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Amyloid precursor protein haploinsufficiency preferentially mediates brain iron accumulation in mice transgenic for the Huntington's disease mutation.

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Abstract

Background: Huntington's disease (HD) is an autosomal dominant disorder caused by a CAG expansion in the huntingtin gene that results in expression of mutant huntingtin protein. Iron accumulates in HD brain neurons. Amyloid precursor protein (APP) promotes neuronal iron export. However, the role of APP in brain iron accumulation in HD is unclear.

Objective: To determine the effects of APP insufficiency on HD in YAC128 mice.

Methods: We crossed APP hemizygous mice (APP^{+/-}) with YAC128 mice that are transgenic (Tg) for human mutant huntingtin (hmHTT) to generate APP^{+/+} hmHTT^{-/-}, APP^{+/-} hmHTT^{-/-}, APP^{+/+} hmHTT^{+/-} and APP^{+/-} hmHTT^{+/-} progeny. Mice were evaluated for behavioral, biochemical and neuropathology HD outcomes at 2–12 months of age.

Results: APP heterozygosity decreased cortical APP 25% and 60% in non-Tg and Tg mice, respectively. Cerebral and striatal iron levels were increased by APP knockdown in Tg mice only. Nest-building behavior was decreased in Tg mice; APP knockdown decreased nest building in non-Tg but not Tg mice. Rota-rod endurance was decreased in Tg mice. APP^{+/-} hmHTT^{+/-} mice demonstrated additional decreases in rota-rod endurance from 4–10 months of age. Tg mice had smaller striatal volumes and fewer striatal neurons but were not affected by APP knockdown.

Conclusions: APP heterozygosity results in greater decreases of cortical APP in Tg versus non-Tg mice. Mutant huntingtin transgenic mice develop brain iron accumulation as a result of greater suppression of APP levels. Elevated brain iron in Tg mice was associated with a decline in motor endurance consistent with a disease promoting effect of iron in the YAC128 model of human HD.

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Author contributions

JF and AB conceived the experiments. SA completed iron analyses with assistance from JH. SA completed biochemical analyses. KB completed behavioral analyses. RN and Julia Fox completed stereologic analyses. JF wrote the paper with assistance from SA, AB, and KB.

Author conflict statement

The authors declare that there are no conflicts of interest.

Keywords

Huntington's disease; neurodegeneration; iron; amyloid precursor protein

Introduction

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disease caused by a triplet CAG-repeat expansion in exon-1 of the huntingtin gene (*HTT*) (1). Polyglutamine-expanded mutant huntingtin protein (mhtt) misfolds and forms aggregates primarily in neurons. Misfolded mhtt interferes with normal cellular processes that result in neurodegeneration primarily affecting striatum, cerebral neocortex and sub-cortical white-matter (2) but also other brain areas (3). As a result, HD patients develop progressive motor symptoms dominated by chorea, cognitive decline, and varied psychiatric manifestations. Despite the single gene cause, a large number of downstream pathways are implicated in HD pathogenesis including transcriptional dysregulation, oxidative stress, and decreased neurotrophic signaling (4–6). Therapeutic development efforts in the HD field are currently focused on approaches to decrease mhtt levels in brain and also targeting of pathways distal to mhtt known to be important for disease progression.

Iron is linked with the pathogenesis of several neurodegenerative diseases including HD (7, 8). Promotion of oxidative stress through catalysis of reactive oxygen species generation is one mechanism by which iron is thought to promote neurodegenerative processes. Brain iron accumulation is a consistent feature of human HD and in mouse HD models (9–11) and suggests a role in promoting brain oxidative stress and neurodegeneration. Studies have demonstrated that brain iron accumulation in HD models occurs at a number of cellular sites. We reported that in R6/2 mice striatal iron accumulation occurs in neurons as shown by x-ray fluorescence (9). Firdaus et al (8) reported that mhtt aggregates are iron-dependent centers of oxidative events in cell culture. Simmons et al (12) reported, in R6/2 mice, that there is microglial iron accumulation based on a histochemical approach. Furthermore, a magnetic resonance imaging study in human HD patients demonstrated early iron accumulation in areas of white matter degeneration (13). Iron accumulation in HD brain could therefore potentiate progression by a number of mechanisms.

