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The Effect of Anti-aging Treatment on Expression of Aging Markers in a Mouse Model of Huntington Disease

Mary Isabelle E. Guerra
University of Central Florida

THE EFFECT OF ANTI-AGING TREATMENT ON EXPRESSION OF AGING MARKERS IN A MOUSE MODEL OF HUNTINGTON DISEASE

by

MARY ISABELLE GUERRA

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Thesis Chair: Amber Southwell, Ph.D.

ABSTRACT

Huntington disease (HD) is a fatal neurodegenerative disease caused by CAG tract expansion in the huntingtin (*HTT*) gene, which results in production of mutant huntingtin (mtHTT) protein. Although mtHTT is expressed throughout life, onset of HD symptoms typically begins in mid-life, around 35 to 50 years of age. Characteristic HD symptoms include motor, cognitive, and psychiatric abnormalities. The emergence of symptoms in adulthood suggests that aging may play a role in HD pathogenesis. Furthermore, markers of accelerated aging can be observed in HD patients, including telomere attrition, epigenetic alterations, and mitochondrial dysfunction. Our lab has previously observed that induction of age-like changes by treatment with progerin, the mutant protein that causes Hutchinson-Gilford Progeria Syndrome, enhances HD phenotypes and contributes to pathogenesis in HD neurons. Taken together, these findings suggest a link between aging and HD, with implications for potential therapeutic benefits from anti-aging treatment. Our lab has conducted a young blood anti-aging trial in which aged HD and wild-type (WT) mice were injected with plasma from young WT or HD mice. Previous work in our lab confirmed that cortical aging markers decline with age at the protein level and are differentially affected by young blood treatment. In this study, we observed a significant effect of age on striatal expression of aging markers, *Grin1* and *Lmnb1*. Varying effects of young blood anti-aging treatment were observed on the genes of interest.

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INTRODUCTION

Huntington disease (HD) is an inherited, progressive neurodegenerative disease characterized by motor, cognitive, and psychiatric symptoms (Ghosh & Tabrizi, 2018). It is caused by the expansion of a polyglutamine encoding CAG tract in the huntingtin (*HTT*) gene to more than 35 repeats (The Huntington's Disease Collaborative Research Group, 1993). This results in production of mutant HTT (mtHTT) protein, which causes neuronal dysfunction and death. The mtHTT protein disrupts cellular processes such as metabolism, vesicular trafficking, and gene transcription (Zuccato et al., 2010). Striatal medium spiny neurons (MSNs) are the first population of neurons to die and show the greatest magnitude of loss in HD (Vonsattel & DiFiglia, 1998). The length of the CAG tract is inversely correlated with age of onset, explaining approximately 50-70% of its variability among individuals (Snell et al., 1993; Wexler & Project, 2004). However, age of onset is also influenced by other genetic and environmental factors, such as somatic instability of the CAG repeat (Swami et al., 2009).

Currently, there are no disease modifying therapies for HD. Treatments are primarily symptomatic and do not affect the underlying pathogenesis (Jimenez-Sanchez et al., 2017). Symptomatic therapies include Tetrabenazine and Deutetrabenazine, the only FDA approved medications specifically for HD, which treat chorea, as well as antipsychotics and antidepressants for psychiatric symptoms (Rodrigues & Wild, 2018; Shannon & Fraint, 2015).

Although mtHTT is ubiquitously expressed throughout life, HD onset typically occurs in mid-life, from 35 to 50 years of age. The disease progresses fatally over the course of 15-20 years, from symptom onset to death (Ghosh & Tabrizi, 2018). The emergence of symptoms in

adulthood suggests that aging may play a role in HD pathogenesis. Furthermore, aging is known to be a primary risk factor for many neurodegenerative diseases, including Alzheimer's, Parkinson's, and HD (Azam et al., 2021). The exact mechanisms through which aging affects and contributes to neurodegeneration have not yet been identified. With regards to HD, some hypothesize that the passage of time allows mtHTT-related micro-toxic events to accumulate until disease onset, while others believe that the aging process actively contributes to HD.

Aging can be distinguished into two categories: chronological and biological. Chronological age refers to the amount of time passed since birth. In contrast, biological age, also known as physiological age, refers to the decline in tissue and organismal function over time (Hamczyk et al., 2020). Biological aging can be defined at the cellular and molecular level by 9 hallmarks, shown below in **Figure 1** (Lopez-Otin et al., 2013). Many of these biological aging hallmarks are also observed in HD, further supporting the link between aging and HD onset/progression (Machiela & Southwell, 2020).

