Targeting DNA Mismatch Repair as a Potential Therapeutic Strategy for Huntington's Disease

Huntington's disease (HD) is a devastating and fatal neurodegenerative disorder affecting movement, thinking, and behavior. In the United States alone, there are approximately 40,000 people with symptomatic Huntington's disease, and more than 200,000 are at risk of inheriting it. The fatal disease often leaves a devastating family legacy, as a child of someone with the HD mutation has a 50% chance of inheriting it.

While everyone has the Huntingtin gene (*HTT*), in those with the disease, the C-A-G codons surpass the standard 15-35 repeat length, expanding to more than 40. In someone with Huntington's, this three-letter sequence undergoes somatic instability over time — a phenomenon in which the expanded region of CAG repeats increases even further in specific brain cells (known as somatic expansion). The sequence eventually surpasses 150 repeats and triggers cell death, causing patients to lose neurons in brain areas responsible for movement control, motor skill learning, language, and cognitive function. This process doesn't occur in all cells and is more common in medium spiny neurons (MSN).

Patients usually live 15 to 20 years after diagnosis and currently, no cure or therapy can slow or reverse the disease. Somatic instability has long been a focus in the field (1, 2), and recent research may expand the field's knowledge with additional insight into the progression of somatic expansion and what genes could be targeted to slow the disease's progression.

New research published in Cell centers on how the triple repeat expansion mutation causes Huntington's disease and provides insight into what avenues researchers could focus on to delay the disease (3). The study — led by a team of researchers from the Broad Institute, Harvard Medical School, and McLean Hospital — also tries to link somatic instability to individual cells and demonstrates the

timing of individual cell vulnerability as the pernicious mutation gradually morphs into a toxic form that causes specific brain cells to die.

The researchers, co-led by Steve McCarroll, PhD and Sabina Berretta, MD, focused on how the mutated HTT undergoes somatic expansion. Notably, once the repeats reach 80, which would typically take decades, a tipping point occurs, and the buildup of codons accelerates, reducing the time it takes to reach 150 repeats from decades to a matter of years. After that, the expansion to hundreds of CAG repeats could happen within months.

This expansion is typical when the symptoms of the disease appear, which is generally between the ages of thirty and fifty, and include difficulty with thinking and planning, lack of impulse control that can result in outbursts, involuntary movements (chorea), and impairment of voluntary movements, making work, communication, and daily activities extremely challenging. The symptoms worsen over time, often resulting in dementia and the inability to walk, speak, or feed oneself.

The authors describe their data as an armadillo-shaped curve, with the CAG lengths of most MSNs falling under the curved body part of the animal in the earlier decades of life and moving to the long, flat tail of the armadillo later in life. The researchers explain that over decades, the unstable alleles expand to more than ten times their initial length.

The authors conclude that the therapeutic implications are the most essential aspect of their findings, noting that almost all advanced clinical developments for Huntington's disease focus on reducing the levels of the mutated *HTT* gene. However, this may just be one approach that can be taken and, as demonstrated, there is another mechanism that may be considered. First, at any time, very few MSNs may have a toxic HTT protein that would benefit from being lowered, and second, once the MSN becomes toxic and would benefit from HTT lowering, its expected lifespan may only be months (3).

"In short, HTT-directed therapeutic efforts will need to address the possibility that HTT toxicity is brief, asynchronous, and intense rather than long, synchronous, and indolent," the authors state (3).

Instead, the authors suggest developing a treatment that stops or slows CAG-repeat expansion in the *HTT* gene, which could delay toxicity in a larger number of cells and postpone or prevent the onset of the disease (3).

"A lot was known about Huntington's disease before we started this work, but there were gaps and inconsistencies in our collective understanding," said Bob Handsaker, of Broad Institute, and co-author of the study. "We've been able to piece together the full trajectory of the pathology as it unfolds over decades in individual neurons, and that gives us potentially many different time points at which we can intervene therapeutically."

However, more work supporting this hypothesis will be needed to determine if it can be replicated and stand the test of time.

MSH3: a mismatch repair protein that could delay or halt the disease

A new study published in Science Translational Medicine grows the field's understanding of how the three-letter repeat expands over time and areas that could be potential targets to slow or stop the disease (4).

The research, led by Professor Sarah Tabrizi of University College London, in collaboration with Professor Gabriel Balmus of Cambridge University and others, focused on genes involved in cell DNA repair systems, specifically *MSH3*, which codes for a mismatch repair protein that is designed to correct DNA errors. While its role is to repair, evidence from multiple mouse and human cell models demonstrates that in its attempt to fix the abnormal structures formed by long CAG repeats, it inadvertently expands the repeat tract, making the disease worse (4). While models have shown that lowering MSH3 reduces repeat expansion — making it an ideal therapeutic target — the research team set out to determine the degree to which MSH3 reduction would influence disease progression (4).

"Establishing the degree of MSH3 reduction required for therapeutic benefit is vital to inform future clinical development strategies in patients," the authors state. To explore this, the researchers used medium spiny neurons taken from a person with Huntington's. The neurons were treated with an antisense oligonucleotide (ASO), which binds to *MSH3* RNA and degrades it before it can be translated into a protein, thus lowering MSH3 levels (4).

The researchers found a dose-dependent relationship between reducing the MSH3 levels and the CAG repeat expansion in the Huntingtin gene. Their results found that lowering MSH3 by 41% halved the expansion rate, and lowering it by 83% was estimated to completely halt expansion (4). Additionally, they showed that lowering MSH3 did not interfere with DNA repair pathways or activate cancer signaling pathways.