Modulation of iron status by nutritional and pharmacologic approaches alters the course of HD. Increased neonatal iron intake potentiates neurodegeneration, energetic dysfunction and oxidative stress in the R6/2 mouse model of HD which expresses a mhtt fragment and has advanced disease by 12-weeks of age (14). The YAC128 HD model expresses full-length mhtt and has slowly progressive disease that first manifests around 9–12 months of age (15). In this model, neonatal iron supplementation potentiates neurodegeneration based on brain morphometry in 1-year-old mice (16). Interestingly, these studies did not demonstrate a correlation between HD potentiation by iron supplementation and elevated brain iron. Therapeutic studies in HD mouse models have demonstrated that compounds with iron-chelating activity provide some protection (9, 17). However, despite evidence supporting effects of iron-modulating interventions in HD models, associated mechanisms are incompletely understood.

Amyloid precursor protein (APP) is a multifunctional protein (18–20) that also has an important role in brain iron homeostasis (21). APP is not known to interact directly with huntingtin; however, huntingtin-associated protein 1 interacts with both huntingtin and APP supporting the presence of an indirect interaction (22, 23). Knock-out of APP increases brain iron by 12-months of age; in contrast, overexpression of APP in transgenic mice opposes the age-dependent elevation of brain iron (24, 25), as well as the pathological iron accumulation that mediates nigral toxicity in the MPTP model of Parkinson's disease (26). Ferroportin is the only known cellular iron exporter and is expressed by neurons and other cell types in brain (27). APP is expressed in neurons but not glial cells in brain (28) and is important for stabilizing the structure and cell surface localization of ferroportin and thereby promotes neuronal iron export (29, 30). We have previously reported that APP levels are decreased in R6/2 mouse brain (31). To test whether the decrease in APP lies upstream in the accumulation of iron in the model, we studied the effects of APP suppression (by heterozygosity) on the HD phenotype in the YAC128 mouse model.

Materials and Methods

Materials

Chemicals were from Sigma unless otherwise stated.

Mouse husbandry and maintenance

YAC128 mice were obtained from Jackson Labs (stock #4938) and maintained on the FVB-inbred background. These mice express full-length human huntingtin with 128 CAG repeats (hmHTT). Tails snips were obtained at 3 weeks of age and mice were genotyped by PCR as previously described (32). Male and female mice were used for experiments. Mice were maintained on a 12-hour light-dark cycle and were provided with access to cereal-based rodent chow (LabDiet®–6673) and water *ad libitum*. Each cage had a mouse igloo (Bio-Serv) and sani-chip bedding (Harlan Laboratories). Amyloid precursor protein (APP) global homozygous knockout mice were obtained from Jackson Labs (stock #4133). Due to difficulties breeding APP mice were maintained on the hemizygous background. APP double knockout mice have been reported to demonstrate a phenotype characterized by elevated brain iron in response to dietary iron challenge, gliosis and impaired motor function (21, 33). We are unaware of reports of effects of APP hemizygosity in these mice.

Genotyping used a three-primer system: APP knockout forward: 5' - AAG TGG CTC TGC AAA AAG AA - 3'; APP knockout reverse: 5' - GCC AGA GGC CAC TTG TGT AG - 3'; common: 5' - AAA ACC AAA CCA GGA AAT GC - 3'. All procedures were approved by the Institutional Animal Care and Use Committee and were in compliance with NIH guidelines.

Experimental design:

The experiment was a 2 × 2 factorial design with hmHTT and APP genotypes as the main factors and sex used as a covariate. To obtain the required genotypes we crossed YAC128 males with APP+/- females. We also utilized progeny from crosses between YAC128 males and C57BL/6 wild-type females. Progeny were genotyped then weaned into study cages at 4 weeks of age. Mice of the same sex and different genotypes were grouped together to

minimize age differences. Cage cards were blinded as to genotype by a person not involved in the behavioral analyses. Mice were sacrificed at 1-year of age for quantitative pathology and biochemical analyses.

