

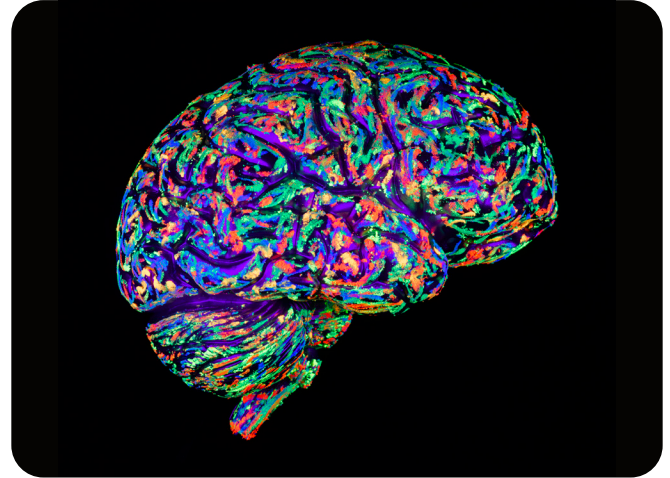
Huntington's disease therapeutic research

Selective targeting of mHTT presents novel therapeutic approach to Huntington's disease

Huntington's disease (HD) is a fatal monogenic, autosomal dominant neurodegenerative disorder that affects around 30,000 people in the US, with another 200,000 at risk of developing the condition. The disease is characterized by progressive motor, psychiatric, and cognitive impairments, with symptoms commonly developing between ages 30 and 50. The disease progresses slowly, and a person may live for another 15-20 years after the onset of symptoms.

In 1993, the causal mutation of HD was mapped to an expanded CAG repeat within exon 1 of the huntingtin gene (*HTT*)¹ that exceeds 35 units. Affected individuals produce an altered form of the huntingtin protein (mHTT), which in turn causes gradually damaging effects to neurons. The onset and severity of disease has been shown to correlate with the number of CAG repeats.

Despite extensive research into HD, there are currently no approved disease-modifying therapies available to slow or halt disease progression. Dr. Ignacio Muñoz-Sanjuan, VP Biology at CHDI Foundation and President at [Factor-H](#), believes the ideal therapeutic will be one that completely eliminates the expression of mHTT without interfering with normal *HTT* function. "Many in the field think that maintaining a certain level of *HTT* expression is needed for normal brain function," he explained. "Therefore, we must strive for lowering mHTT as much as we can to have a therapeutic effect without lowering too much of the normal *HTT* protein."



