Characterization of Htt-Q130-LEH, a novel heterozygous knock-in rat model of Huntington's Disease.

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Introduction

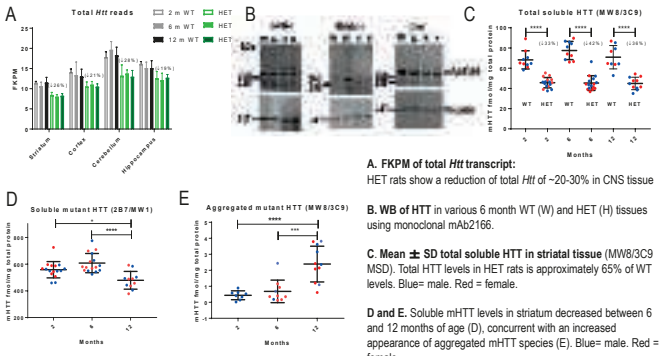
Using a ZFN-mediated gene editing approach, a knock-in Long-Evans rat model of Huntington's disease (Htt-Q130-LEH) was developed. This model carries a pure ~130 *CAG* repeat followed by C**AA**CAGCAGCAGC**AA**CAG in Exon 1 of the endogenous rat *Htt* gene.

Here we provide the first phenotyping information from heterozygote knock-in (HET) Q130 LEH rats.

Results

Quantitation of HTT levels in Htt-Q130-LEH rats

MSD and Western blot quantitation of mutant (mHTT) and total HTT levels, and immunohistochemical assessment of mHTT aggregation.



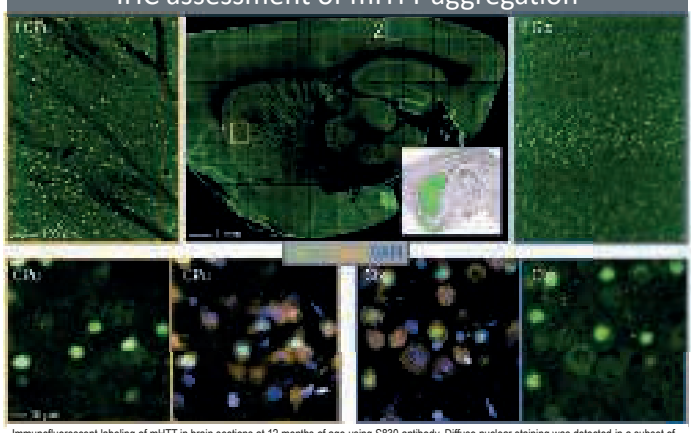
A. FKPM of total *Htt* transcript: HET rats show a reduction of total *Htt* of ~20-30% in CNS tissue

B. WB of HTT in various 6 month WT (W) and HET (H) tissues using monoclonal mAb2166.

C. Mean ± SD total soluble HTT in striatal tissue (MW8/3C9 MSD). Total HTT levels in HET rats is approximately 65% of WT levels. Blue= male. Red = female.

D and E. Soluble mHTT levels in striatum decreased between 6 and 12 months of age (D), concurrent with an increased appearance of aggregated mHTT species (E). Blue= male. Red = female.

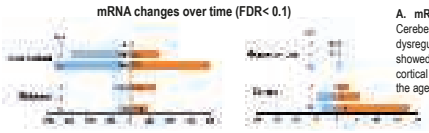
IHC assessment of mHTT aggregation



Immunofluorescent labeling of mHTT in brain sections at 12 months of age using S830 antibody. Diffuse nuclear staining was detected in a subset of NeuN-positive cells within striatum **1 Cpu** , cortex **2 Ctx** , as well as olfactory tubercle and dentate gyrus. No mHTT staining was detected yet at 6 months of age.

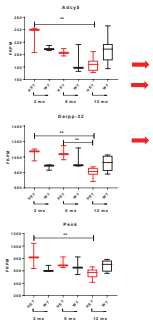
Gene expression profiling

Striatal, cortical, hippocampal and cerebellar samples from 2, 6 and 12 month old male and female WT and HET rats (5M/5F per genotype) were subjected to Stranded mRNAseq (40M reads, Paired End 100bp)



A. mRNA changes over time (HET v WT)
Cerebellar tissue showed the greatest number of dysregulated mRNAs with increasing age. Striatal tissue showed an early and late signature. Hippocampal and cortical tissue showed negligible or minor dysregulation over the ages studied.