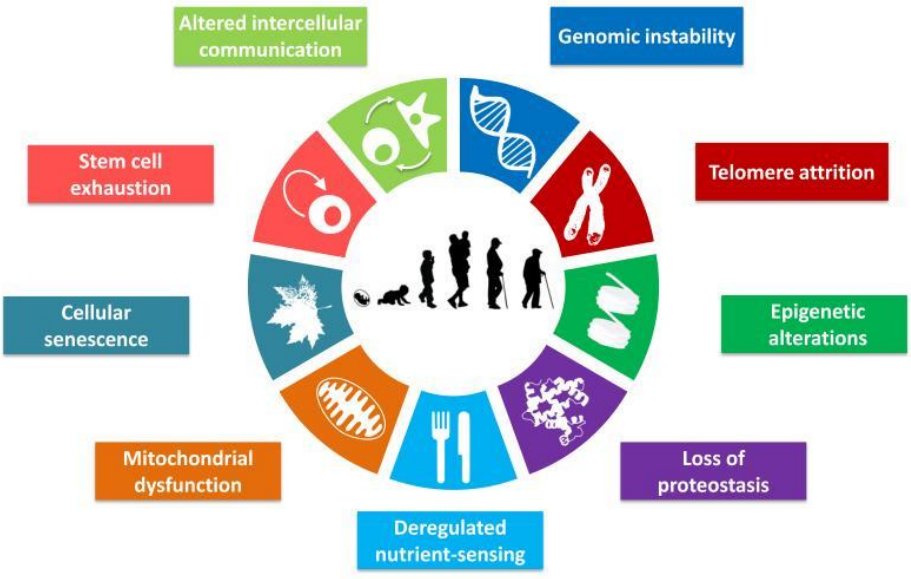

Figure 1: The nine hallmarks of aging

The nine hallmarks of aging as defined by Lopez-Otin et al., 2013: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.

Furthermore, there is evidence of advanced biological age in HD. Telomere attrition, or telomere shortening, is observed during natural aging in both humans and mice (Blasco, 2007). Studies in HD leukocytes reveal shortened telomere length compared to age-matched controls (Kota et al., 2015), suggesting accelerated aging of peripheral cells in HD. In addition, HD postmortem brains exhibit accelerated epigenetic age based on DNA methylation levels (Horvath et al., 2016), suggesting that accelerated age in HD is global. Another hallmark of aging observed in HD is cellular senescence. Previous studies have shown increased expression of $p16^{INK4a}$, a marker of cellular senescence, in human HD neural stem cells and MSNs (Baker et al., 2016; Voisin et al., 2020).

Building upon this, our lab previously induced age-like changes in HD and control neurons using progerin treatment. Progerin is the mutant protein that causes Hutchinson-Gilford Progeria Syndrome, a disease of premature aging (Eriksson et al., 2003). We found that progerin treatment resulted in enhanced mtHTT misfolding in HD neurons, which suggests that biological age can affect mtHTT folding and aggregation (**Figure 2**). Additionally, progerin treatment exacerbates HD-related phenotypes, including increased susceptibility to stress-induced DNA damage and cell death (**Figure 3**) (Machiela & Southwell, 2020).

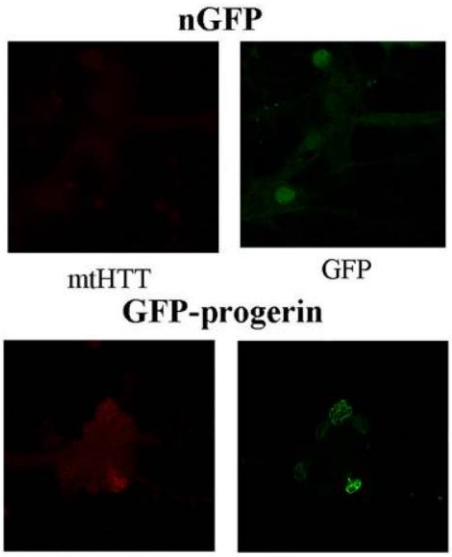

Figure 2: Progerin-induced age-related changes causes mtHTT oligomerization.

Mutant HTT aggregation was assessed by EM48 immunoreactivity. EM48 is an anti-HTT antibody that only binds aggregated or oligomeric mtHTT. Mature primary HD mouse neurons were treated with either nGFP or GFP-progerin. The HD neurons treated with nGFP lack EM48 immunoreactivity, which suggests that the mtHTT is only in its soluble and monomeric form. The HD neurons treated with GFP-progerin, which induces age-like changes, revealed EM48 immunoreactivity. This suggests that biological age can affect mtHTT folding and aggregation. Reproduced from **(Machiela & Southwell, 2020).**

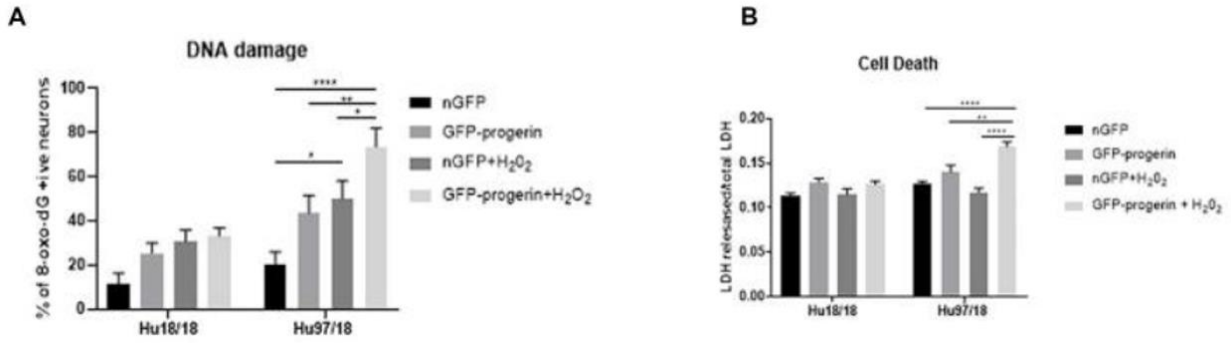

Figure 3: Progerin-induced age-related changes enhances HD phenotypes.

