Novel HD mouse models enabling new pathogenic mechanisms discovery

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EHDN 2021

Abstract

Background: Experiments with HD mouse models allow for insight into detailed molecular pathways disrupted in the course of disease development. New aspects of HD pathology need to be determined to bring additional targets and hints for therapeutic approaches.

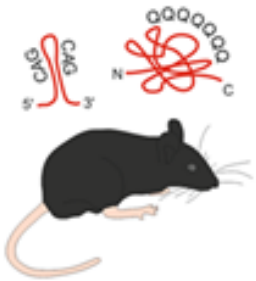
Aims: New HD mouse models have been created due to limitations of existing ones. Our models allow for separation of pathogenic pathways caused by mutant RNA alone from overall pathogenesis and enable to carry out a number of precise molecular analyses.

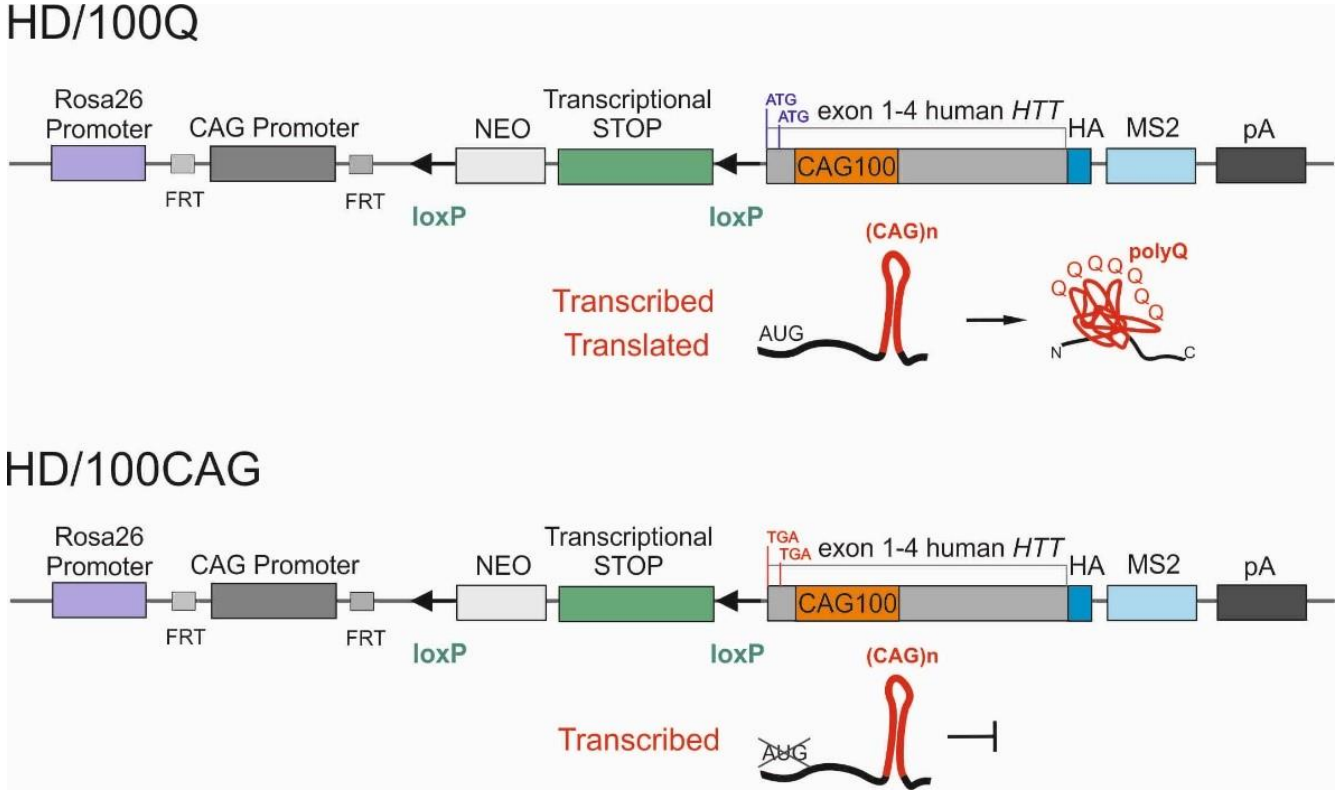
Methods: We have generated two HD mouse models that contain four first exons of *HTT* gene and mutation of ~100 CAG repeats: in non-translated version (HD100CAG) and in translated version (HD100Q). The advantage of these models is the Cre/lox system to induce the mutant *HTT* expression either in whole organism or in tissue/cell type-specific manner. Additionally, visualization of mutant RNA and protein is enabled with the use of specific tags. We used a broad spectrum of molecular, behavioral and cognitive methods at 4, 8 and 12-month time points. Some of further analysis are still ongoing including lifespan and end-stage of the disease progression.

