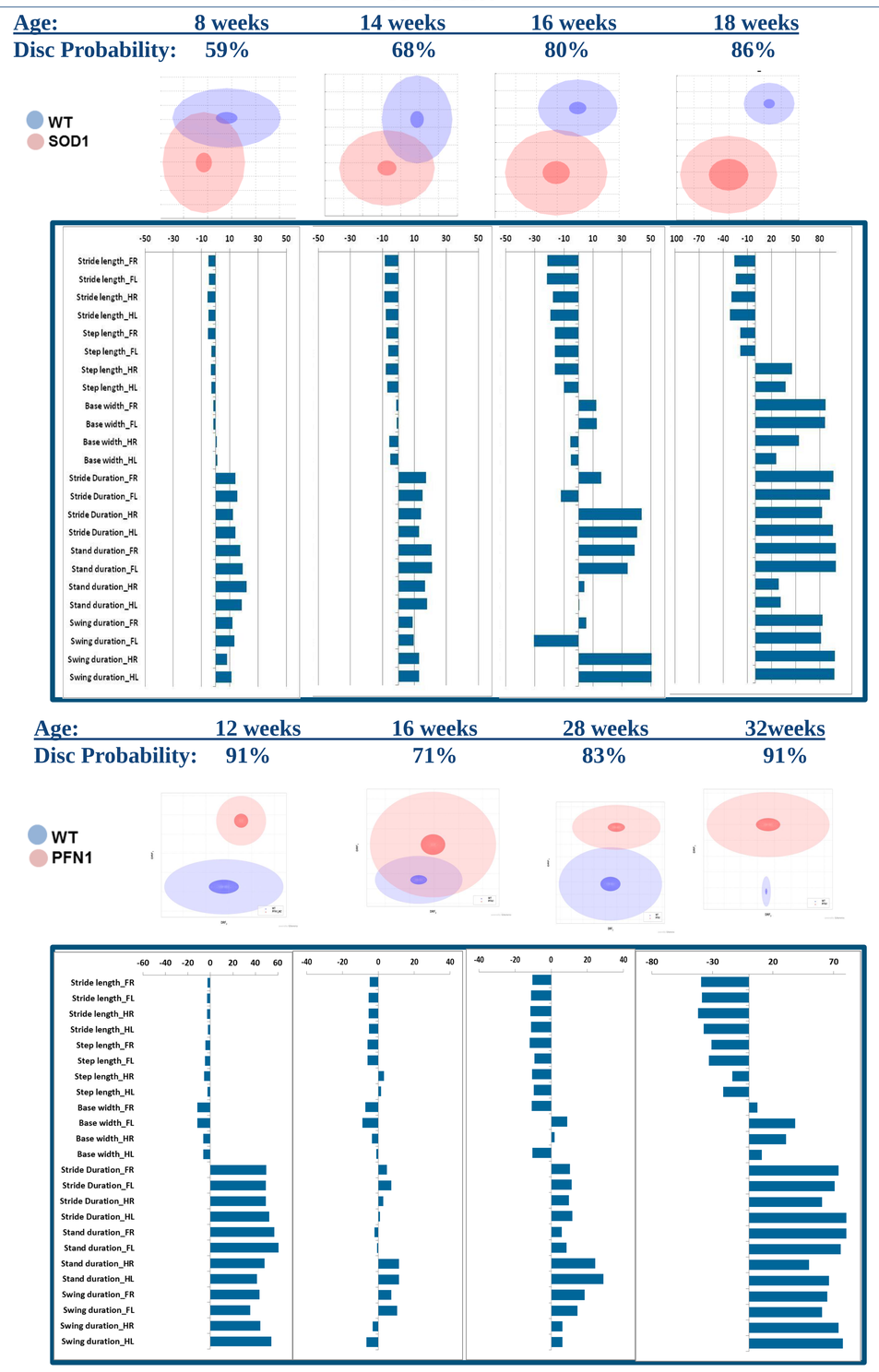


Figure 4: Disease Progression of Gait Feature Using NeuroCube ® Technology in SOD1 and PFN1 Mice



ABREVIATIONS: HL (Hind Limb); FL (Fore Limb), X axis represents discrimination probability as a % of WT mice.

