The Role of HAP1 in the Progression of Huntington's Disease in Middle-aged Patients

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Introduction

Huntington's Disease (HD) is a rare genetic disease that causes neurodegeneration. This disease is genetically linked and inherited in a dominant manner, however it affects females more than males[1]. There is currently no mechanism elucidated as to why females are affected more than males. (6) HD is an adult-onset disease, with symptoms starting to present at around 40 to 50 years of age. Interestingly, the severity of HD symptoms increases throughout the generations and the age of onset lowers.(7) While HD typically presents in middle aged adults, there is a low chance of a juvenile onset occurring (<21 years old). HD is caused by an enlarged CAG repeat number in the huntingtin gene (HTT) resulting in a polyglutamine repeat on the protein. The HTT gene on chromosome 4 is responsible for the instructions needed to create the huntingtin protein. Healthy individuals typically present with a repeat number less than 26 while affected individuals have a CAG repeat above 40s.(7) While it is known that the enlarged repeat results in HD, the function and exact disease pathway remains unknown. Research has shown that degeneration begins by affecting voluntary movement. While HTT is expressed cytoplasmically in all neurons throughout the brain, the exact regions of the brain that are affected first remain unknown. However, leading hypotheses predict that HD affects the cerebellum and basal ganglia due to their involvement in motor function.(8)

Research on HD has focused on HTT interacting proteins. This includes Fanconi anemia group D2 (FANCD2) and FANCI associated nuclease 1 (FAN1) and Huntingtin associated protein 1 (HAP1). FAN1 is one of many proteins that interact with HTT, and is what many believe to be the most important huntingtin-interacting protein. FAN1 binds to the polyglutamine repeat on the HTT protein to stabilize the repeat number. Without the presence of FAN1, the CAG repeat increases drastically. (5) This suggests that FAN1 is important for lessening the severity of HD. HAP1 is a protein found in neurons and the digestive system and is integral for neuronal survival. (3) Interestingly, while HAP1 has similar localization and density patterns in human versus monkey brains, the onset of HD drastically differs in humans compared to other primates, raising many questions abouts its true functions. HAP1 is also a HTT binding protein. When FAN1 is knocked out and there is a resulting higher CAG repeat, studies show that HAP1 has a higher binding affinity to HTT. This affinity likely plays a role in the progression and/or severity of HD. However, since HAP1 is found in different cell types throughout the body, it likely has multiple cellular functions.

Researchers remain unsure of the exact disease mechanism of HD and how this pathway can cause issues in the central nervous system. Additionally, it remains unknown as to which parts of the brain are affected and the order in which they are affected. However, levels of HAP1, a commonly found protein in the nervous system, has been shown to be correlated to the time of onset of HD. I hypothesize that HAP1 is the causative protein that when interacting with mutated HTT, begins the onset of the disease mechanism cascade. In order to evaluate my hypothesis, I will analyze data from multiple papers on HD and HAP1 interaction with HTT and compare those results. I hope that the conclusions drawn from this paper will aid in creating a disease mechanism for HD.

Definitions and Literature Review

HD - Huntington's Disease, an autosomal dominant inherited neurodegenerative disease which shows first symptoms at ~40 years old and is fatal 10-30 years after first diagnosis. Onset of HD symptoms starts earlier as the number of generations increases.

HTT - gene that provides instructions for creating the Huntingtin protein.

CAG repeat - Cytosine Adenine Guanine repeats found in HTT. The higher the repeat number, the higher the risk for developing HD.

HAP1 - Huntington's Associated Protein 1, the most studied protein in the HD field and widely hypothesized to be a main catalyst for HD.

FAN1 - FANCI Associated Nuclease 1, a nuclease that plays a role in DNA cross-link repair. This protein has also been found to interact with HAP1.

I used PubMed to begin my initial search finding papers related to HD and the proteins involved, such as HAP1 and FAN1. However, I found that many papers were focused on the genetics of HD and its symptom progression. Thus, I had to narrow down my search to focus on papers that specifically evaluated the relationship and related symptoms between HAP1/FAN1 and HTT (Figure 1).