Results: Behavioral testing suggests a mild phenotype of created models. In different time points rotarod and static rod revealed some motor deficits during light phase while ActiMot indicated hyperkinesis during dark phase. Furthermore, expression of untranslated mutant transcript induced cardiac hypertrophy and decreased spleen weight.

Conclusions: Generated models are slightly symptomatic and mostly useful for precise *in vivo* molecular analyses. These models are also suited for investigation of cell- and tissue-specific HD initial pathogenic pathways. This will allow not only to understand overlapping relations between dysfunctions in astrocytes, microglia or neurons within central nervous system but can further advance studies determining peripheral aspects of HD.

Design of novel mouse models for HD





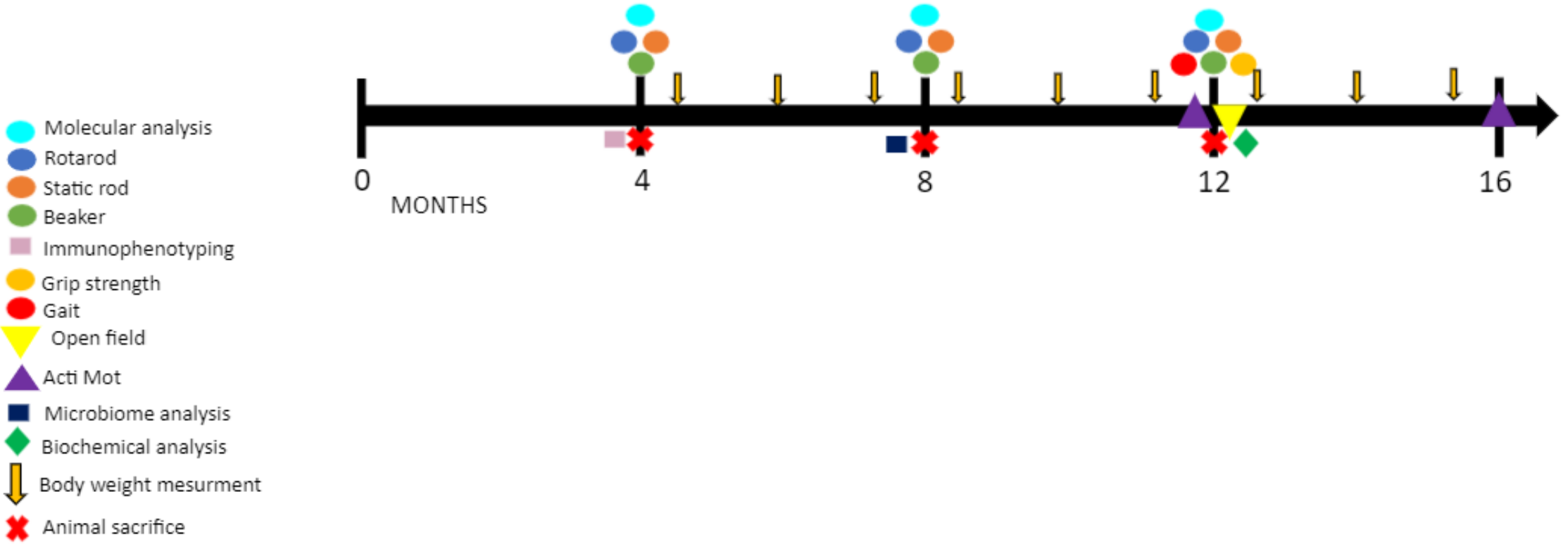
These knock-in models have sequence of first four exons of human *HTT*, with additional HA tag and MS2 label, introduced into murine Rosa26 locus and are characterized by ~100 CAG repeats in the first exon of huntingtin gene.

The translated HD100Q model expresses both mRNA of the *HTT* fragment and protein product with the elongated polyglutamine stretch.