Western blot analysis:

Frozen brain samples were homogenized in 9 volumes of a buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% triton X 100 and a protease inhibitor cocktail). Samples were sonicated and centrifuged at 16,000X g for 15 min at 4°C. Supernatant was collected and stored at -80°C. Protein concentrations of the samples were measured using Bradford-based protein assay (Bio-Rad) with BSA as a standard. Sample (20 µg) were mixed with 1 X Laemmli buffer, heated at 95°C for 5 min, chilled then loaded onto a 12 % Novex™ Tris-glycine mini protein gel (Invitrogen). Samples were loaded into gel well in an order that reduced the risk of data bias due to uneven transfer or membrane antibody staining. Standard methods were used for protein separation, transfer to PVDF membrane and protein detection. Anti-amyloid precursor protein (ab32136) and anti-β-actin (AC40) were used at 1:1000 dilution. HRP-conjugated secondary antibodies were used at 1:2000. Chemiluminescent detection used substrate Western blot luminol reagent (Santacruz Biotechnology) and CL-XPosure™ film (Thermo Scientific). Densitometric analysis was conducted using Image-J software. APP expression was normalized to β-actin.

Behavioral analyses

Accelerating Rota-rod: We used a rota-rod (IITC Life Science) set to accelerate from 5 to 25 rpm over 15 minutes. Mice were given one training day followed by three consecutive test days. For training, mice were placed on the rotating rod; each time the mouse fell off it was replaced onto the rod for up to 5 minutes. During testing, each mouse was placed on the rota-rod and the latency to fall was recorded. Trials were terminated at 15 minutes of testing.

Nest-building activity: Individual mice were placed into cages containing intact nestlets and lacking an igloo about 2 hours before the beginning of the dark cycle. Twenty-four hours later the level of nestlet building was scored. Blinding was retained throughout. Scoring of nest building was on a nine-point scale (1–5 with 0.5 increments) as described (34) with higher scores indicating more sophisticated nest building and lower scores indicating failure to build a nest or disorganized building. Cages were re-used once only for mice of the same sex.

Quantitative neuropathology:

To prepare tissues for stereological analyses, 12-month-old mice were sacrificed and perfused transcardially with 0.9% (w/v) heparinized (125 units/ml) saline for two minutes followed by 200 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 12 ml / minute. Brains were extracted and post-fixed for 48 hours in the same solution. They were then weighed before transfer to cryoprotectant (10% glycerol, 2% DMSO, 0.1M phosphate buffer pH 7.4) solution for 5–10 days at 4° C. Serial 40 µm sections representing the entirety of the striatum were obtained with a freezing microtome. Sections were mounted on glass slides such that every 12th section through the entirety of the striatum was present on a single slide. Mounted sections were allowed to dry overnight, stained with a 0.5% (w/v)

thionin solution then dehydrated and mounted in permount solution. Stereo Investigator Software (MBF Bioscience, Williston, VT) was used to quantify striatal volumes, cell body volumes and neuronal numbers as described previously (14, 16, 35). Briefly, in a single section at the level of the anterior commissure, the optical fractionator workflow and the nucleator method were used for estimation of neuronal cell body volume. For neuronal number estimates, each section on a slide that contained the striatum was analyzed using the optical fractionator workflow. Slides were coded such that the operator did not know the genotypes and were analyzed in a systematically randomized order.

Biochemical assays:

Glutathione (GSH), oxidized glutathione (GSSG) and lactate were measured as previously described (31, 36). Tissue iron analysis was carried out essentially as previously described (25) but using an Agilent Technologies 7700 series inductively-coupled plasma mass spectrometer.

Statistical analysis:

Analyses were performed using SAS software (version 9.4, Cary, NC). For behavioral analyses with repeated measures the MIXED procedure was used; this method is robust against unequal group sizes as were present. For all other data, the general linear model (GLM) procedure was used. All main effects, including sex, and interactions were investigated in the initial model. When there were significant main effects or interactions present in the overall model, we performed pre-planned pairwise comparisons on all non-confounded pairs from the same time point. Results are reported as means $\pm$ standard error of the mean (SEM). Unless otherwise indicated, significance bars on graphs indicate pairwise comparisons. Significant differences between confounded comparisons are not shown. P-values less than 0.05 were considered significant. P-values between 0.05 and 0.10 were considered trends while those >0.10 are reported as not significant. All group sizes including sexes are provided in figure legends.