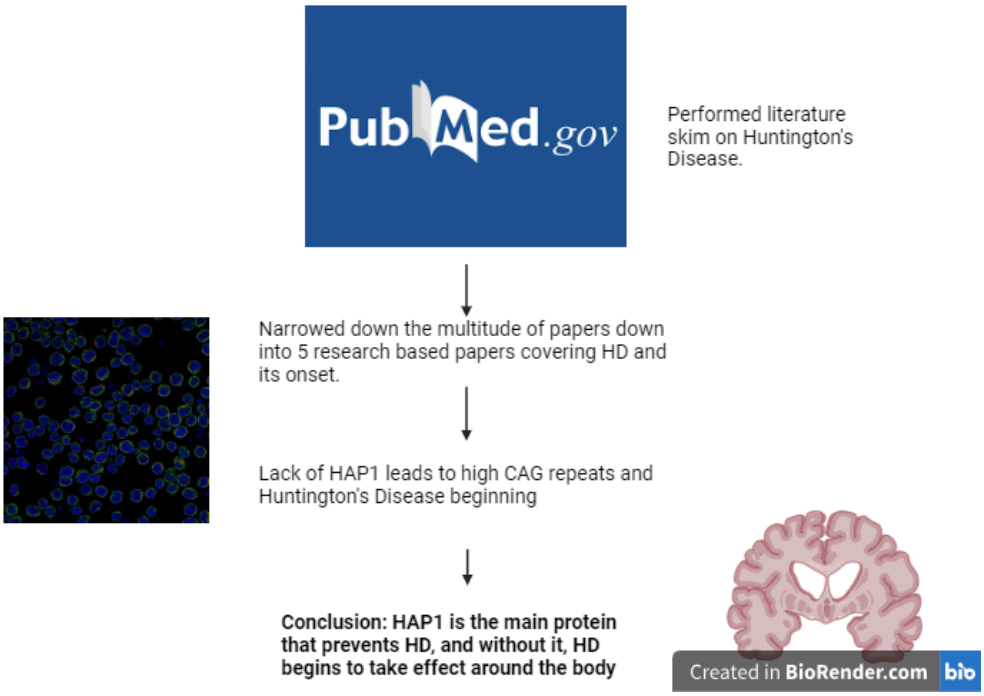

Figure 1. Literature Review

I used Pubmed to research papers involving HD and its onset, then finalized multiple papers into 5 that related specific proteins with their interactions with HTT. Using those papers, I drew a conclusion that HAP1 was the main protein necessary for avoiding HD.

Results/Evidence

HTT is expressed throughout all regions of the brain and specifically, in all neuronal and glial cells. Liu et al. 2023 summarized histological evidence showing HTT expression throughout the different brain regions in primate, human, and rodent brains. This showed that there are

differential expression patterns of HTT in the different brain regions in humans and monkeys compared to rodents[4]. HAP1 is a protein that is predicted to bind to HTT and affect the cell transport through organelles such as the vesicles, which reduces the amount of nutrients taken to certain areas of the brain. HAP1 binding also is hypothesized to reduce the number of repeat expansions in the HTT gene. HAP1 has been widely studied and is hypothesized to play a role in the onset of HD. Interestingly, when mutant HTT is delivered to monkey and rodent models, the distribution of HTT and HAP1 seemingly plays a role in the progression of HD. Expression and localization of HTT and HAP1 in humans and monkeys is similar, and so is their progression of HD. In contrast, rodents have different expression and localization patterns of HAP1 as well as a different disease progression, indicating that HAP1 could contribute a large amount to HD disease progression (Figure 2 A,B). However, which specific regions of the brain are first affected by HD remains unknown.