Mature primary HD neurons were treated with nGFP, GFP-progerin, nGFP + H_2O_2, and GFP-progerin + H_2O_2. H_2O_2 treatment was used to assess sensitivity to oxidative stress. (A) DNA damage was assessed by Oxo-8-dG immunoreactivity. The combination of progerin and oxidative stress resulted in a significant increase in DNA

damage in HD neurons only, greater than GFP-progerin or nGFP + H_2O_2 alone. (B) Cell death was assessed by released/total lactate dehydrogenase (LDH). The combination of progerin and oxidative stress had no significant effect on cell death in control Hu18/18 neurons. However, this combination (GFP-progerin + H_2O_2) resulted in a significant increase in cell death in HD neurons. This suggests that induced aging by progerin treatment can increase the susceptibility of HD neurons to stress-induced DNA damage and cell death. Reproduced from **(Machiela & Southwell, 2020).**

These findings further support the link between biological age and HD - increasing biological age contributes to HD pathogenesis. Thus, anti-aging treatments may be a potential therapeutic strategy to slow HD onset/progression.

One anti-aging therapy of interest to our lab is young blood. Young blood anti-aging treatment uses plasma from young mice to rejuvenate and reverse aspects of aging in old mice. Evidence has shown that administration of young blood plasma in aged mice improves aged mice hippocampal-dependent learning and memory, and enhances synaptic plasticity (Villeda et al., 2014). A previous study has also shown that factors in young blood can induce vascular remodeling and enhance neurogenesis in aged mice (Katsimpardi et al., 2014). These findings demonstrate that young blood plasma can be used to reverse cognitive impairments observed in aging and other age-related deficits. Consequently, we decided to investigate the therapeutic potential of young blood in HD.

Our lab previously conducted a young blood anti-aging trial in aged HD mice and wildtype littermate controls. Heterozygous Q175FDN (Southwell et al., 2016) HD model mice with exon 1 of human *HTT* knocked-in to the mouse homolog were used for this trial. The Q175FDN

mouse model was chosen because it exhibits earlier onset and greater severity of HD-like phenotypes than any other full-length mtHTT HD mouse model. Furthermore, the Q175FDN mouse displays robust HD-like phenotypes while heterozygous. Heterozygous mice were chosen for this trial to better recapitulate the genetic mutation that causes HD. The HET mice are more representative of human HD because they produce both wild type HTT and mutant HTT protein, similar to human HD patients. Homozygous mice, on the other hand, completely lack wild type HTT and are therefore less relevant to human HD.

During the young blood anti-aging trial, mice were injected with saline control or plasma from young WT or HD mice – WT yb or HD yb, respectively. Treatment began at 6 months of age, around the time of motor phenotype onset, and continued until 12 months of age, when phenotypes are known to be advanced **(Figure 4).** At 12 months of age, treated mouse brains were micro dissected by region and snap frozen for analysis.

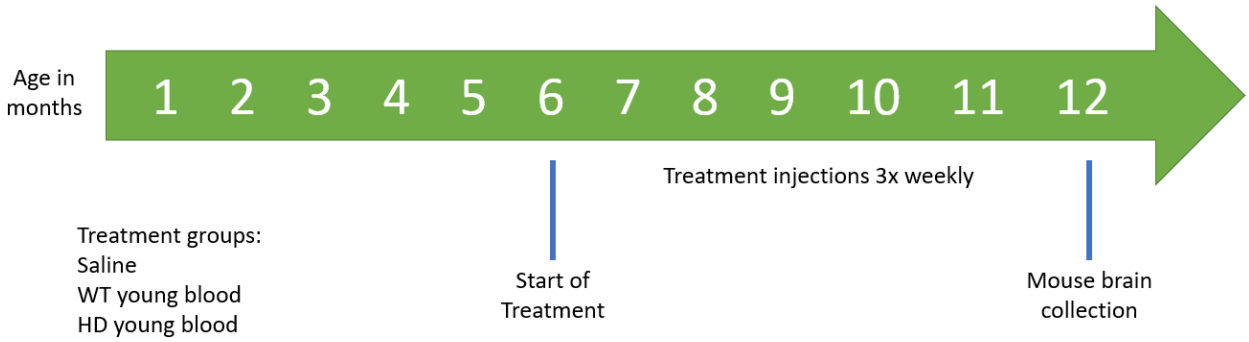

Figure 4: Design of young blood anti-aging trial in Q175FDN mice.

Young blood anti-aging treatment involved administration of saline control or plasma from young WT or Q175FDN HET mice. Treatment injections were 3 times a week from 6 months of age until 12 months of age.