Results

Effect of mhtt expression and genetic APP knockdown on cerebral cortical APP levels in 1-year-old mice:

APP levels were significantly decreased by hmHTT transgene expression (Tg) ($F_{1,20} = 10.45$, $p=0.0042$) and APP knockdown ($F_{1,20} = 10.21$, $p=0.0045$) (Fig. 1). Pair-wise comparisons demonstrated that APP levels were decreased by APP knockdown in Tg mice ($p=0.0172$); there was a trend to decreased APP in non-Tg mice hemizygous for APP ($p=0.0689$). APP levels were only decreased by 25.5% in APP^{+/-} non-Tg mice but were markedly decreased by 60.5 % in APP^{+/-} Tg mice. These results indicate that in APP^{+/-} mice there is some compensation for hemizygosity by overexpression of the remaining APP allele, but this is lost in the presence of hmHTT transgene expression. There was no effect of gender on APP levels. APP levels were not assessed in striatum.

YAC128 mice hemizygous for APP have elevated cortical and striatal iron concentrations:

There was a significant effect of APP knockdown ($F_{1,43} = 4.09$, $p = 0.0494$) but not Tg ($F_{1,43} = 2.62$, $p = 0.1128$) on cerebro-cortical iron concentrations at 1-year of age (Fig. 2A). Tg mice hemizygous for APP had 19% more cortical iron compared to Tg mice homozygous for APP ($p = 0.0154$). Tg and APP hemizygous mice also had 17% more cortical iron compared to non-Tg APP hemizygous mice ($p = 0.0302$). There was a significant effect of Tg ($F_{1,43} = 4.15$, $p = 0.0477$) on striatal iron concentrations. There was also a trend towards increased striatal iron concentrations when comparing all APP hemizygous with all APP homozygous mice ($F_{1,43} = 3.13$, $p = 0.0838$) (Fig. 2B). Tg mice hemizygous for APP had 13% more striatal iron as compared to Tg mice homozygous for APP ($p = 0.0202$). Tg APP hemizygous mice had 9% more iron compared to wild-type APP hemizygous mice ($p = 0.0127$). There was no effect of gender on iron levels. We also measured copper, zinc and manganese. Copper concentrations were significantly elevated in striatum ($F_{1,43} = 4.96$, $p = 0.0312$) but not in cerebral cortex in Tg mice. There was no effect of APP hemizygosity on these three elements in cerebro-cortical or striatal brain regions (data not shown).

APP hemizygosity has different behavioral effects in YAC128 Tg versus non-Tg mice:

Overall, APP hemizygosity had a moderate inhibitory effect on the combined motor and cognitive task of nest-building, but inhibitory effects on the motor endurance task of rota-rod were only notable in the Tg mice. For nest-building, on a 9-point scale from 1–5 all mice received scores of 3.5, 4.0, 4.5 or 5. The presence of Tg ($F_{1,117} = 36.10$, $p < 0.0001$) and APP $^{+/-}$ ($F_{1,117} = 8.11$, $p = 0.0052$) resulted in decreased nest-building activity; there was also a significant Tg x APP interaction ($F_{1,117} = 9.08$, $p = 0.0032$) (Fig. 3A). Slicing this interaction revealed that there was an effect of APP knockdown only in non-Tg mice ($F_{1,117} = 16.16$, $p < 0.0001$); APP hemizygosity in Tg mice did not further adversely impact nest building ($F_{1,117} = 0.00$, $p = 0.9455$). Male mice demonstrated better nest building than females ($F_{1,574} = 8.61$, $p = 0.0035$). Nest building also decreased significantly with age ($F_{5,574} = 2.97$, $p = 0.0117$). Ten and 12 month-old mice had significantly reduced nest-building activity compared to 2-month-old animals ($p = 0.0004$ and 0.0239 , respectively).

Forced motor activity on the rota-rod demonstrated that Tg ($F_{1,120} = 48.91$, $p < 0.0001$) and APP hemizygous ($F_{1,145} = 5.00$, $p = 0.0272$) mice had significantly decreased endurance (Fig. 3B). Female mice demonstrated better rota-rod endurance than males ($F_{1,575} = 5.08$, $p = 0.0246$). The Tg x APP interaction effect was not significant. Pair-wise comparisons revealed that Tg mice with APP knockdown had significantly decreased motor endurance at 4, 8 and 10 months of age compared to Tg mice homozygous for APP; there was also a trend at 6 months ($p = 0.0956$). As we have reported previously Tg mice were significantly heavier than wild-type mice ($F_{1,145} = 16.21$, $p < 0.0001$) (16); however, there was no effect of APP hemizygosity on body weight ($F_{1,145} = 2.015$, $p = 0.1545$). Male mice were heavier than females ($F_{1,1501} = 52.72$, $p < 0.0001$) and there was a significant decrease in body weight with age ($F_{11,1501} = 30.15$, $p < 0.0001$) (data not shown).