"MSH3 ASO treatment reduced MSH3 abundance in all cell clusters, suggesting that the ASO was taken up successfully by all cell types within the culture," the researchers state. "Moreover, the broader effects of ASO treatment did not appear to be cell type specific" (4).

To further test the safety and efficacy of the drug, the team developed a mouse model with the human *MSH3* gene and injected their brains with either the MSH3-ASO or a nonspecific ASO. After two weeks, MSH3 expression had dropped throughout several areas, with the highest ASO dose dropping MSH3 by 77% in the brain stem, 74% in the spinal cord, 49% in the cortex, and 46% in the striatum (2). Notably, they found that MSH3-deficient mice are not more tumor-prone than their wild-type counterparts and showed a normal lifespan (4).

"Targeting MSH3 is exciting not only because it's directly involved in the CAG repeat expansion, but also because genetic studies suggest that loss of MSH3 function is relatively well-tolerated in humans," Tabrizi said in a recent article. "Our findings emphasize the potential for MSH3 suppression as a safe and effective way to delay Huntington's disease."

The findings complement another recent study published in Cell, led by Dr. X. William Yang of UCLA, which showed that a distinct subset of mismatch repair genes (*MSH3* and *PMS1*) are key drivers of Huntington's disease and how the disease impacts specific neurons (5).

To start, Yang and his colleagues used Huntington's disease model mice with 140 CAG repeats to observe the features of the disease. The researchers questioned if altering nine HD modifier genes — DNA variants that could speed up or slow the disease onset — could change any disease phenotypes. The researchers found that *MSH3* and *PMS1* — another mismatch repair gene — sped up the CAG-repeat expansion rate in vulnerable neurons and that targeting these genes could improve deficits caused by the disease (5).

"We were surprised to see the potent and sustained effects of targeting these mismatch repair genes in HD mice — the benefit lasts up to 20 months of age in a mouse, which would be comparable to about 60 years in humans," said Yang. "Our study suggests that these genes are not just disease modifiers, as suggested by the previous studies, but are genetic drivers of Huntington's disease."

Additional efforts targeting MSH3

While these recent studies highlight the role MSH3 has in driving the disease, previous research has targeted the protein in mouse models.

A 2023 paper published in Molecular Therapy demonstrated that silencing *MSH3* with a single dose of divalent short interfering RNA (siRNA) blocks somatic repeat expansion for up to four months in mouse models of Huntington's Disease (6). The study notes that somatic repeat expansion is a key feature of other trinucleotide repeat disorders, meaning the findings could present promising treatment methods for patients with Huntington's and other diseases with the repeat (6).

A 2024 study in Nature also used divalent siRNA to silence mouse *MSH3* mRNA expression. The study results support MSH3 as a method to limit somatic expansion, noting that a complete termination of somatic expansions would require almost 100% reduction in MSH3 levels, which may not be achievable in humans. However, the authors state that reducing MSH3 levels by 50% could lead to a corresponding 50% decrease in somatic instability (7). The study also states that despite a 75% reduction in MSH3 protein levels, striatal nuclear HTT aggregates remained unchanged (7).

"Thus, our data suggests that pharmacological reduction of MSH3 activity can effectively prevent further expansions but may not significantly reduce existing expansions" (7).

Beyond MSH3, teams are also looking at other parts of the DNA mismatch machinery (including MSH2, MLH1, or PMS1), as well as additional triplet repeat disorders (8, 9). Skyhawk Therapeutics, Rgenta, LoQus23 Therapeutics, and Harness Therapeutics are among those working on this as part of their therapeutic mechanisms.

Skyhawk Therapeutics has developed an oral small-molecule RNA splicing modifier which is in phase 1 trials. It interacts with the splicing machinery to introduce pseudoexons in *PMS1* and *mHTT* transcripts, leading to downregulation by nonsense-mediated decay (9).

Rgenta has designed a small molecule that targets *PMS1* RNA, reducing its expression, which causes a reduction in PMS1 protein levels, halting repeat expansion.

LoQus23 Therapeutics is developing small-molecule allosteric inhibitors of the MutSβ complex (MSH2–MSH3) and has "spent several years identifying allosteric inhibitors that potently bind sites outside MSH3's ATP pocket with high selectivity" (9). The company plans to initiate Investigational New Drug application-enabling studies later this year.

Harness Therapeutics is targeting *FAN1*, a DNA repair enzyme shown to retard somatic expansion, and therapeutic intervention of this target requires upregulation. They are "developing an antisense oligonucleotide (ASO) modality that boosts *FAN1* expression by blocking inhibitory microRNA binding sites at the 3' end of the gene" (9).

Relieving suffering: the future of Huntington's Disease treatment

Described as a simultaneous combination of Alzheimer's disease, Amyotrophic lateral sclerosis, and Parkinson's disease, Huntington's has a devastating impact on families affected by it. While previous clinical trials have focused on targeting the mutated gene, new research has unveiled promising targets for future therapies to focus on. Not only do the studies highlight that targeting *MSH3* significantly slows the progression of Huntington's disease, the research also provides insight into how the disease progresses. As Sabina Berretta, MD, of Harvard Medical School and McLean Hospital states, "the point of our work — what we all do — is relieving suffering caused by disease."

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