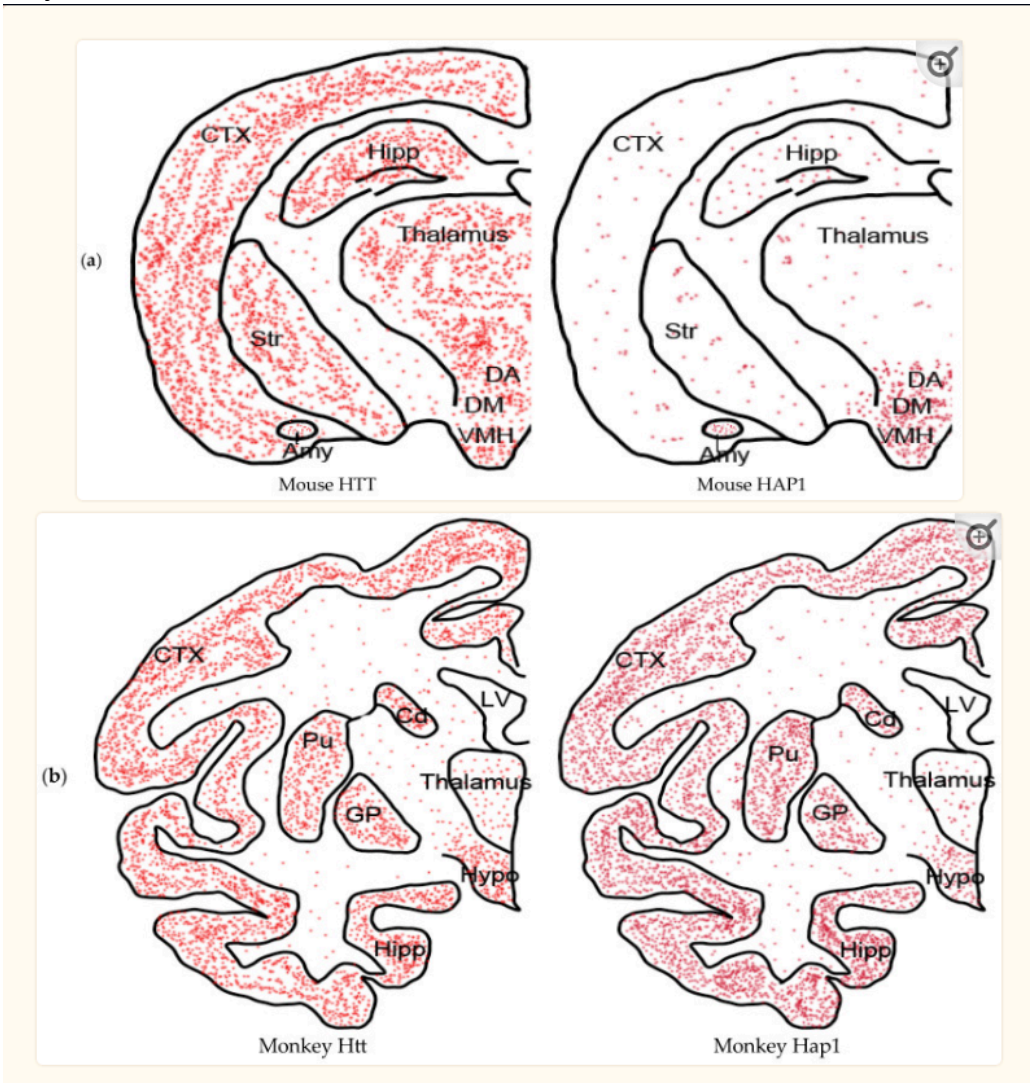

Figure 2. HTT and HAP1 localization in mouse and monkey brains
Images showing the density and localization of HTT and HAP1 in **a)** mouse brains **b)** primate brains. Figure taken from Liu et al, 2023.

Another important gene predicted to be related to HD is FAN1. In Goold et al, the authors hypothesize that a high FAN1 expression is linked to slower progression of HD in affected patients[5]. This was tested by using a transcriptome-wide association study (TWAS) to identify specific genes that alter the age-at-onset (AAO) of HD. The authors analyzed the transcriptome of 452 diseased human dorsolateral prefrontal cortex samples. The results were analyzed for significance using Bonferroni correction statistics. From these results, authors showed that FAN1 transcription levels are highly associated with AAO of HD. The CAG repeat levels in all areas of the CNS and peripheral nervous system were impacted, showing levels of 100+ CAG repeats, which is 60 over the threshold for HD, showing a late-onset case[5]. Further trends suggest a decrease in FAN1 mRNA levels resulting in an earlier onset of HD. From this data, it can be hypothesized that HD patients have a lower concentration of FAN1 in particular brain regions. However, what causes this lower concentration of FAN1 is unknown.