APP hemizygosity decreases brain weights in non-Tg but not in Tg mice:

We evaluated a number of structural markers of neurodegeneration. There was a significant APP x Tg interaction ($F_{1,56} = 7.47$, $p = 0.0084$) for brain weight; however, main effects of

APP ($F_{1,56}=0.73$, $p=0.3969$) and Tg ($F_{1,56}=0.99$, $p=0.3252$) were not significant (Fig. 4A). Pairwise comparisons revealed that non-Tg and APP hemizygous mice had significantly lower brain weights than non-Tg APP homozygous mice ($p=0.0076$). There was no effect of APP knockdown in Tg mice ($p=0.2882$). Striatal volumes measured unilaterally were significantly decreased by Tg ($F_{1,21}=5.64$, $p=0.0272$) but not APP status ($F_{1,21}=0.02$, $p=0.8845$) (Fig. 4B). Pair-wise comparisons revealed that Tg mice had decreased striatal volumes in APP wild-type animals only ($p=0.0088$). We found no effect of Tg ($F_{1,52}=0.01$, $p=0.9329$) on striatal neuronal cell body volume in 12-month YAC128 mice consistent with our previous findings (16). Further, there was no effect of APP genotype ($F_{1,52}=0.02$, $p=0.8849$) on neuronal volume (Fig. 4C). Striatal neuronal numbers were significantly decreased by Tg ($F_{1,21}=6.04$, $p=0.0228$) but not APP status ($F_{1,21}=2.56$, $p=0.1244$). Pair-wise comparisons revealed that Tg mice wild-type for APP had decreased striatal neuronal numbers ($p=0.0231$) (Fig. 4D).

Brain glutathione and lactate concentrations in APP hemizygous and Tg mice:

Promotion of oxidative stress is an important mechanism by which iron potentiates neurodegeneration. We measured levels of the major aqueous phase antioxidant glutathione (GSH) and also oxidized glutathione (GSSG) in cortex and striatum. There was no effect of Tg or APP on GSH concentrations (Fig. 5A–B). However, in cortex there was a strong trend to increased GSSG in APP^{+/-} mice ($F_{1,43}=3.94$, $p=0.0535$); pair-wise comparisons were not significant. We have previously shown that in R6/2 mice, neonatal iron supplementation increases brain lactate a marker of energetic dysfunction (14). We therefore measured cortical and striatal lactate but found no effects of either Tg or APP on this outcome (Fig. 5E–F).

Discussion

Amyloid precursor protein (APP) levels are closely linked with brain iron status (21, 24). Recently, APP has been shown to promote neuronal iron export by stabilizing the cellular iron export protein ferroportin (29, 30). Ferroportin is expressed widely in brain including endothelial cells and neurons (37). APP is expressed significantly in neurons and also endothelial cells (21, 28, 38). Therefore, based on current knowledge of brain iron metabolism impaired APP iron export function will increase neuronal iron and also interfere with iron trafficking at the blood-brain barrier. YAC128 mice demonstrate slowly progressive disease, resembling human HD, first evident around 9–12 months of age (15). Therefore, our study addressed the effect of APP suppression throughout the pre-clinical to early-middle disease stages in this mouse HD model.

Genetic knockdown of APP in this model, as expected, resulted in decreased cortical APP levels (Fig. 1). However, the extent of APP suppression was 25% in non-Tg ($p=0.0689$) but 60% in Tg mice ($p=0.0002$), as compared to non-Tg controls. APP levels are regulated by iron mainly at the level of protein translation by an iron-response element (IRE) located within the 5'-untranslated region of APP mRNA (39). In R6/2 mice, brain APP levels are decreased while transcript is not changed supporting such a mechanism (31). Iron-regulatory protein expression is increased by iron deficiency, which binds the APP mRNA IRE