Figure 5: Gait Deficits in SOD1 and PFN1 mice During Late Stage Disease Using NeuroCube ® Technology

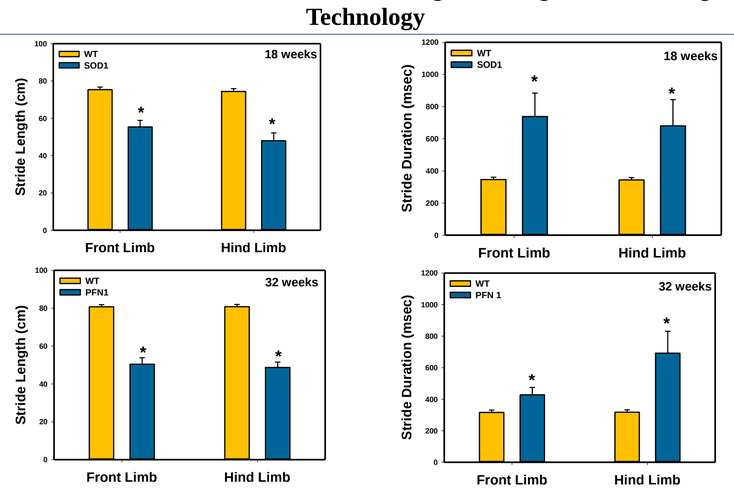
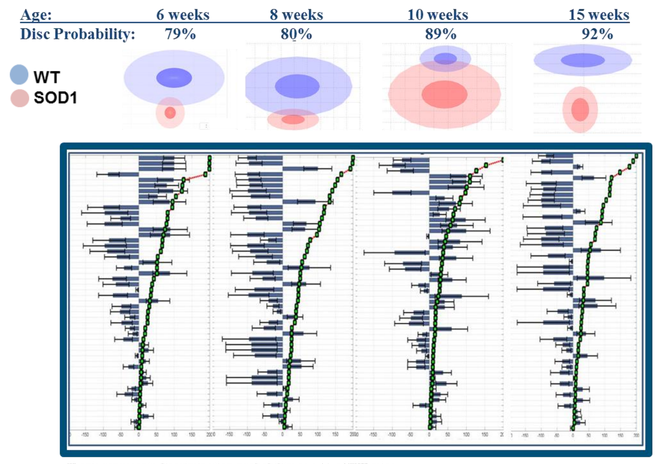
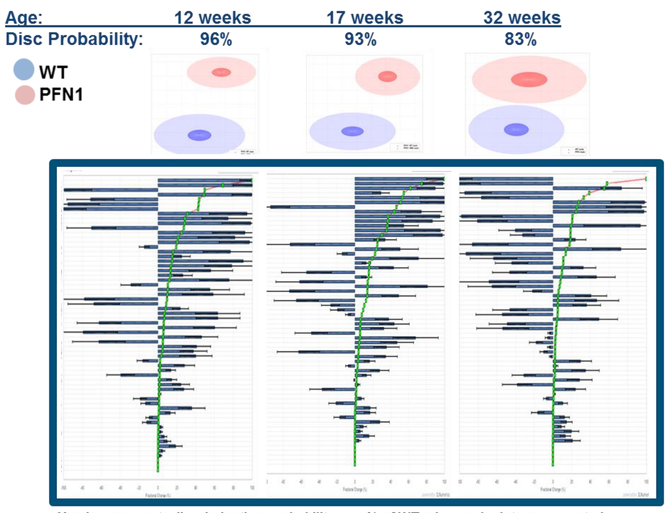


Figure 6: Disease Progression with Age Using SmartCube ® Technology in SOD1 and PFN1 Mice



X axis represents discrimination probability as a % of WT mice



X axis represents discrimination probability as a % of WT mice, male data represented

DISCUSSION

➢ The tests employed here demonstrate similar neurological and motor function deficits in SOD1 and PFN1 mice at early to mid-disease level, with a clear decline in behavior starting at 13 and 28 weeks of age, respectively.

➢More advanced computer vision systems such as NeuroCube® and SmartCube® are able to identify distinctive behavioral patterns and discriminate the disease phenotype as early as 6-8 weeks of age in SOD1 mice and 12 weeks of life in PFN1 mice.

➢This earlier period of disease identification in both mutant lines with our SmartCube ® and NeuroCube ® systems presents a valuable model for improving future assessment of potential therapeutic approaches for ALS.

➢ **One main distinction between the two models is noted with disease progression:** In contrast to the SOD1 mouse model that demonstrates a rapid phenotype of paralysis between 16-18 weeks of age, the longer life-span and much slower disease progression seen in PFN1 mice more closely mimics the clinical scenario and presents a larger window for potential therapeutic intervention.

➢Histological analyses are now in progress comparing the neuro-inflammatory processes in these two models during late disease stages.

ACKNOWLEDGEMENTS

➢We would like to thank the Behavioral Pharmacology Team for their help in performing the behavioral experiments and the IT department for their continuous support and innovation of SmartCube ® and NeuroCube ®Technologies.

REFERENCES

1. *Wu, C. H. et al. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature **488**, 499-503 (2012).*

2 *Alkam, D., Feldman, E. Z., Singh, A. & Kiaei, M. Profilin1 biology and its mutation, actin(g) in disease. Cellular and Molecular Life Sciences, doi:10.1007/s00018-016-2372-1 (2016).*