B. Some mRNAs appear to show a biphasic pattern of dysregulation in the striatum.



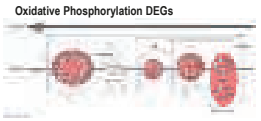
C. IPA: striatal DEGs.

Canonical Pathways	2 Months	12 Months
Opioid Signaling Pathway	3.43E-05	4.87E-11
Calcium Signaling	5.17E-01	7.36E-11
Neuroinflammation Signaling Pathway	2.07E-01	2.52E-09
Leukocyte Extravasation Signaling	9.14E-02	1.34E-07
Superpathway of Inositol Phosphate Compounds	1.35E-03	4.23E-05
Role of NFAT in Cardiac Hypertrophy	4.76E-02	1.61E-06
3-phosphoinositide Biosynthesis	1.18E-03	6.73E-05
RhoA Signaling	2.59E-02	3.78E-06
cAMP-mediated signaling	2.34E-01	4.94E-07
NF-κB Signaling	1.25E-04	1.14E-03
Role of NFAT in Regulation of the Immune Response	2.51E-01	5.79E-07
Adrenomedullin signaling pathway	9.31E-02	1.73E-05
Type I Diabetes Mellitus Signaling	1.00E+00	1.63E-05
Gap Junction Signaling	9.61E-03	1.85E-05
Protein Kinase A Signaling	3.27E-02	6.15E-06
Dendritic Cell Maturation	2.78E-01	7.34E-07
Gαs Signaling	5.97E-01	8.87E-07
Signaling by Rho Family GTPases	1.74E-02	3.06E-06

D. IPA: cerebellar DEGs.

Canonical Pathways	6 Month	12 Month
Mitochondrial Dysfunction	2.63E-08	1.13E-23
Oxidative Phosphorylation	5.16E-06	1.72E-23
EIF2 Signaling	0.537402	4.8E-20
Sirtuin Signaling Pathway	3.3E-08	1.31E-08
Regulation of eIF4 and p70S6K Signaling	1	1.03E-09
mTOR Signaling	0.184386	1.33E-08
Protein Ubiquitination Pathway	0.050115	6.59E-07
Huntington's Disease Signaling	0.001273	7.26E-05
NGF Signaling	0.00132	0.000129
VEGF Signaling	0.089971	6.53E-06
Gαq Signaling	0.054961	1.29E-05
B Cell Receptor Signaling	0.017569	4.52E-05
Cathrin-mediated Endocytosis Signaling	0.110004	1.04E-05

Oxidative Phosphorylation DEGs

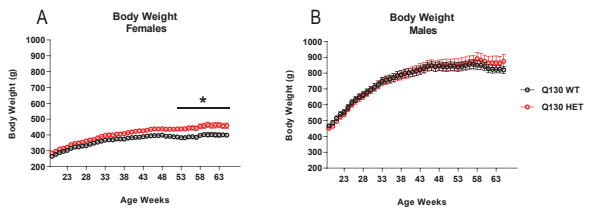


C-D. Ingenuity pathway analysis DEGs in striatum (C) and cerebellum (D). **C**. Striatal differentially expressed genes (DEGs) are predominantly involved in cAMP/ PKA –, RhoA-, calcium and phosphoinositide- signaling pathways. Some genes and pathways appear to show biphasic age-dependent changes (arrows, see also B), where certain signaling pathways were upregulated, converting to downregulation (blue) by 12 months of age. Upregulation of NF-kB signaling activation is an early and sustained signature. **D**. In the cerebellum, mitochondrial dysfunction, increased Oxidative-Phosphorylation and enhanced EIF2 ribosomal translation signaling pathways predominate. Red= upregulated, blue= downregulated, grey – inconsistent prediction for sign of dysregulation (IPA).