resulting in translational inhibition (39). APP heterozygosity is expected to result in dysregulation of brain iron homeostasis with elevated iron, which may feedback to result in a compensatory increase in APP. Therefore, finding only a 25% suppression of cortical APP in APP^{+/-} mice can be explained by a compensatory response to iron elevation and results in normalization of iron homeostasis by 1-year of age (Figs. 1–2). Interestingly, these findings suggest a role of mhtt in interfering with the compensatory APP response thus explaining significantly lower cortical APP levels and elevated brain (striatal and cortical) iron in Tg mice (Figs. 1–2). However, other potential mechanisms exist as APP protein levels are also regulated by other mechanisms (40, 41). We did not evaluate other iron homeostatic proteins in this study. However, in the R6/2 HD model there are decreased brain levels of iron-regulatory protein 1 and the transferrin receptor; further, ferroportin levels are increased (9) indicating significant changes in the iron-homeostatic machinery.

Our prior studies in R6/2 mice with advanced disease have demonstrated elevations of brain iron and decreased APP (9, 31). Here APP^{+/+} Tg mice had a 25% decrease in cortical APP ($p=0.0656$) but no increase in cortical or striatal iron (Figs. 1–2). This finding is consistent with a prior study of YAC128 mice where at 1-year of age Tg mice on the same cereal-diet as used in this study did not have elevated brain iron. However, another cohort of mice on a casein-based diet, with approximately the same iron content as the cereal diet, had elevated cortical iron (16). These different results may be explained by different levels of iron bioavailability between the two diets and suggests that at 1-year of age YAC128 mice are on the threshold of developing brain iron elevation. APP suppression by genetic knockdown potentiates this effect, but is not sufficient to induce iron elevation in wild-type mice. Therefore, APP suppression greater than ~50% appears to be necessary to result in brain iron elevation. This is reasonable since there are several other proteins that participate in iron homeostasis in the brain e.g. ceruloplasmin and tau (42, 43).

We incorporated well established (motor endurance, striatal morphometry) and novel (nest-building behavior) outcomes in our assessment of YAC128 mice. APP hemizygosity resulted in decreased rota-rod forced motor endurance in Tg mice from ~4–10 months of age (Fig. 3B). This effect was not confounded by body mass as APP suppression did not influence body weight in Tg mice (not shown). At 12-months of age cortical and striatal iron levels were elevated in Tg APP hemizygous mice; however, there was not a decrement in rota-rod endurance. We have previously shown that phenotypic effects of iron manipulation in mouse HD models do not always correlate with changes in brain regional iron levels (14, 16). This can be explained as elevated iron can exist in non-toxic forms such as bound to ferritin (14) or in a labile toxic form, and this balance may change with disease progression. Conversely, changes in cellular and subcellular iron distribution can also occur without changes in total brain regional iron levels. Therefore, our findings are not incompatible with an effect of iron on the disease phenotype. Despite this, effects of APP in YAC128 mice unrelated to iron metabolism are possible. APP is reported to modulate brain copper homeostasis through mechanisms that are not well understood (24). We quantified brain copper status in this study. While striatal copper concentrations were elevated in Tg mice, there was no effect of APP knockdown on cortical or striatal copper (see results). APP has effects on axonal transport, brain synaptic morphology / plasticity and is also present at the neuromuscular junction (18–20). These APP effects could have contributed to the phenotypes observed;

how the role of APP in iron homeostasis relates to these other functions is not clear. Mice naturally build nests and a disorganized nest may indicate cognitive and / or motor dysfunction (34, 44). We found that Tg mice demonstrate a decrement in nest building activity from 2 through 12 months of age (Fig. 3A). Interestingly, this deficit did not progress with age indicating that it is not part of the progressive disease phenotype. Possibly, there is a developmental effect of mhtt on this process that is not affected by progression. The failure of APP heterozygosity to further suppress nest building in Tg mice suggests that this outcome is not influenced by elevated brain iron.

Brain APP levels are decreased and iron levels increased in both YAC128 (Fig. 1) and R6/2 HD mouse models (14, 16). We show here that the presence of mhtt interferes with the compensatory elevation of brain APP in response to genetic APP suppression. Taken together, these findings suggest that an inadequate response of APP to brain iron accumulation contributes to loss of iron homeostatic control in YAC128 mouse brain and may exacerbate disease progression. Despite the effect of decreased APP on increasing brain iron we found effects of the genetic intervention on motor endurance but not markers of neurodegeneration such as striatal volume. In contrast, we have previously shown in the same HD model that increased neonatal iron intake potentiates striatal atrophy at 1-year of age even though there was no increase in brain iron (16). These apparent discrepancies between elevated brain iron and HD progression underscores the importance of understanding the specific iron pool(s) that are important in promoting HD neurodegeneration; this is the focus of ongoing studies.