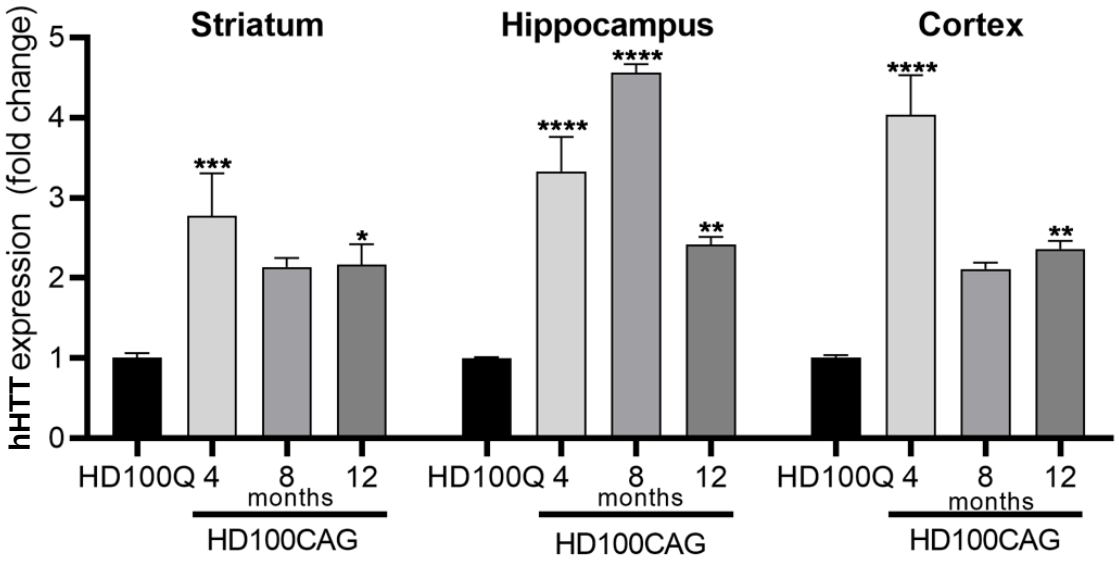
Non-translated HD100CAG model shows only the expression of mRNA of the *HTT* fragment due to the point mutations in the two ATG codons.

Transgene has additional STOP cassette flanked by loxP sites to allow for overall or cell-specific expression of the transgene after crossing of parental lines STOP-100CAG and STOP-100Q with specific Cre mouse model. Moreover, the CAG promoter is flanked by FRT-sites, thus after Flp-mediated recombination it is possible to use endogenous Rosa26 promoter (with 10-100 lower expression level than from CAG promoter).

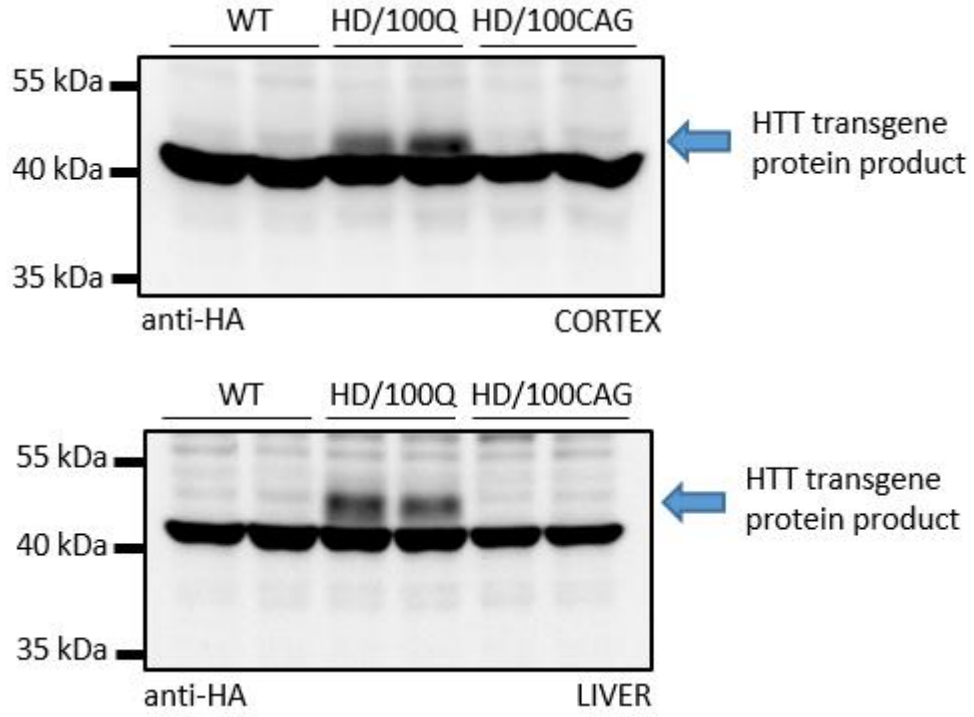
Workplan - time points of tests performed