Allele-selective transcriptional repression of mHTT

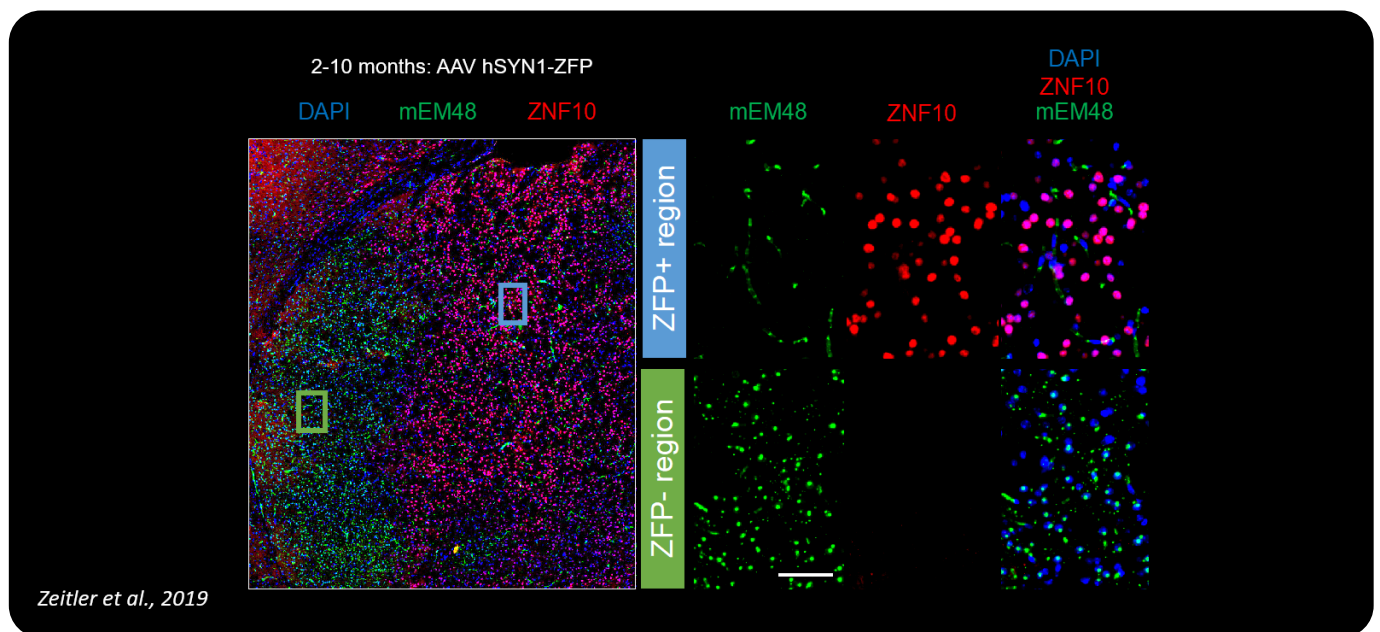
Since joining CHDI Foundation, Dr. Muñoz-Sanjuan has been exploring technologies that would achieve allele selectivity and lower mHTT expression through binding and modulating the expanded CAG repeats that are characteristic of HD. One technology that caught his attention was zinc finger proteins (ZFPs) – a class of DNA-binding proteins that recognize specific DNA target sequences through non-covalent interactions of each zinc finger motif with three or four base pairs. Researchers can engineer ZFPs to bind to virtually any DNA sequence, including CAG, thereby targeting specific locations in the genome. "The fact that the CAG tract in the *HTT* gene is located in exon 1 suggested to me that ZFP repressors might work well, as the transcriptional start site in *HTT* is close to the mutation," said Dr. Muñoz-Sanjuan. "I identified Sangamo Therapeutics as a leading organization that had technology valuable to the mission of CHDI Foundation and approached them with the goal of directing some of their efforts to this problem."

"The Zinc Finger platform provides an especially attractive therapeutic approach for allele-selective targeting of *mHTT*," said Dr. Zeitler, Director of Gene Regulation at Sangamo Therapeutics and first author of the study. "Sangamo's engineered ZFP-TFs are derived from endogenous human transcription factors and can achieve high potency, excellent genome-wide specificity, and durable activity following a single administration. Our strategy was to design a large number of potential ZFP-TF solutions using different CAG recognition architectures and binding affinities. This proved to be particularly important for achieving robust allele selectivity."

Together, the team worked to engineer ZFP transcription factors (ZFP-TFs) to target the pathogenic CAG repeat, with the aim of selectively lowering *mHTT* as a therapeutic strategy for HD. For their study,² 41 distinct poly-CAG-targeted ZFPs were first screened for selective repression of *mHTT* in patient fibroblasts. Of these, three were chosen for further study in neural stem cells and neurons differentiated from embryonic stem cells. The team's analysis revealed that these ZFP-TFs selectively repressed more than 99% of HD-causing alleles over a wide dose range while discriminating against at least 86% of normal *HTT* alleles. Microarray analysis (n = 18,149 genes queried) also revealed limited levels of off-target gene repression.

To further confirm selective *mHTT* repression, the researchers tested whether one of the selected ZFPs (ZFP-B) could reduce *mHTT* *in vivo* using het Q50 (HdhQ50/Hdh+) mice. Although this model is phenotypically normal, the authors note that it harbors a *mHTT* allele (CAG48) that is typical of patients with HD and equivalent in length to that tested in human neurons. AAV ZFP-B or GFP was administered by intrastriatal injection to 11-week-old Q50 het mice at two doses. The team reports that after seven weeks, Q50 transcripts were reduced by 55% (low dose) and 67% (high dose), whereas endogenous mouse *HTT* remained unchanged. "This is an important result because it demonstrated that the allele selectivity we screened for in our *in vitro* models translates to a therapeutically relevant *mHTT* allele *in vivo*," said Dr. Zeitler.