The goal of my project is to assess the effects of young blood anti-aging treatment on age-related changes in gene expression. While our lab has previously investigated the effects of young blood treatment on protein aging markers, no study has been done on expression of the corresponding genes. More specifically, this study will characterize transcriptional changes that occur during natural aging in the striata. Previous study has shown that the greatest number and magnitude of differentially expressed mRNAs in the human HD brain are observed in the caudate nucleus of the striatum (Hodges et al., 2006). This illustrates the importance of studying striatal gene expression changes.

To provide a basis for evaluating the success of young blood treatment, the effect of age on aging markers was assessed. Relative expression of aging markers was compared between the striata of 3 month old and 12 month old naïve mice. Then, the effects of anti-aging treatment were assessed in the trial mice, to provide insight on how young blood treatment could potentially counteract or reverse the age-related changes in gene expression. This work will further provide insight into the link between biological age and HD, and the potential benefits of young blood anti-aging treatment.

RESULTS

Expression of age-related markers (NMDAR1 and lamin B1) was quantified by RT-qPCR in naïve 3 month old and 12 month old mouse striata. These aging markers are known to decrease with natural aging and with progerin treatment at the protein level (Machiela & Southwell, 2020). Furthermore, preliminary findings from our lab reveal that cortical lamin B1 decreases with age and cortical NMDAR1 may decrease with age in naïve mice (**Figure 5)** (Koilpillai, 2022). It is not known whether these age-related reductions at the protein level results from transcriptional changes, translational changes, or post-translational changes. Additionally, it is unknown whether these age-related reductions occur in the striatum as well, or exclusively in the cortex.

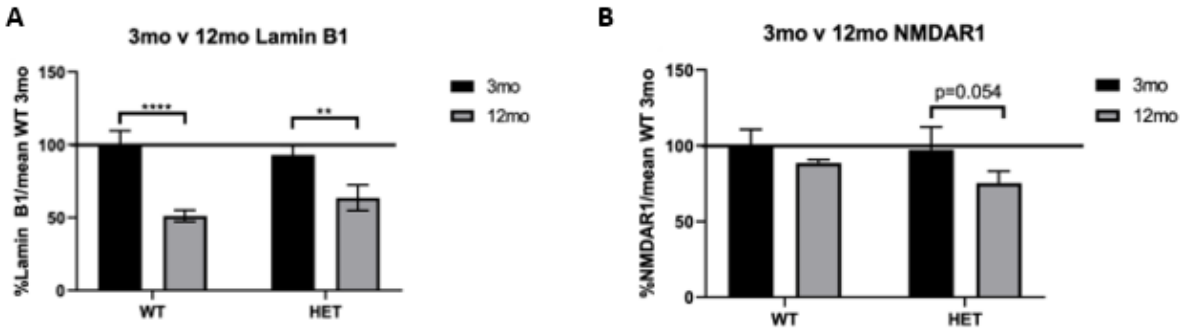

Figure 5: Cortical Lamin B1 decreases with age and cortical NMDAR1 may decrease with age in naïve Q175FDN mice.

Western blot analysis of cortical lysates from naïve 3 and 12 month old Q175FDN mice was performed. Lamin B1 was observed to significantly decrease with age in both genotypes (Two-way ANOVA: age $p<0.0001$, genotype $p=0.5623$, interaction $p=0.0548$. Sidak's multiple comparison *post hoc* test: WT $p<0.0001$, HET $p=0.0028$). NMDAR1 demonstrated an overall age-related reduction (Two-way ANOVA: age $p=0.0201$, genotype $p=0.2083$, interaction $p=0.3887$) which did not reach significance for either genotype. N=3, *=Difference between indicated bars,**= $p<0.01$, ****= $p<0.0001$.

Striatal expression of *Grin1* may increase with age

To address these outstanding questions, striatal expression of *Grin1*, the mouse gene that encodes NMDAR1, was evaluated by RT-qPCR. Striatal expression of *Grin1* revealed a significant effect of age but not genotype (**Figure 6,** Two-way ANOVA: age p=0.0472, genotype p=0.2274, interaction p= 0.1545). *Post hoc* analysis revealed a trend towards increased *Grin1* mRNA in WT 12mo mice compared to WT 3mo mice (Tukey's multiple comparison test: WT p=0.0729, HET p=0. 0.9720), which was absent in HET mice.

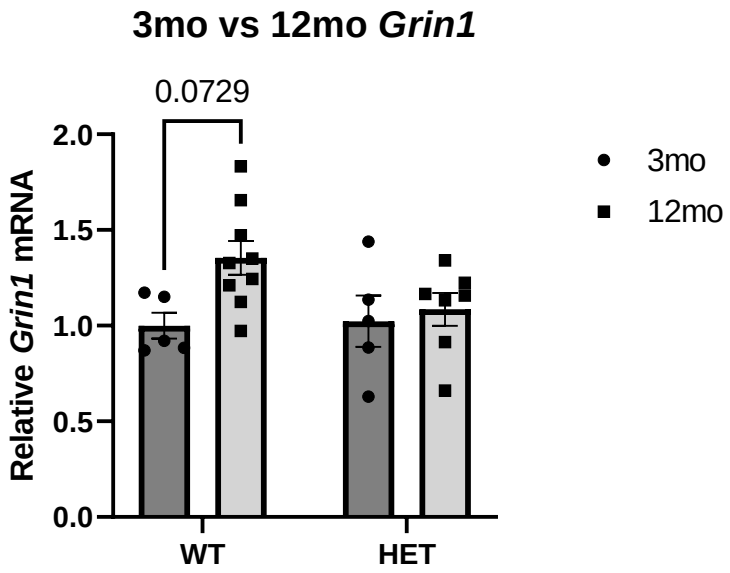

Figure 6: Striatal expression of *Grin1* may increase with age in naïve mice.