Molecular analysis of novel HD mouse models expressing mutant translated or non-translated fragment of huntingtin

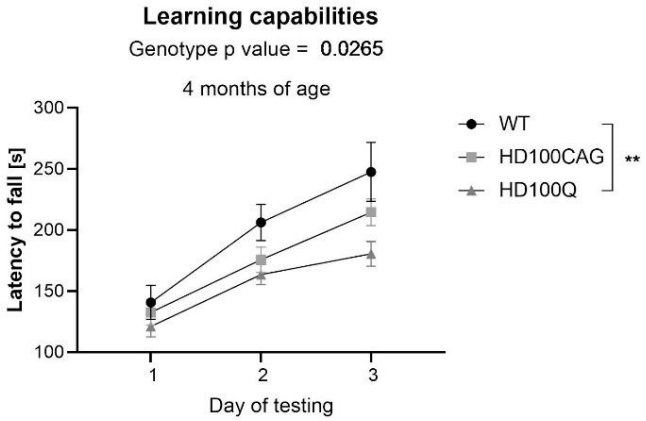


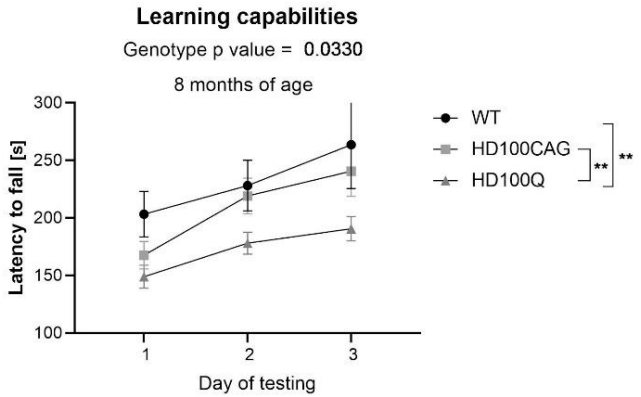
***HTT* transgene expression analysis in striatum, hippocampus and cortex by RT-qPCR at 4, 8, 12 months of age.**
hHTT expression is higher in HD100CAG model compared to HD100Q at all time points for tested brain tissues.

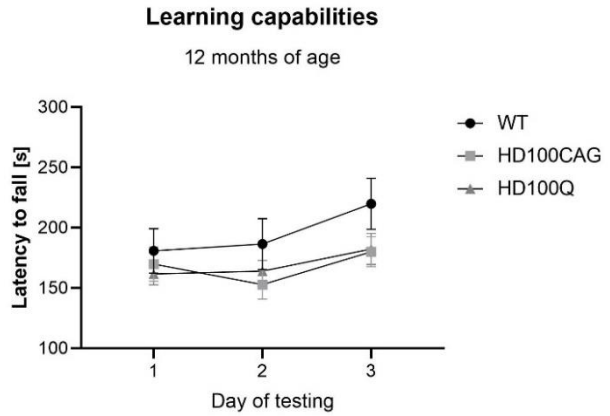


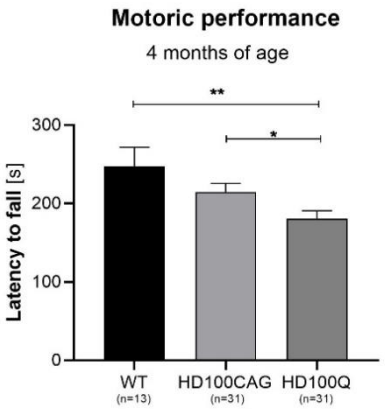
***HTT* transgene expression at protein level in cortex and liver**.
The specific western blot product is indicated by an arrow and present only in the HD/100Q model.