The researchers then used a slow-progressing zQ175 knock-in model (~188 CAG repeats) to examine whether ZFP-mediated *mHTT* repression could improve neuropathological deficits in mice. EM48 immunoreactivity was used to monitor *mHTT* inclusions in cohorts treated before (two months) or after (six months) neuropathological onset. Subsequent aggregation was evaluated using high-content imaging at four and 10 months, respectively (Figure 1). Analysis revealed that ZFPs resulted in significant improvements in neuropathological deficits in the zQ175 het mice following treatment, as observed using bAP protocols (Figure 2).



Zeitler et al., 2019

Figure 1: Taken from the On Demand Nature Webinar: [Advances in neurological rare disease therapeutic research](#) (June 2021). *mHTT* inclusion (mEM48) were significantly decreased with treatment of ZFP-B delivered via intrastriatal AAV injections (ZNF10) in regions with high AAV distribution compared to regions without.

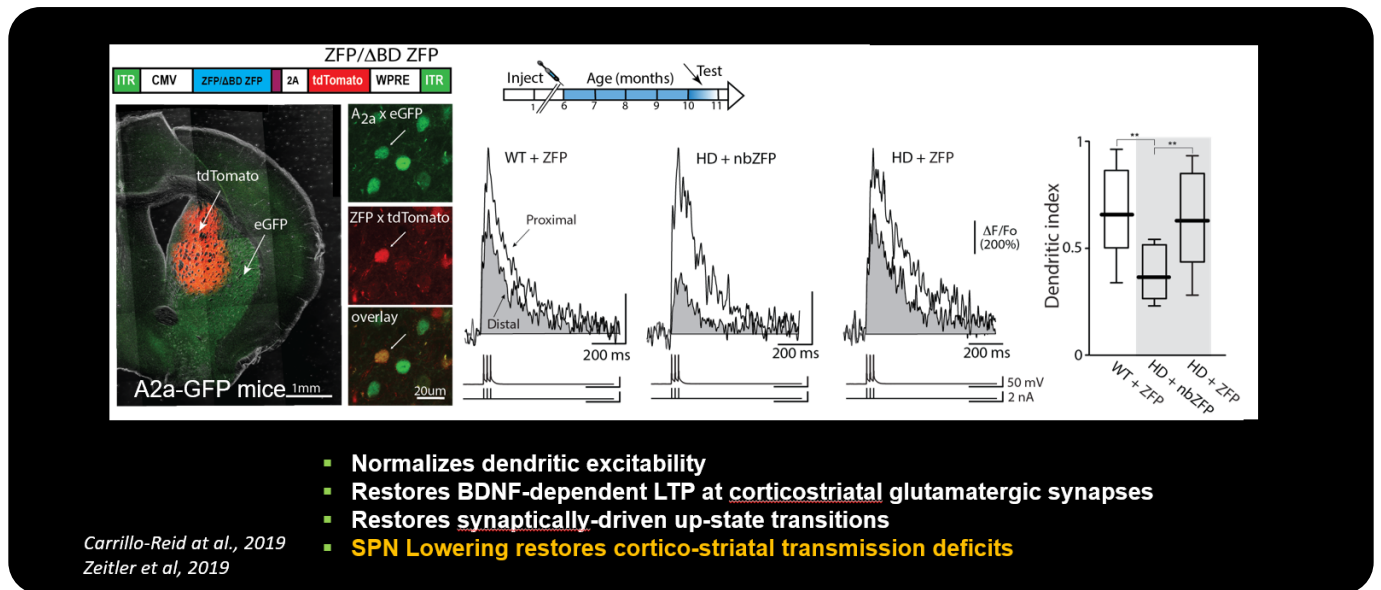


Figure 2: Taken from the On Demand Nature Webinar: [Advances in neurological rare disease therapeutic research](#) (June 2021). ZFP-mediated mHTT suppression restores iSPN alterations to near WT levels assessed via Ca²⁺ imaging from a simplified bAP (back-propagating action potential) burst protocol.