RT-qPCR was used to evaluate expression levels of *Grin1* in striata of naïve 3 and 12 month old Q175FDN mice. Relative quantity of *Grin1* was normalized to housekeeping gene *EIF4A2*. Values were then normalized to the mean value for 3mo WT mice. There is a significant effect of age on striatal expression of *Grin1* (Two-way ANOVA: age p=0.0472, genotype p=0.2274, interaction p= 0.1545). *Post hoc* analysis revealed a trend toward age-related increase of *Grin1* expression in WT mice (Tukey's multiple comparison test: WT p=0.0729, HET p=0. 0.9720). N=5-9. Error bars ±SEM.

Significant effect of age on striatal expression of *Lmnb1*

Striatal expression of *Lmnb1*, the mouse gene that encodes lamin B1, revealed a significant effect of age but not genotype (**Figure 7,** Two-way ANOVA: age p=0.0440, genotype p=0.9148, interaction p=0.2975), though this did not reach significance in either WT or HET mice by *post hoc* analysis (Tukey's multiple comparison test: WT p=0.1234, HET p=0.8805).

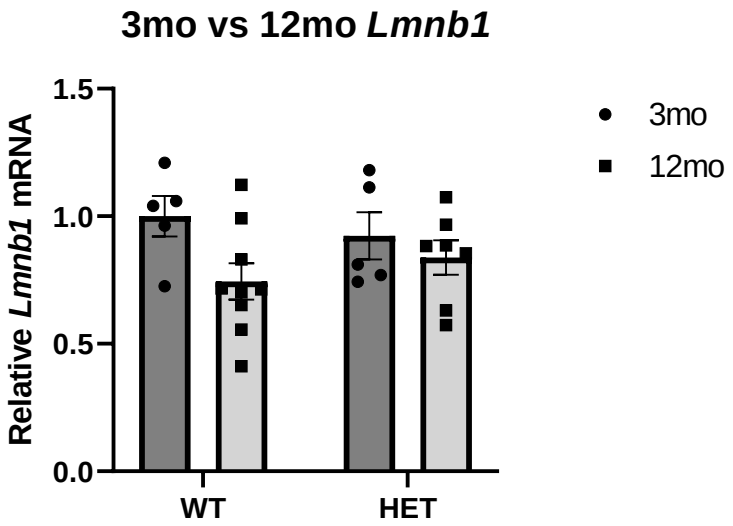

Figure 7: There is a significant effect of age on striatal expression of *Lmnb1* in naïve mice.

RT-qPCR was used to evaluate expression levels of *Lmnb1* in striata of naïve 3 and 12 month old Q175FDN mice. Relative quantity of *Lmnb1* was normalized to housekeeping gene *EIF4A2*. Values were then normalized to the mean value for 3mo WT mice. There is a significant effect of age on striatal expression of *Lmnb1* (Two-way ANOVA: age p=0.0440, genotype p=0.9148, interaction p=0.2975) that did not reach significance for either genotype by *post hoc* analysis (Tukey's multiple comparison test: WT p=0.1234, HET p=0.8805). N=5-9. Error bars ±SEM.

Anti-aging treatment had varying effects on expression of aging markers

RT-qPCR was used to evaluate the effects of young blood anti-aging treatment on striatal expression of *Grin1* and *Lmnb1*. Failed assays were excluded from the data, leaving an N=2 for some treatment groups. Statistical analysis was performed, however, the following results cannot be considered conclusive due to the small sample size. Two-way ANOVA revealed a trend towards a significant effect of genotype and treatment on striatal *Grin1* mRNA levels (**Figure 8,** Two-way ANOVA: treatment p=0.0717, genotype p=0.0585, interaction p=0.2063). *Post hoc* analysis revealed significantly lower levels of *Grin1* mRNA when comparing HD yb-treated WT mice to HD yb-treated HET mice (Sidak's multiple comparison test: saline p=0.9983, WT yb p=0.7902, HD yb p=0.0405). There were no significant effects of treatment in WT mice (saline vs. WT yb p=0.9166, saline vs. HD yb p=0.9985). In HET mice, there is a trend towards decreased striatal *Grin1* when comparing saline vs. HD yb treatment (saline vs. WT yb p=0.9992, saline vs. HD yb p=0.0688).