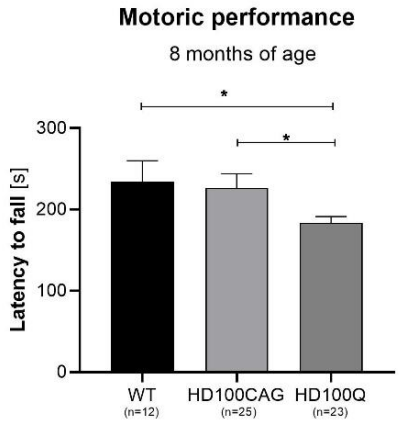
Behavioral characteristics of novel HD mouse models

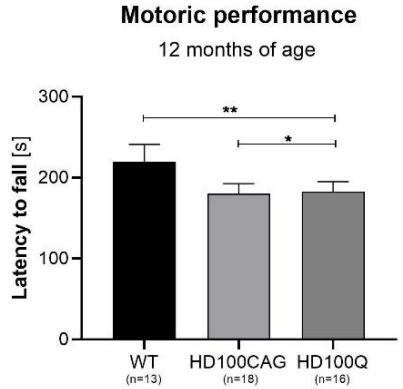






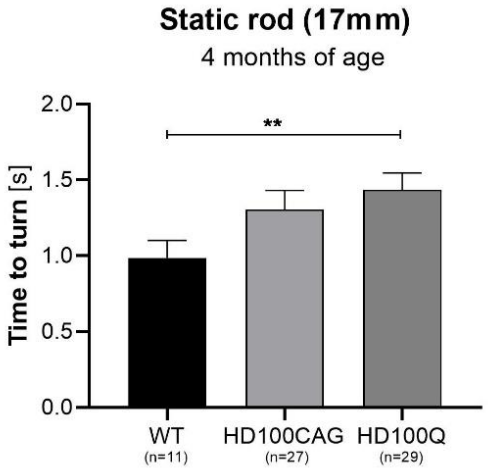


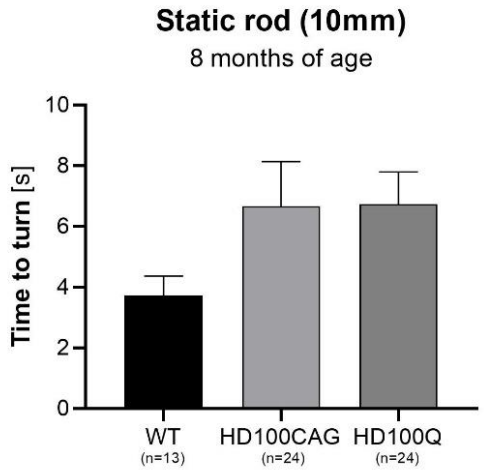


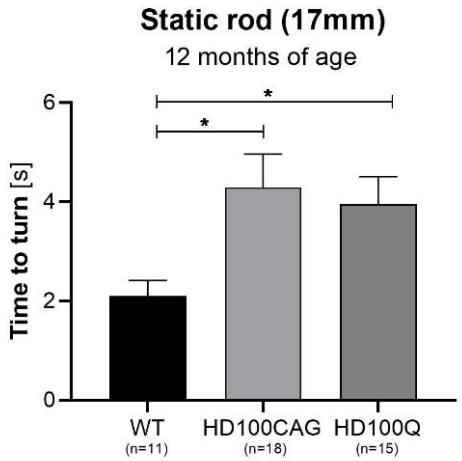


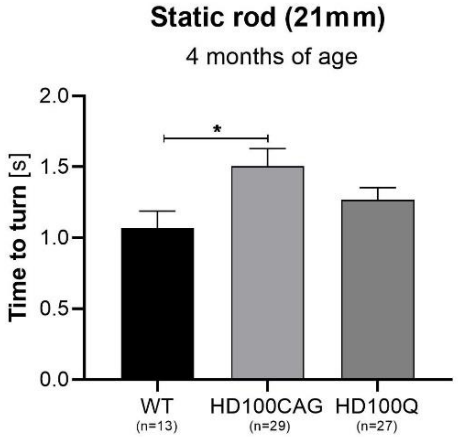
Rotarod test

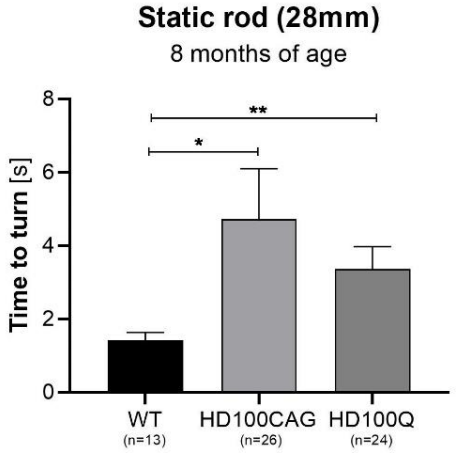
Three-day trials of the accelerated rotarod test showed cognitive decline represented by slower learning capabilities for HD100Q model at 4 and 8 months of age . Disturbed motoric function on third day of trial on rotarod for HD100Q mice was observed at all time points.