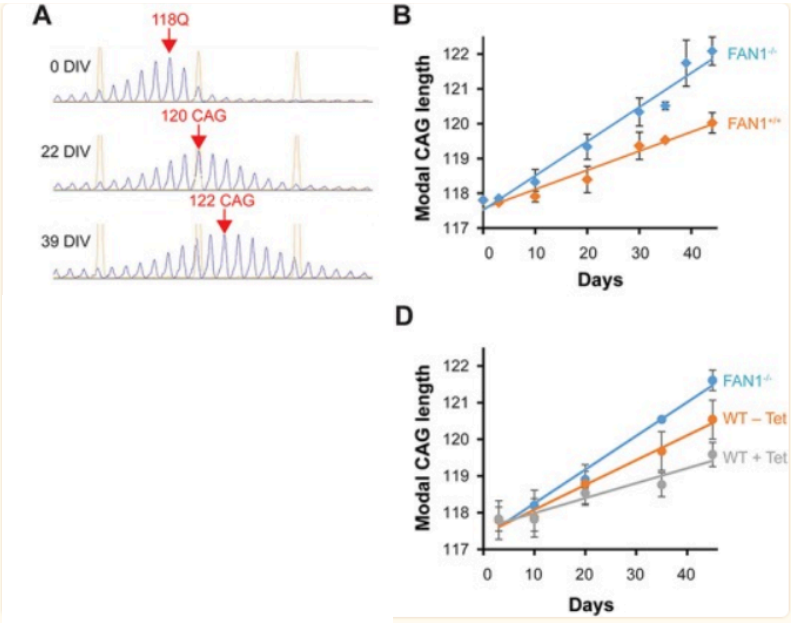

Figure 3. FAN1 expression stabilizes CAG repeat levels
A. Visual representations showing the number of modal CAG repeat levels after cell divisions. CAG repeats greatly increase between 0 and 22 divisions. **B**. Modal CAG lengths are shown over 40 days in cells with (orange) and without FAN1 (blue). Cells lacking FAN1 have a significant increase in modal CAG length. **C.** Modal CAG repeat size in cells expressing exon 1 of HTT. Data of 3 replicants for each $FAN1^{GFP-WT}$ condition and six for $FAN1^{-/-}$ are shown. Cells lacking FAN1 have the longest CAG length and cells with exon 1 of HTT (gray) have the shortest CAG length. Figure taken from Goold et al, 2018.

An increase of CAG repeats causes instability within the 4th chromosome[1]. This instability affects the HTT gene, which likely begins the onset of HD. Pinto et al. performed comprehensive quantitative analyses of CAG expansion of around 50 central nervous system (CNS) and peripheral nervous system postmortem patients to evaluate the different CAG repeat amounts. This was examined by extracting DNA and determining the stability of the 4th chromosome and comparing it to the number of CAG repeats of the subjects. As seen in Figure

3A, cells lacking FAN1 have high CAG repeats after division, possibly contributing to HD. Additionally, cells lacking FAN1 have an increase in CAG lengths compared to cells with FAN 1 (Figure 3B, C). The authors' results on GeneMapper, an automated genotyping program, showed higher CAG repeats in similar parts of the brain, further showing that these higher CAG repeats are likely associated with the onset of HD and the severity of its symptoms (Fig 4A,B). Some of these brain regions presenting with high instability include the accumbens and the putamen, both of which are linked to movement. Involuntary movement is another characteristic symptom of HD, further supporting the authors' hypothesis that high CAG repeats are linked to a faster onset of HD.