Behavioral analysis

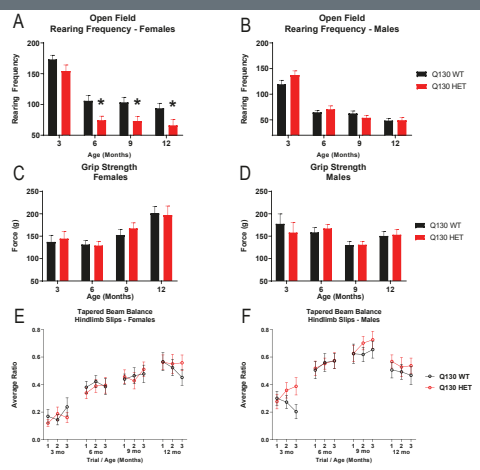
Heterozygote (HET) Htt-Q130-LEH rats (20M / 20F) and Wild type (WT) littermates (20M / 20F) undertook a battery of motor behavioral tests every 3 months starting at 3 months of age, including fine motor kinematic analysis, open field, tapered beam balance, grip strength, and weekly bodyweight monitoring.

Body weight



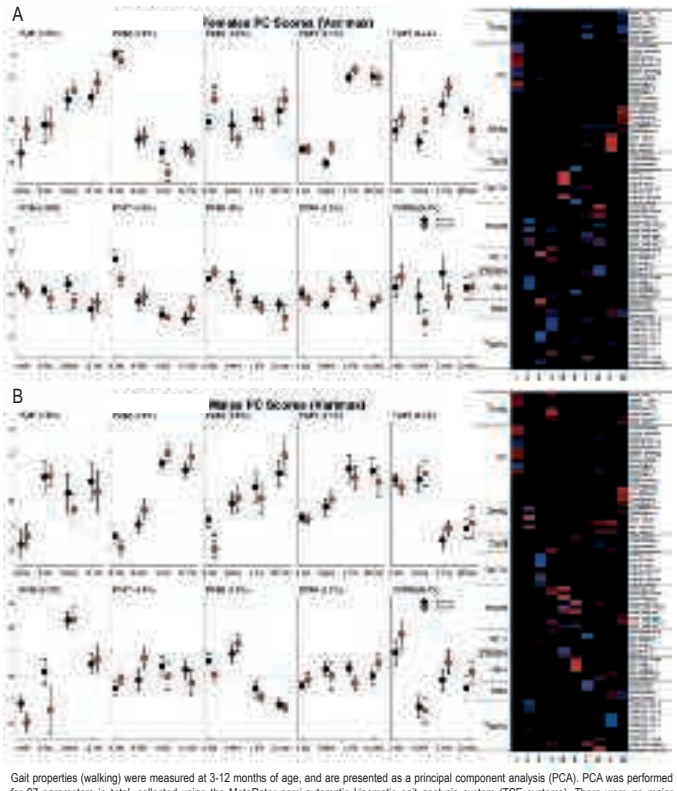
Female HET rats show increased body weight starting from 52 weeks of age (A), whereas there are no significant differences among males (B) (*p < 0.05, Mixed-effects analysis).

Motor evaluation



In the open field, female HET rats show decreased rearing activity from 6 months of age (A; *p < 0.05, Mixed-effects analysis), however this was not seen in males (B). Open field total distance and velocity were equivalent in WT and HET. Grip strength and tapered beam balance tests did not reveal any genotype differences at 3-12 months of age (C-F) in either sex (p > 0.05).

MotoRater gait kinematic analysis

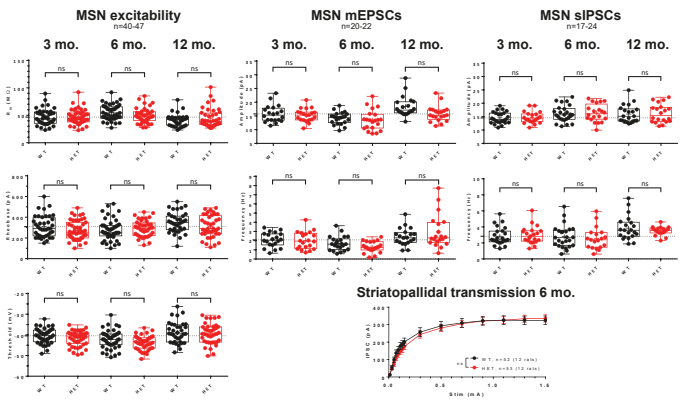


Gait properties (walking) were measured at 3-12 months of age, and are presented as a principal component analysis (PCA). PCA was performed for 97 parameters in total, collected using the MotoRater semi-automatic kinematic gait analysis system (TSE systems). There were no major significant genotype differences. Female HET rats were significantly different at 3 months of age in features related to eg. front paw trajectories (A, PC#3), and male HET rats showed elevated tail position (B, PC#3) at the same age, compared to WT rats (#p < 0.05, Student's t-test). There were no clear progressive genotyep deficits observed by 12 months of age.