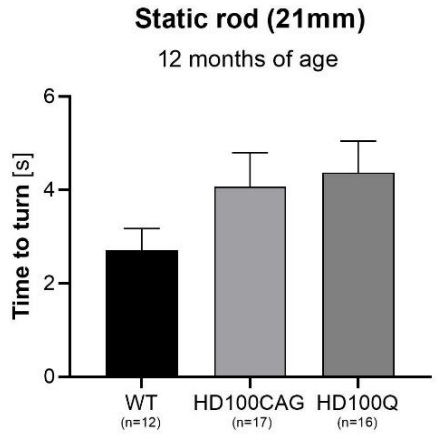






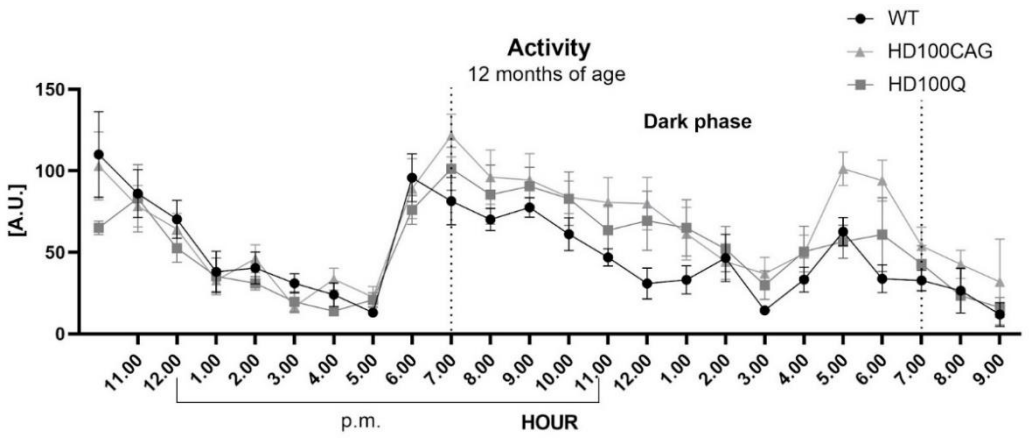


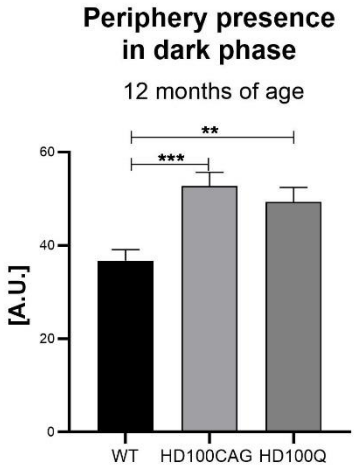


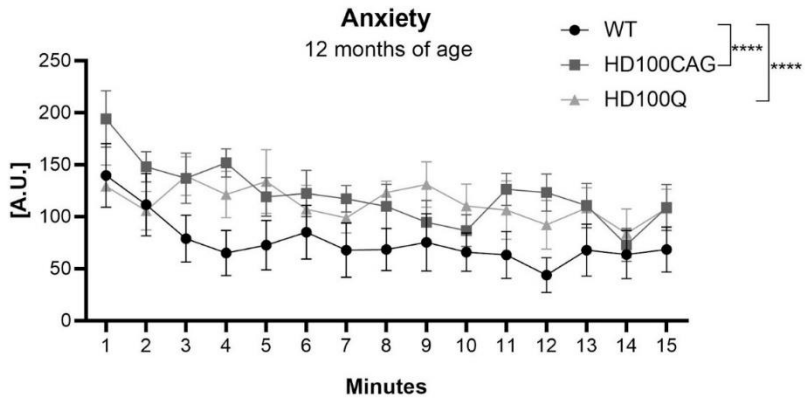


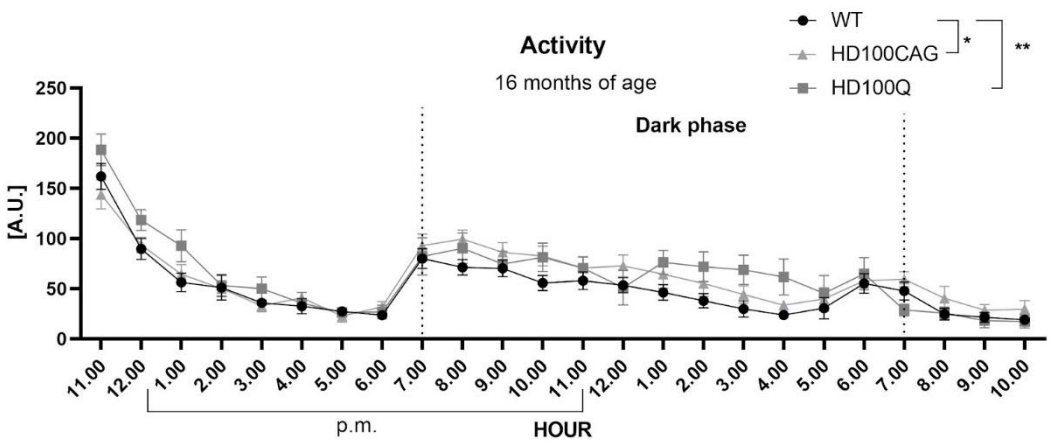
Static rod test

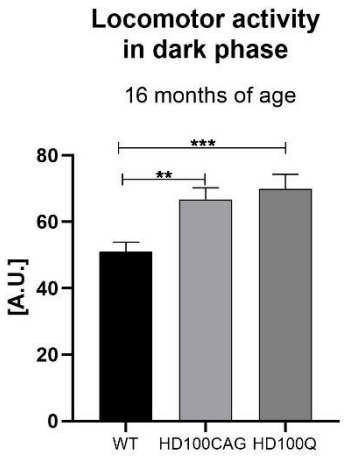
Significant differences were reported for static rod test represented by Time to turn on 10, 17, 21, 28 mm diameter rod for both models as compared to WT animals at different time points.