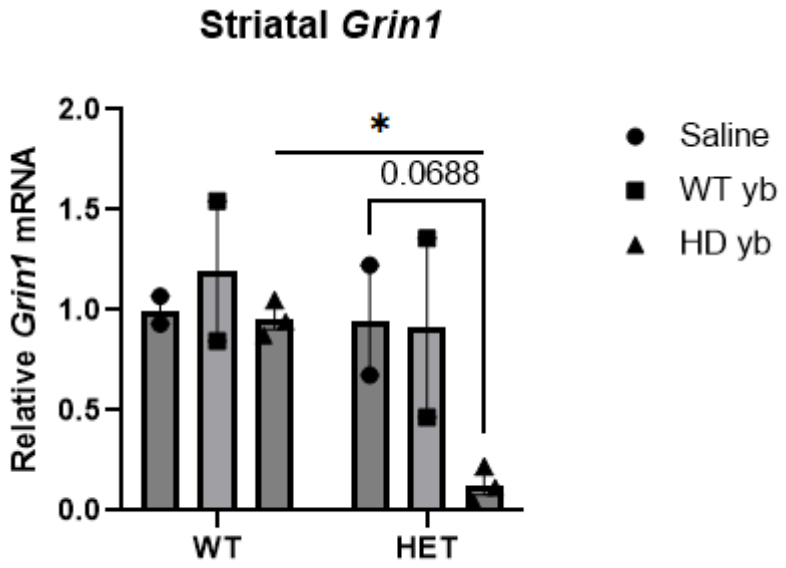

Figure 8. Striatal expression of *Grin1* is significantly lower in HET mice treated with HD young blood.

RT-qPCR was used to evaluate expression levels of *Grin1* in striata of 12 month treated mice. Treatment groups included saline, WT youngblood, and HD youngblood. Relative quantity of *Grin1* was normalized to housekeeping gene *EIF4A2*. Values were then normalized to the mean value for WT saline treated mice. There is a trend towards a significant effect of genotype and treatment on striatal *Grin1* mRNA levels (Two-way ANOVA: treatment p=0.0717, genotype p=0.0585, interaction p=0.2063). *Post hoc* analysis showed that compared to WT mice, HD yb-treated HET mice had significantly lower levels of *Grin1* (Sidak's multiple comparison test: saline p=0.9983, WT yb p=0.7902, HD yb p=0.0405). There were no significant effects of treatment in WT mice (saline vs. WT yb p=0.9166, saline vs. HD yb p=0.9985). In HET mice, there is a trend towards decreased striatal *Grin1* when comparing saline vs. HD yb treatment (saline vs. WT yb p=0.9992, saline vs. HD yb p=0.0688). N=2-3. Error bars ±SEM.

Striatal *Lmnb1* levels were not significantly affected by young blood anti-aging treatment. No significant effects of treatment or genotype were observed by Two-way ANOVA (**Figure 9,** treatment p=0.4437, genotype p=0.1820, interaction p=0.9851). *Post hoc* analysis showed no significant effect of treatment in WT mice (Sidak's multiple comparison test: saline vs. WT yb p=0.9986, saline vs. HD yb p=0.9020). Similarly, no significant effects of treatment were observed in HET mice (saline vs. WT yb p=0.9927, saline vs. HD yb p=0.8269). Additionally, when comparing WT mice to HET mice of the same treatment groups, no significant differences were observed (saline p=0.8080, WT yb p=0.8899, HD yb p=0.7143).

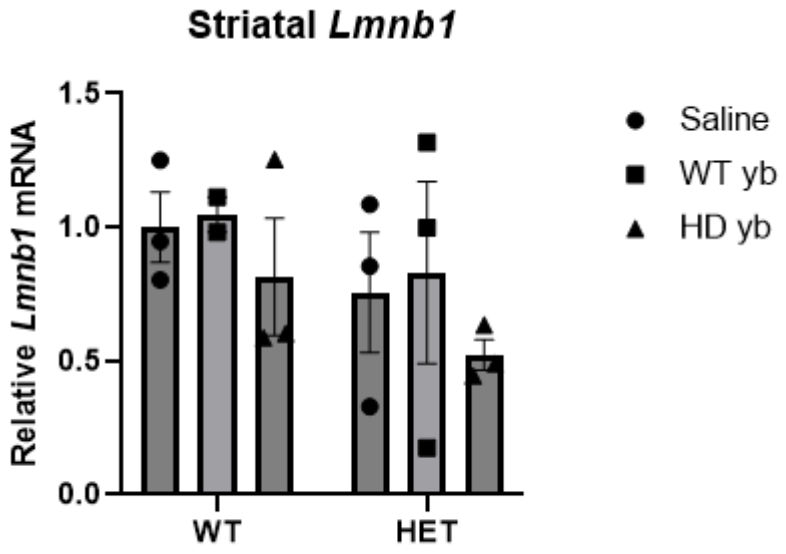

Figure 9: Striatal expression of *Lmnb1* is not significantly affected by young blood treatment.