Electrophysiology

Ex vivo electrophysiology

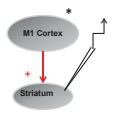
Medium spiny neuron (MSN) excitability (membrane resistance (Rm), rheobase, and action potential threshold), mEPSC and sIPSC inputs, and striatopallidal transmission were assessed in acute brain slices prepared from 3, 6 and 12 month old HET and WT rats by patch clamp. All parameters showed no significant genotype differences at these ages.



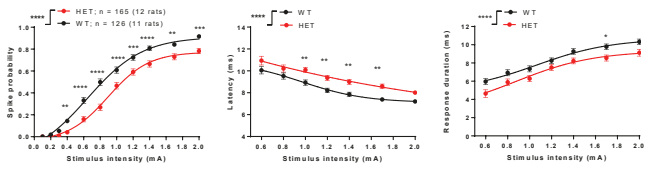
In vivo electrophysiology

In vivo recordings of cortico-striatal and cortico-subthalamic nucleus (STN) transmission were assessed in 12 month old urethane-anesthetized male HET and WT rats for evidence of altered cortico-basal ganglia function.

Corticostriatal transmission is impaired

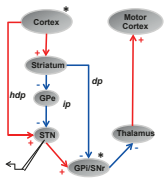


- M1 cortical stimulation.
- Neuronal firing recorded in dorsal striatum using extracellular linear multi-electrode arrays.
- Spike probability and response duration are significantly impaired, and latency to spike is enhanced in 12 month old HET rats.

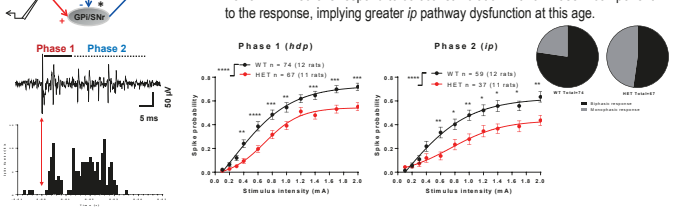


Mean ± SEM. Statistics by 2-way ANOVA for genotype comparison, with Bonferroni's multiple comparison test. * P < 0.05, ** P < 0.01, *** P <0.001, **** P<0.0001

Cortico-STN transmission is impaired



- M1 cortical stimulation.
- Neuronal firing recorded in STN using tetrodes.
- Both the excitatory short-latency Phase 1 (putative monosynaptic hyperdirect pathway - ***hdp***) and polysynaptic Phase 2 (putative indirect pathway - ***ip***) responses were significantly impaired in 12 month HET rats.
- Fewer HET neurons respond to cortical stimulation with a Phase 2 component to the response, implying greater *ip* pathway dysfunction at this age.



Mean ± SEM. Statistics by 2-way ANOVA for genotype comparison, with Bonferroni's multiple comparison test. * P < 0.05, ** P < 0.01, *** P <0.001, **** P<0.0001

Conclusions

- Until 6 months of age, HET Htt-Q130-LEH rats appear to be largely equivalent to their WT counterparts on most investigated parameters.
- From 6 months, female HETs start to display very mild motor deficits, most notably as decreased rearing in the open field. Male HETs were aphenotypic in the open field, beam walking and MotoRater tests from 3-12 months.
- At 12 months, cortico-striatal and cortico-STN transmission are significantly impaired, and striatal and cortical aggregates start to become detectable. However, MSN active and passive membrane properties, mEPSCs and mIPSCs are unaltered at this time.
- By 12 months, RNAseq revealed alterations in striatal genes predominantly involved in GPCR / cAMP-mediated and calcium signaling, as well as neuro-inflammation. Cerebellar DEGs revealed predominantly mitochondrial dysfunction, and upregulation of oxidative phosphorylation.
- Later ages are being monitored for further phenotype progression.