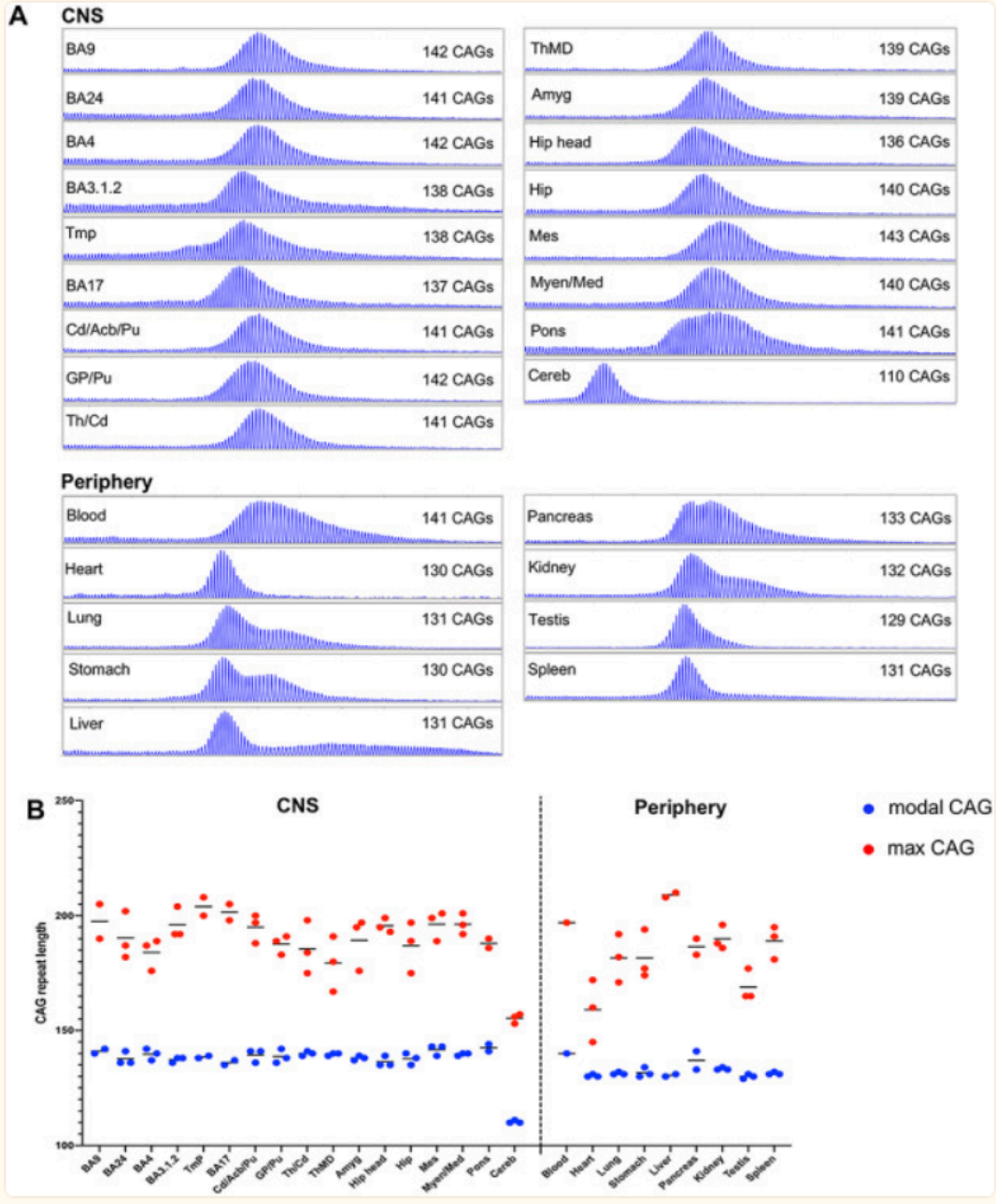

Figure 4. CAG Repeat levels in juvenile-onset HD

A. GeneMapper results from expanded allele HTT products from areas in the nervous system. Cells split up into CNS and PNS locations, repeat number shown for each. **B**. Modal (blue) and maximum (red) CAG repeats plotted to compare CAG repeat lengths between different cells in the CNS and PNS. Figure taken from Pinto et al, 2020.