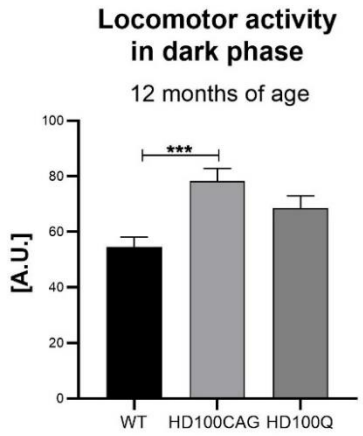








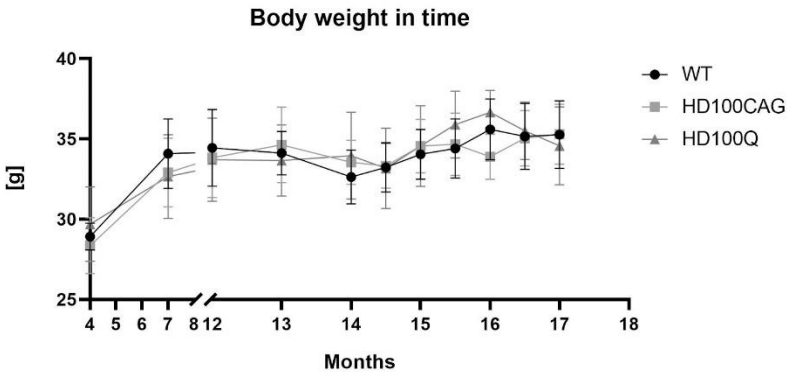


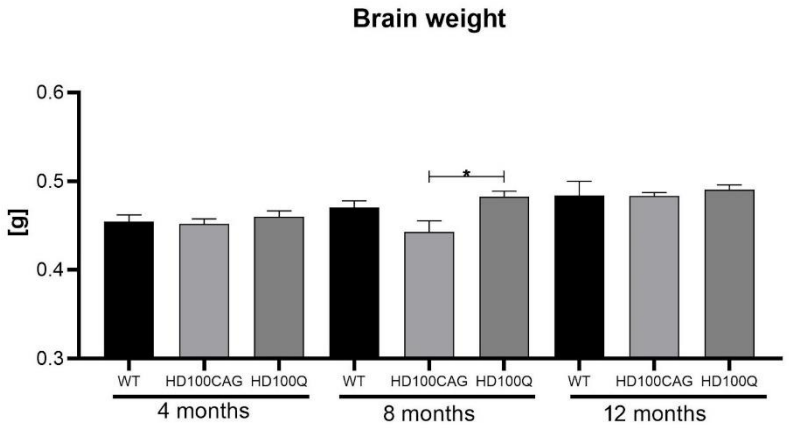


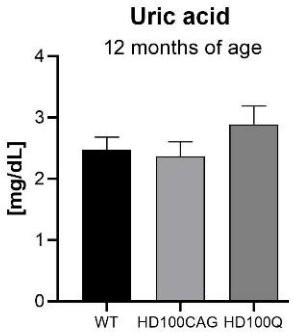
ActiMot and Open field tests

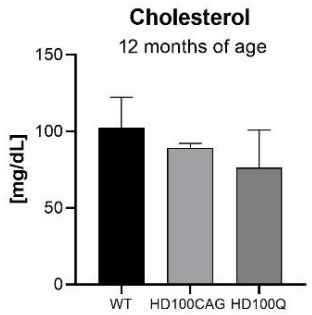
Home-cage system (ActiMot) shows hyperkinesis during dark phase in 12 and 16 months of age. Open field testing shows increased anxiety of the models.

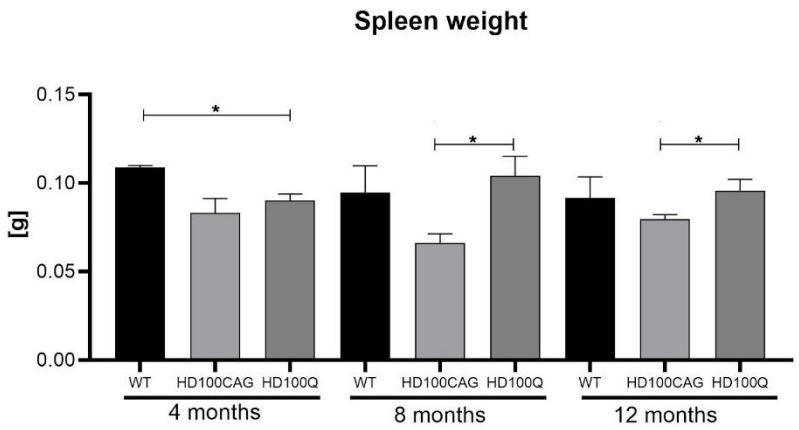
Body and organ weight

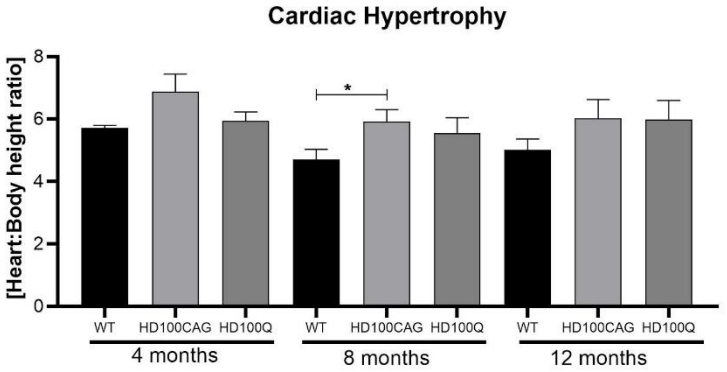


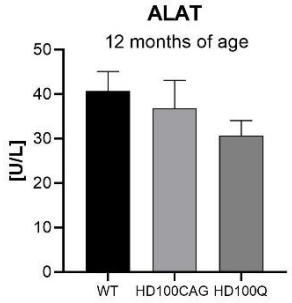


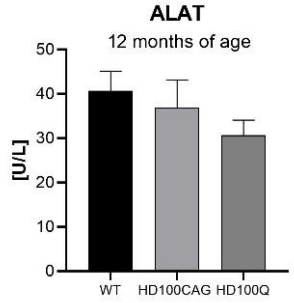












Comparison of clinical biochemical parameters in mouse serum at 12 months of age.

Conclusions

- HD/100CAG is the very first *in vivo* model which allows direct investigation of RNA toxicity in HD.
- HD/100CAG and HD/100Q are unique set of HD mouse models.
- Molecular analyses are are now being completed and full characteristics is to be finished by the end of the year.
- The results so far show slow disease progression reflecting human pathophysiological condition and indicate greater usefulness of the models for molecular analyses.
- Characteristics of these models open up a broad spectrum of possibilities for investigating cell- and tissue-specific HD pathogenic pathways.