Finally, to determine whether allele-selective ZFP-TFs could prevent and reverse the loss of translational markers of HD progression, the researchers used *in vitro* autoradiography (ARG) of striatal brain sections and longitudinal imaging in live animals. zQ175 het mice were either injected at two months and analyzed at six months (early) or injected at four months and analyzed at 10 months (late). The team demonstrated restoration of imaging biomarkers in both the early and late models.

Commenting on their findings, the researchers say these results indicate that a one-time striatal AAV-ZFP infusion can correct histopathological, electrophysiological, and biomarker deficits that are characteristic of human HD pathology. “The ability to modulate mHTT expression selectively, in specific cells and circuits, as animals age and disease progresses, has enabled us to understand what disease markers respond to the intervention and whether core features of the disease can be halted, or altogether reversed, after mHTT suppression,” added Dr. Muñoz-Sanjuan.

Implications for Huntington’s research

The collaboration between CHDI Foundation and Sangamo proved to be a success, and the program was licensed to Shire for additional preclinical and clinical development. “Currently, Takeda is driving the IND enabling studies to bring this therapy to the clinic,

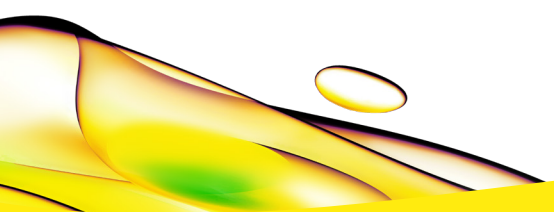
after they acquired Shire in 2019. CHDI continues to work with Takeda to enable their efforts and to see this program get to patients,” said Dr. Muñoz-Sanjuan. One challenge for the researchers, which still remains unsolved, relates to distribution. “Like other gene therapy agents, the delivery of DNA-based therapies requires AAV viruses delivered directly into brain parenchyma via neurosurgery. These have a restricted distribution depending on the viral vector used. Whether this is a serious issue in the context of clinical efficacy remains to be investigated,” he said.

In addition to ZFPs, several other modalities are currently being explored to treat neurodegeneration at the level of the DNA, including CRISPR-mediated *HTT* deletion. However, according to Dr. Muñoz-Sanjuan, the realization of CRISPR-based editing for brain disorders is still pending. “The complication for HD is the fact that targeting both alleles of *HTT* might lead to untoward side effects; therefore, companies need to develop a gene editing strategy that either deletes the mutant allele specifically, or that leads to contractions in the CAG repeat. This is being explored academically, but not to my knowledge as a therapeutic program.” He added that the ZFP transcriptional repressors are the most advanced for the treatment of HD at present. “All other molecular therapies target the mRNA, not the DNA,” he said. “The unique advantage of the current ZFP program is its allele specificity - they only modulate transcription from the mutant allele, leaving the unexpanded normal allele untouched.”

Only one other therapeutic program aims to decrease mHTT expression selectively. This uses antisense oligonucleotide (ASO) therapeutic agents that can decrease expression of the mutant allele through specific sequence differences (single nucleotide polymorphisms [SNPs]). "The issue with these therapies is that only a subset of patients with HD would benefit from these therapeutic agents, as the frequency of the SNPs associated with the mutant allele vary, and only about 20 - 40% of all subjects of European descent have the SNPs being targeted," asserted Dr. Muñoz-Sanjuan, adding that the first two ASOs targeting mHTT via two distinct SNPs were stopped in Phase 1/2a due to lack of a sufficient pharmacodynamic response. "At the moment, the ZFP program is the only program that would target mHTT expression in the majority of HD patients, because it recognizes expanded CAG repeats," he concluded.

References

1. Macdonald M et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. 1993;72(6):971-983.
2. Zeitler B, Froelich S, Marlen K, Shivak D, Yu Q, Li D et al. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington's disease. *Nature Medicine*. 2019;25(7):1131-1142.



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