Calcium release channels in the CNS play an important role in neuronal signaling and dysfunction of these channels is associated with HD. Tang et al. identified a calcium release channel in neurons, $InsP_3R1$, that complexes with HAP1A and HTT. The authors found that mutated HTT activates $InsP_3R1$ in the lipid bilayer of medium spiny striatal neurons promoting erroneous release of Ca^{2+}. This signaling is hypothesized to quicken cell death within these medium spiny striatal neurons. This theory was tested by studying mice that had targeted disruption of both HAP1 alleles and dissecting the striata from the brains to determine how mutant HTT interacts with different proteins like $InsP_3R1$ in the brain. They found that HAP1 plays a role, but is not necessary, for the interaction between HTT and $InsP_3R1$ and subsequent Ca^{2+} release. Even in mouse cells without HAP1, the $InsP_3R1$ levels were unaltered, showing HAP1 is not necessary for the interaction between HTT and $InsP_3R1$ (Fig. 5). If the CAG repeat in HTT is long enough, HTT can bind to $InsP_3R1$ without needing HAP1. In HD cases, there is a significant lack of HAP1 within neurons and a large CAG repeat, resulting in excess HTT $InsP_3R1$ binding but not Ca^{2+} release. HAP1 is necessary to alter basal Ca^{2+} levels, even with HTT genes with large expansions. Interestingly, it is hypothesized that erroneous Ca^{2+} levels could be the earliest symptom of HD, supporting HAP1 as a pivotal protein in the onset of HD. Even with these promising results the mechanism for what causes neuronal death in HD patients is still largely unknown and is mostly speculatory as many proteins may be contributing and no disease mechanism has been elucidated.

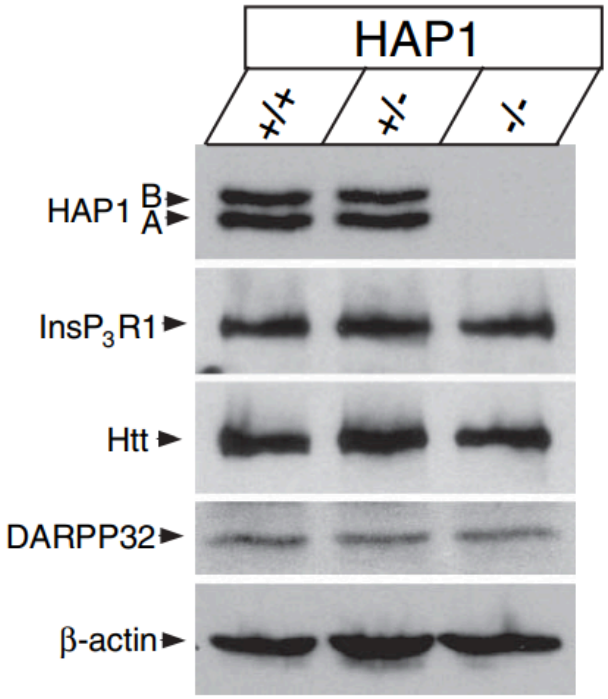

Figure 5. Western Blotting of cultured mice medium spiny neurons (MSNs).

16 MSNs from different HAP1 variants of mice (HAP1 -/-, HAP1 -/+, HAP1+/+) were collected and blots were performed to determine protein concentrations of HAP1, $InsP_3R1$, Htt, DARPP32, and B-actin (control). Figure taken from Tang et al, 2004.

Discussion

Most evidence points to the presence of HD stemming from mutations of certain proteins. Many proteins seem to be attributed to this, such as FAN1 and HAP1. Altered levels of these proteins can cause changing levels of CAG repeats within the Huntingtin gene, which is known to cause HD symptoms. Liu et al. showed that HAP1 was necessary to maintain the number of CAG repeats within HTT. Thus, a decrease or knockout of HAP1 would result in an increase in the number of CAG repeats, implicating it as a potentially HD-causing gene. Similarly, FAN1 has been associated with a slower onset of HD in patients, suggesting a disease mechanism between FAN1 and HAP1 working together to restrict HD from forming. Tang et al. showed that mutated HTT also binds to calcium release channel $InsP_3R1$, causing unnecessary calcium to be released into cells, resulting in cell death. Excessive cell death will eventually result in organismal death. Interestingly, HD patients present with high Ca^{2+} levels at the beginning of disease onset, which suggests that elevated CA^{2+} levels are an early symptom of HD. HAP1 is also responsible for the interaction between $InsP_3R1$ and HTT. HAP1 seems to be related to most of the relationships between HD and its symptoms. This prevalence leads to the conclusion that HAP1 is the most important protein involved in preventing HD and its onset. This research could be used to possibly slow down HD by theoretically adding new HAP1 proteins into the CNS, thus balancing the CAG repeats. However, it will likely be quite a while until technology makes this idea a reality.

References

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