RT-qPCR was used to evaluate expression levels of *Lmnb1* in striata of 12 month treated mice. Treatment groups included saline, WT youngblood, and HD youngblood. Relative quantity of *Lmnb1* was normalized to housekeeping gene *EIF4A2*. Values were then normalized to the mean value for WT saline treated mice. No significant effects of treatment or genotype were observed (Two-way ANOVA: treatment p=0.4437, genotype p=0.1820, interaction p=0.9851). *Post hoc* analysis revealed no significant effects of treatment in WT mice (Sidak's multiple comparison test: saline vs. WT yb p=0.9986, saline vs. HD yb p=0.9020) or in HET mice (saline vs. WT yb p=0.9927, saline vs. HD yb p=0.8269). No significant differences were observed between WT and HET mice of the same treatment groups (saline p=0.8080, WT yb p=0.8899, HD yb p=0.7143). N=2-3. Error bars ±SEM.

DISCUSSION

In this study, the effects of young blood anti-aging treatment on age-related changes in gene expression were assessed. Previous studies have shown evidence of accelerated age in HD (Horvath et al., 2016; Kota et al., 2015), and many hallmarks of biological age are shared with HD (Machiela & Southwell, 2020). Thus, reversal of aging, through anti-aging treatment, could potentially slow HD onset and progression. My aim is to investigate the effects of young blood therapy on striatal expression of previously identified aging markers.

Preliminary findings from our young blood anti-aging trial have validated that aging markers – NMDAR1, lamin B1, and dynamin 1 – decrease with natural age in the Q175FDN mouse cortex (Koilpillai, 2022). However, it is not known whether these age-related reductions at the protein level are a result of transcriptional changes, translational changes, or post-translational changes. Errors in mRNA translation or increased protein degradation are examples of post-transcriptional mechanisms that could explain the decrease in protein levels. Additionally, it is unknown whether these age-related reductions occur exclusively in the cortex or if they can be observed in the striatum as well. Therefore, we first characterized striatal gene expression changes during natural aging in the naïve study.

In the naïve Q175FDN mice, there was a significant effect of age on striatal expression of *Lmnb1*, the gene that encodes lamin B1. These findings suggest that striatal *Lmnb1* expression may decrease with natural age. If such is the case, it is possible that the age-related decline of lamin B1 protein may be the result of decreased gene expression. It should be noted that while changes at the mRNA level are generally informative for changes at the protein level, they are far from predictive. Previous study in human circulating monocytes demonstrated that mRNA

levels were generally correlated with protein levels for the 71 genes studied (Guo et al., 2008). However, the observed correlation between mRNA and protein expression varied largely depending on the gene and gene category. Additionally, my project investigated *Lmnb1* expression changes in the striatum, whereas our previous findings on lamin B1 protein levels were in the cortex (Koilpillai, 2022).

There was also a significant effect of age observed on striatal expression of *Grin1*, the gene that encodes NMDAR1. Interestingly, there was a trend towards an age-related increase of *Grin1* mRNA levels in WT mice. In WT mice, there was a 35.45% mean increase in *Grin1* expression, whereas in HET mice, there was a 6.22% mean increase. These findings suggest that striatal *Grin1* may increase with natural age. This differs from a previous study that observed a reduction in cortical and striatal *Grin1* expression in aged mice (Magnusson, 2000). Furthermore, cortical NMDAR1 levels were previously observed to decrease during aging. One possible explanation is upregulation of the *Grin1* gene to compensate for reduced protein levels. NMDAR1 plays a key role in long-term memory consolidation and learning (Riedel et al., 2003). Therefore, it is possible that reduced levels of NMDAR1 protein is detected and signals the striatum to upregulate *Grin1*. This may explain the mean increase observed in striatal *Grin1* expression. Additionally, there is a greater mean increase in WT (35.45%) compared to HET (6.22%) mice. If *Grin1* levels increase as a compensatory mechanism, these findings indicate that the HET mice may be less successful than WT mice at compensating for changes in NMDAR1 levels. Striatal levels of *Grin1* and *Lmnb1* mRNA were used to evaluate the success of the young blood anti-aging trial.

In our young blood anti-aging trial, we observed significantly lower levels of *Grin1* mRNA when comparing HD yb-treated WT mice to HD yb-treated HET mice. Additionally, there is a

trend towards decreased *Grin1* mRNA when comparing saline vs. HD yb treatment that is only seen in the HET mice. Taken together, these findings suggest that HET mice may be more susceptible to reduction in *Grin1* mRNA when administered HD young blood. It is possible that toxic factors in HD youngblood, due to accelerated age in HD, are responsible for decreased *Grin1* mRNA. These effects observed in the HET mice but not WT mice further demonstrate that WT mice may be better equipped at compensating for changes in gene expression. There were no significant effects of treatment or genotype observed on striatal expression of *Lmnb1*.

