Longitudinal comparison of disease progression in three knock-in HD mouse models with a similar CAG repeat expansion size: z_Q175KI, z_Q175KIDN and Mc_Q170KI lines.

S. Ramboz[1], K.R. Walker[1], K.Cox[1], M. Bansal[1], M. Kwan[1], J. Beltran[1], A. Ghavami[1], L. Park[2], S. Kwak[2] and D. Howland[2]

[1] PsychoGenics 215 College Rd, Paramus, NJ, 07652

[2] CHDI Management/CHDI Foundation 155 Village Blvd, Princeton NJ 08540

Introduction

Huntington's disease is caused by an expansion of a polyglutamine tract in exon 1 of the huntingtin (HTT gene). Of the many mouse models available for pre-clinical testing, heterozygous knock-in mice most closely resemble the genetic mutation responsible for Huntington's disease. Knock-in mouse lines have expanded polyglutamine (CAG) tracts in exon 1 of HTT (of either mouse or human origin) which are targeted to the mouse HTT allele. We set out to examine the disease progression in three knock-in mouse lines with comparable CAG repeat expansions (approximately 190, see Figure 1) but who differ in sequences surrounding the HTT locus.

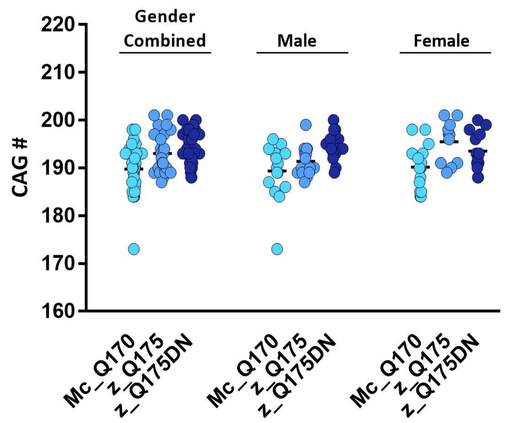


Figure 1: CAG repeat expansion sizing from the three lines tested z_Q175KI (C57BL/6J; CHDI-81003003; z_Q175), z_Q175KI (neo-) (C57BL/6J; CHDI81003019; z_Q175DN) and Mc_Q170KI (C57BL/6J; CHDI-81003022; Mc_Q170). No significant difference was detected in repeat length between the three lines.

Heterozygous and wild-type mice from two newly described lines: the z_Q175DN (z_Q175 line with 5' flanking neo^R cassette excised) and the Mc_Q170 line (generated at MGH) were compared to mice from the well characterized z_Q175 line. Animals were subjected to longitudinal behavioral analysis utilizing PsychoGenics Cubes platform technologies as well as standard behavioral testing paradigms (Open Field and Tapered balance beam).

Methods

Animals:

Heterozygous and Wild-type mice from three lines z_Q175KI (C57BL/6J; CHDI-81003003; z_Q175), z_Q175KI (neo-) (C57BL/6J; CHDI81003019; z_Q175DN) and Mc_Q170KI (C57BL/6J; CHDI-81003022; Mc_Q170) were generated at Jackson Laboratories and shipped to PsychoGenics for behavioral evaluation.
Animals were housed in homogenous genotype and line groups in OptiRat cages (n=10 per cage) for the duration of the study. Behavioral assessments were performed at 2, 4, 6 and 10 months of age.

Behavioral Analysis:

Phenocube®

PhenoCube® is a high-throughput platform that assesses circadian, cognitive, social and motor behavior exhibited by group-housed mice.

Smartcube®

SmartCube® is a platform that employs computer vision to detect changes in body geometry, posture and behavior both spontaneous and in response to particular challenges.

Neurocube®

The Neurocube® system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics. Mice were tested for 5minutes in a rectangular Neurocube® chamber where mice were allowed move freely back and forth through the rectangular walkway. Complex bioinformatics algorithms are employed to subtle phenotypes related to gait.

Open Field

Locomotor activity was measured over a 30 minute interval in a plexiglas square chamber (27.3 x 27.3 x 20.3cm with 16 x 16 x 16 infrared photobeam sources (Med Associates Inc., St Albans, VT). Horizontal activity (distance traveled) and Vertical activity (rearing) were measured by consecutive beam breaks.

Tapered Balance Beam

The tapered balance beam test consisted of a training session (five trials) followed 24hrs later by a testing session (3 trials with an inter-trial interval of 2-3minutes). The tapered balance beam consisted of a tapered angled beam elevated from the floor with a goal box located at the steepest end. Video recordings of each mouses' three test session traversals were later manually scored for foot-slips.

Data Analysis:

Cubes analysis: data was only included from animals who completed all three cubes testing paradigms at 2, 4 and 6mths of age (10mth animals were not analyzed due to testing attrition).
Open Field and Tapered balance beam: data was only included from animals who completed testing at 2, 4, 6 and 10mths of age.