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Figure 1. Cortical amyloid precursor protein (APP) expression in 12-month Tg and APP hemizygous mice.

Tg mice have significantly lower APP than wild-type mice. APP levels are significantly decreased in Tg APP^{+/-} mice compared to Tg APP^{+/+} mice. Each group contained 3 male and 3 female mice. ME = graph bar shows main effect of Tg. P-values: * <0.05 , ** <0.01 , $a=0.0689$

Figure 2. Cortical and striatal iron levels are increased by APP knockdown in YAC128 Tg mice. Mice were sacrificed at 12-months of age and brain regional iron levels determined by ICP-MS (see methods). YAC128 Tg mice have significant elevations of cortical (A) and striatal (B) iron when hemizygous for APP. There is no effect of APP knockdown in wild-type mice on brain iron levels. $n=11-12$, Group sizes (male, female): non-Tg / APP^{+/+} (6, 6); non-Tg / APP^{+/-} (6, 6); Tg / APP^{+/+} (6, 6); and, Tg / APP^{+/-} (7, 4).

Figure 3. Behavioral and motor effects of APP knockdown in wild-type and YAC128 Tg mice.

Nest-building (A) and motor endurance (B) were evaluated throughout the year-long study.

A. Nest-building behavior is decreased in Tg mice from 2 months of age. APP knockdown results in significant decreases in nest-building; however, the effect is only in wild-type mice.

B. Rota-rod endurance is decreased by Tg and APP knockdown in a time-dependent manner. Group sizes (male, female): non-Tg / APP^{+/+} (9, 19); non-Tg / APP^{+/-} (10, 13); Tg / APP^{+/+} (17, 27); and, Tg / APP^{+/-} (9, 13). P-values: * <0.05 , ** <0.01 and *** <0.001 ;

a=0.0956

Figure 4. Brain morphometric changes in YAC128 Tg and APP hemizygous mice.

Outcome measurements were at 12-months of age. **A.** Whole brain weights are decreased in Tg compared to wild-type mice that are homozygous for APP. Knockdown of APP results in decreased mean brain weights in mice without Tg. Groups sizes (male, female): non-Tg / APP^{+/+} (5, 10); non-Tg / APP^{+/-} (5, 10); Tg / APP^{+/+} (8, 16); and, Tg / APP^{+/-} (2, 5). **B.** Striatal volumes (unilateral) are decreased in Tg mice homozygous for APP. There is no effect of APP knockdown on striatal volume. Groups sizes (male, female): non-Tg / APP^{+/+} (2, 4); non-Tg / APP^{+/-} (2, 5); Tg / APP^{+/+} (3, 4); and, Tg / APP^{+/-} (2, 3). **C.** Striatal neuronal cell body volume is not altered by the presence of Tg or APP knockdown. Groups sizes (male, female): non-Tg / APP^{+/+} (2, 10); non-Tg / APP^{+/-} (2, 10); Tg / APP^{+/+} (6, 14); and, Tg / APP^{+/-} (6, 6). **D.** Striatal neuronal numbers are decreased by the presence of Tg in APP homozygous mice, but not by Tg in APP hemizygous mice. Group sizes are as for 4C. P-values: * <0.05 , and ** <0.01

Figure 5. Brain GSH, GSSG and lactate in Tg and APP knockdown mice.

Outcome measurements were at 12-months of age. Glutathione (A-B) and oxidized glutathione (C-D) concentrations were determined in cerebral cortex (A, C) and striatum (B, D). There is a trend to increased GSSG in the cortices of APP^{+/-} mice; main effect (ME) $p=0.0535$. Lactate concentrations were measured in cortex (E) and striatum (F). Groups sizes (male, female): non-Tg / APP^{+/+} (6, 6); non-Tg / APP^{+/-} (6, 6); Tg / APP^{+/+} (6, 6); and, Tg / APP^{+/-} (7, 4)