One limitation to note is the small sample size (N=3) analyzed in the trial, resulting in great variation within treatment groups. Therefore, one sample can significantly skew the mean for the entire treatment group. In the future, use of a larger sample size could reduce this variability. Additionally, there were some failed assays in the trial that had to be excluded, which was not seen in the naïve study. Statistical analysis was performed; however, due to the small sample size (N=2), the trial results cannot be considered conclusive. The failed assays could be the result of differing length of storage of the naïve and treated samples. Striatal samples for the naïve study were collected in 2021, whereas samples for the trial were collected in 2018. Thus, potential loss of RNA integrity due to the length of storage could explain the failed assays in the trial. Furthermore, striatal RNA extraction for the trial was performed with a different protocol than the naïve study. For samples from the trial, extraction was performed on striata from the left hemisphere using Qiagen's RNeasy mini kit, compared to the naïve study in which RNA was isolated from both striata using TRIzol reagent. It is possible that lower RNA purity resulted in the failed assays observed in the trial.

Another possible explanation may be low expression of *Lmnb1* and *Grin1* in the striatum. Lamin B1 mRNA is expressed in almost every cell type and can be found in both the cortex and

striatum (Alcalá-Vida et al., 2021). Previous studies have also found that NMDAR1 mRNA is widely distributed throughout the brain regions – including the striatum – with highest expression in the hippocampus, cerebral cortex, cerebellum, and olfactory bulb (Moriyoshi et al., 1991). It is possible that initially low expression of these genes in the striatum, as well as RNA extraction of only one half of the striata, may have resulted in low RNA concentrations and the failed assays seen in the trial.

Furthermore, it would be of interest to study changes in NMDAR1 and lamin B1 protein levels in the striatum, as opposed to the cortex. Therefore, more direct comparisons can be made between gene expression changes and protein levels in the striatum. However, protein extracted using TRIzol reagent in the naïve study was insufficient for Western blot analysis (Koilpillai, 2022). Therefore, for our naïve study, we only had preliminary data on changes in cortical NMDAR1 and lamin B1 during natural aging.

Overall, there were no significant effects of young blood treatment observed in both WT and HET mice. A future direction of my project would be to assess the effects of young blood therapy using other aging markers. The $p16^{INK4a}$ gene is of interest because its expression is known to be upregulated during cellular aging (Krishnamurthy et al., 2004; Liu et al., 2009; Ressler et al., 2006), serving as a biomarker of aging. There may also be effects of therapy on HD markers in the brain. Therefore, future work should include quantification and study of other genes which may be affected by either age or HD. Altogether, this work contributes to the understanding of the role that aging plays in HD. The potential therapeutic benefit of anti-aging treatments, outside of young blood therapy, should also be explored for HD in the future.

METHODOLOGY

Mice and Housing

Experiments were conducted with Q175FDN HET and WT mice. Mice were maintained on a 12 hour light/dark cycle in a clean facility with ad libitum access to food and water. In the naïve study, an *n* of 5 mice were dissected at 3 months of age for each genotype. An *n* of 7-10 mice were dissected at 12 months of age for each genotype. In the trial, an *n* of 3 mice per genotype per treatment were dissected at 12 months. Brains were micro dissected by region to isolate striatal tissue. Striatal tissue was snap frozen and stored at -80°C. Experiments were performed with the approval of the Institutional Animal Care and Use Committee at the University of Central Florida.

Striatal RNA Extraction

For striatal samples of naïve mice, total RNA was isolated from both striata using the Invitrogen TRIzol reagent, along with DNA and protein extraction. Striatal tissue was lysed and homogenized using a pestle and pestle motor. Samples were sonicated for 5-10 sec at 20 Hz. RNA extraction was completed according to the manufacturer's instructions. An additional ethanol precipitation step was performed to improve RNA purity. For striatal samples of treated mice, total RNA was isolated from striata from the left hemisphere using Qiagen's RNeasy mini kit, according to the manufacturer's instructions. Homogenization was performed by passing the lysate at least 10 times through a syringe and 25-gauge needle. Samples were then sonicated for 5-10 sec at 20 Hz. A Nanodrop 8000 spectrophotometer (Thermo Scientific) was used to assess RNA concentration and purity of striatal samples.

Quantitative PCR

500 ng of total RNA was reversed transcribed to cDNA using SuperScript III reverse transcriptase (Invitrogen, 18080044) according to the manufacturer's instructions. All PCR reactions used TaqMan Fast Advanced Master Mix (Thermo Fisher, 4444556) to a final volume of 10 ul. Quantification of aging markers were performed using TaqMan Gene Expression Assays (Thermo Fisher, 4331182)(*Grin 1* Mm00433790_m1, *Lmnb1* Mm00521949_m1). Briefly, 1 ul of cDNA was amplified using 5 ul of master mix, 0.5 ul of Taqman Gene Expression Assay, and 3.5 ul of water. Each cDNA sample was performed in triplicate on the QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems). The expression of genes were normalized to housekeeping gene *EIF4A2* (Mm01730183_gH). A pooled cDNA sample was run on each plate for normalization of samples between plates. Relative gene expression was determined using the ΔΔCT method by normalizing to the mean value for 3mo WT mice for the naïve study or to WT saline mice for the trial.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism v9 using two-way ANOVA for genotype and age for the naïve study or genotype and treatment for the trial. Tukey's multiple comparison *post hoc* test was used for pairwise comparisons in the naïve study. Pairwise comparisons for the trial were made using Sidak's multiple comparison *post hoc* test. Differences were considered significant for $P < 0.05$.

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