Results

Early detection of phenotypic differences but not necessarily disease progression in all three knock-in lines using a three cubes analysis approach

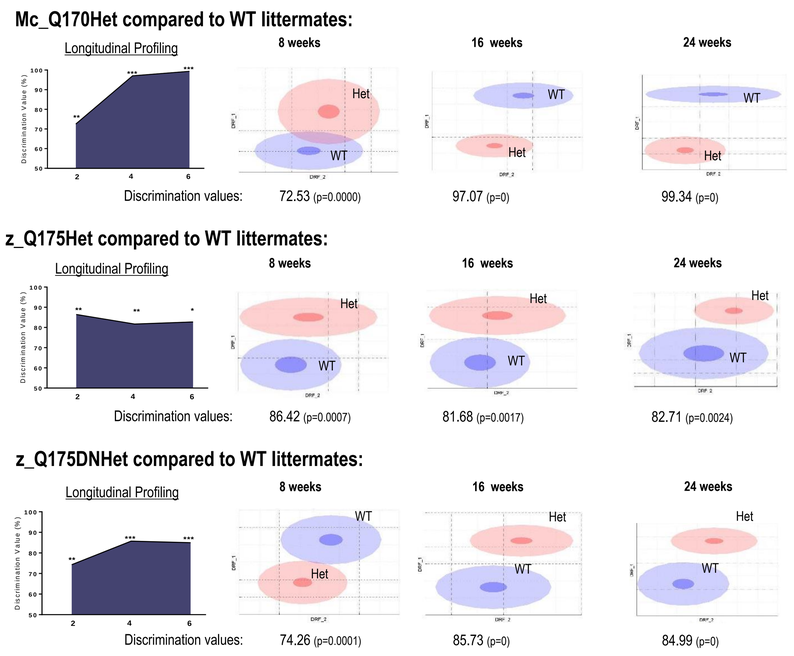
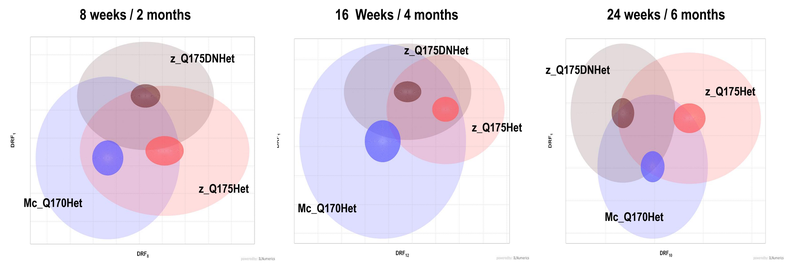


Figure 2: Cloud analysis and discrimination values in combined data assessment of SmartCube®, PhenoCube® and NeuroCube® by the three lines at 2, 4 and 6 months of age: Mc_Q170Het vs WT littermates; z_Q175Het vs WT littermates; z_Q175DNHet vs WT littermates.

❑ All three knock-in lines are significantly different from respective WT littermates as early as 2 months of age when assessed in the three cubes technology. This phenotypic difference remains in all three knock-in lines during aging.

❑ When compared to their WT littermates, the z_Q175Het mice have a stable phenotype through ages while the z_Q175DNHet and Mc_Q170Het mice appear to demonstrate a phenotypic progression with age.

Phenotypic similarity between heterozygous mice from all three knock-in lines using cubes analysis



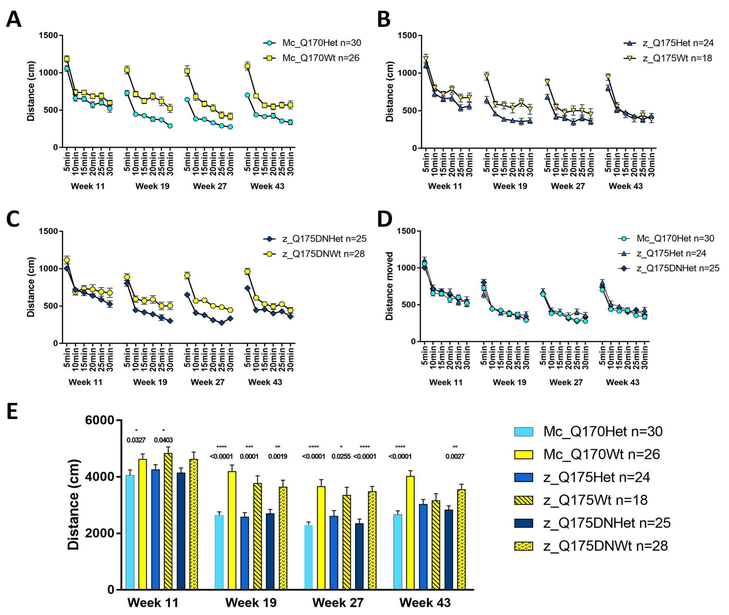
Discrimination Values	8 wks – 2 mths	16 wks – 4 mths	24 wks – 6 mths
z_Q175Het vs Mc_Q170Het	55.93 (p=0.15)	56.04 (p=0.29)	57.06 (p=0.20)
z_Q175DNHet vs Mc_Q170Het	61.95 (p=0.02)	58.72 (p=0.18)	57.24 (p=0.12)
z_Q175Het vs z_Q175DNHet	60.94 (p=0.04)	53.04 (p=0.36)	62.55 (p=0.009)

Figure 3: Cloud analysis and discrimination values in combined data assessment of SmartCube®, PhenoCube® and NeuroCube® by the three lines at 2, 4 and 6 months of age: z_Q175Het vs Mc_Q170Het; z_Q175DNHet vs Mc_Q170Het; z_Q175Het vs z_Q175DNHet.

❑ Combined analysis of SmartCube®, PhenoCube® and NeuroCube® data from the three knock-in Het lines reveals that the disease phenotype of each line is comparable to the other at all ages analyzed. This is reflected by low discrimination values in all pairs-wise comparisons.

Acknowledgments: The authors thank Marcy Macdonald and Vanessa Wheeler at MGH for providing the Mc_Q170 KI and WT littermate mice.

Age related decreases in activity detected in heterozygous mice from all three knock-in lines



Mc_Q170Het	Mc_Q170Wt	z_Q175Het	z_Q175Wt	z_Q175DNHet	z_Q175DNWt
Wk11vsWk19 p<0.0001		Wk11vsWk19 p<0.0001	Wk11vsWk19 p=0.0169	Wk11vsWk19 p<0.0001	Wk11vsWk19 p=0.0066
Wk11vsWk27 p<0.0001	Wk11vsWk27 p=0.0070	Wk11vsWk27 p<0.0001	Wk11vsWk27 p=0.0004	Wk11vsWk27 p<0.0001	Wk11vsWk27 p=0.0012
Wk11vsWk43 p<0.0001		Wk11vsWk43 p<0.0001	Wk11vsWk43 p<0.0001	Wk11vsWk43 p<0.0001	Wk11vsWk43 p=0.0026

Figure 4: Total distance travelled (cm) in 30 minutes of open field testing by the three lines at 2, 4, 6 and 10mths of age A) Mc_Q170Het vs Mc_Q170Wt B) z_Q175Het vs z_Q175Wt C) z_Q175DNHet vs z_Q175DNWt D) Mc_Q170Het vs z_Q175Het vs z_Q175DNHet. Data is presented as mean ± SEM of distance travelled in 5 minute bins. E) Sum of total distance travelled for the three lines at 2, 4, 6 and 10mths of age. Data is presented as mean ± SEM. No differences were detected in velocity (cm/s) or rearing between the three lines.

❑ Age related declines in activity (as measured by distance travelled) are detected in heterozygous mice from all three lines. There is no detectable difference in activity between heterozygous mice from all three lines at any age tested.

❑ Apparent differences in disease progression between the lines (as measured by comparison to their wild-type) is due to age related declines in performance of wild type mice from z_Q175 and z_Q175DN lines.

Robust coordination deficits are detected in Mc_Q170 and z_Q175 lines but not the z_Q175DN line at older ages

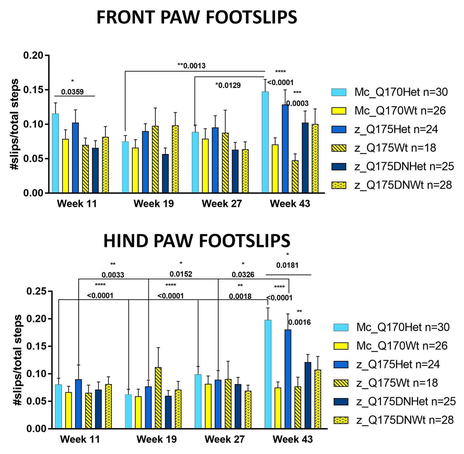


Figure 5: Number of front and hind paw footslips (normalized to total number of steps) at 2, 4, 6 and 10 months of age. Data is presented as mean ± SEM. No statistically significant differences were detected in turning or traverse time between the three lines or their wild-type counterparts at any age tested.

❑ Disease progression (as measured by coordination deficits) is delayed in the z_Q175DN line compared to the Mc_Q170 and z_Q175 lines

Conclusions

❑ Phenotypic differences between Heterozygotes and their Wild-Type littermates can be detected as early as 2mths of age in all knock-in lines .

❑ Apparent differences in disease progression between the three lines (when comparing heterozygotes to their wild-type littermates) is due to reduced performance of wild-type mice particularly those from the z_Q175 and z_Q175DN lines.

❑ Minimal differences are detected between heterozygous mice from all three knock-in lines over aging.