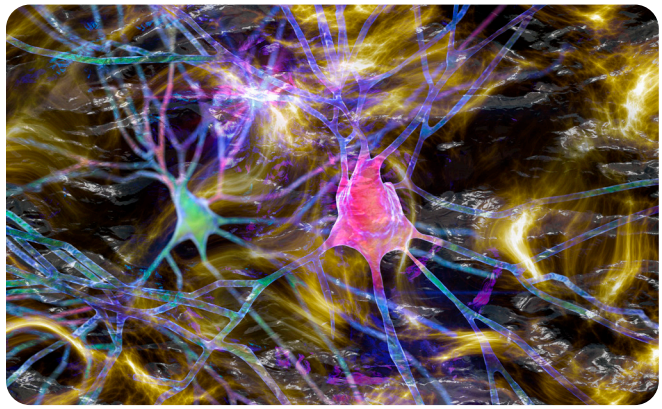
WHITE PAPER

# Huntington's disease: Progress and future perspectives

## An Exploration of biomarkers, somatic instability, and collaborative enablement

Huntington's disease (HD) is a rare, adult-onset, autosomal dominant, progressive neurodegenerative disease caused by an expansion of the CAG trinucleotide sequence in the HD-associated gene, huntingtin (HTT). The resulting mutant huntingtin (mHTT) protein disrupts many cellular processes, ultimately leading to neurodegeneration. Symptoms include loss of motor control, altered personality, and a decline of cognitive function, which typically manifest between 30 and 45 years of age.

HTT was first mapped to human chromosome 4 in 1983 and cloned 10 years later.[1,2] In the 30 years since, researchers have strived to fully understand the molecular drivers of disease onset and progression. Intensive research has provided substantial insight into the pathobiology of HD, culminating in the identification of a multitude of rational targets for therapeutic development. As a result, clinical trials for the treatment of HD have substantially increased whilst the number of recruited participants has grown enormously. Yet despite intensive work there are still no disease-modifying therapeutics for HD. In this article, Dr. Robert Pacifici, Chief Scientific Officer at CHDI Foundation, and Dr. Christian Landles, Senior Research Associate at University College of London, provide insight into recent scientific breakthroughs and expound on the therapeutic approaches currently being pursued by the HD research community.



## Notable breakthroughs in HD research

**HTT-lowering therapies**

One therapeutic approach that has gained significant progress is in the development of agents that target HTT DNA or RNA, with the aim of lowering mHTT levels. Agents acting at the DNA level include zinc finger proteins (ZFPs) and CRISPR-Cas9, whilst antisense oligonucleotides (ASOs) and RNA-interference (RNAi) act at the RNA level. "With a monogenic disease like HD, which has 100% penetrance, it makes sense to remove the insult and hope that it improves the trajectory of disease," explained Dr. Pacifici. However, the risk-benefit of lowering either mHTT or wild-type HTT is a question that is currently being addressed with on-going clinical trials.

In 2021, hopes for HTT-lowering therapies were dealt a significant blow after two pharmaceutical companies announced they were halting their clinical trials of gene-targeting ASO therapeutics for HD.[3,4] "There was a worry that those disappointments would pull the rug out from underneath HTT-lowering as a viable therapeutic

revvity

strategy for HD," said Dr. Pacifici. "But I would convincingly say that's not the case, as evidenced by one company planning to reactivate their trial and the participation of others leveraging their small molecule HTT-lowering agents and gene therapy. I'm very impressed with the breadth, depth, and diversity of HTT-lowering programs that should help us triangulate on whether or not this is a viable mechanistic strategy and what the minimum necessary and sufficient criteria will be for a target product profile."

Dr. Landles agrees: "I have great confidence that this set-back can be overcome. First, this has already initiated much debate as to which types of therapeutic agents are most suited, for example ASOs, RNAi, ZPFs, or CRISPR-Cas9. Second, we are reassessing our strategies to lower only the mutant/cytotoxic isoforms of HTT, as we still do not fully understand if the long-term suppression of the wild-type allele is safe. Third, we have re-evaluated at what levels these isoforms can be safely lowered without causing adverse effects." He added that hypotheses continue to be explored and validated in HD, and interest is still pertinent in the identification of potential small molecule drug targets. Take the small molecule drug, branaplam (LMI070), as an example, which was originally developed as an RNA splicing modulator for the treatment of spinal muscular atrophy (SMA). "Intriguingly, in the process of clinically testing branaplam in SMA, it was also observed that this investigational therapy lowered the levels of the HTT mRNA by a similar mechanism," said Dr. Landles. "With much delight it has now been granted fast track status by the FDA to launch a Phase II clinical branaplam trial to premanifest HD patients."[5]

**Understanding somatic instability**

Another notable development in the field is an improved understanding of somatic instability, a phenomenon whereby the expanded region of CAG repeats in the mHTT gene expands further with time. "The concept of somatic instability has been established and recapitulated in many preclinical mouse models of HD and was already known to be abrogated in the absence of some key DNA mismatch repair genes," explained Dr. Landles. "But recently, the identification of DNA repair genes, namely *FAN1, MSH3, MLH1, PMS1, PMS2, LIG1*, as modifiers of the age-of- onset and/or progression of HD through several genome-wide and/or transcriptome-wide association studies has not only underlined another causative role for somatic CAG instability in the pathogenesis of HD, but could indicate that the pathogenic repeat threshold in brain cells might be much greater than that measured in patient blood and, if correct, such a threshold in the brain remains unknown." Dr. Landles added that any process that increases CAG repeat length could have calamitous consequences for disease prognosis. "Thus, although the HD community has made great breakthroughs in our understandings of the pathogenic consequence associated with somatic CAG expansions of *HTT*, only with further research will this provide us with the mechanistic answers to understand how this pathogenic process contributes to disease."

**Improving the characterization and detection of HD biomarkers**

Although well characterized clinical, cognitive, and neuroimaging biomarkers of HD progression have been established, to date, very few biochemical markers have been identified and, until recently, no predictive biomarkers for assessing neuronal injury, disease progression, or therapeutic responses had been validated. This, according to Dr. Landles, confounds the ability of the HD community to test novel therapeutics. "The development of accessible and trustworthy biochemical markers not only allows us to track disease onset and progression more accurately, but greatly facilitates the development of novel therapeutics for HD," he said.

Blood biomarkers are especially appealing to researchers, in part because patient cerebrospinal fluid is difficult and expensive to obtain, but also because they provide a quick, accessible, non-invasive, unbiased, and reproducible way of quantifying biomarkers of disease progression. Recently, neurofilament light (NfL) has emerged as a potential biofluid marker for neurodegenerative disorders, including HD,[6] due to its exclusively neuronal expression, and release into the extracellular space following axonal degeneration or neuronal damage. "Through recent advancements in ultrasensitive immunoassays, this has enabled the quantification of NfL from blood plasma and/or serum," said Dr. Landles. "For the HD community, this observation has been revolutionary. With the identification and validation of NfL as a suitable biomarker, which can be measured in HD patient blood to predict onset, report current disease status, and track the progression of HD in the brain, this could profoundly help us in the development and assessment of any successful therapeutic treatments for HD.

There is also a need to improve the characterization and detection of HTT protein biomarkers to not only better understand the molecular pathogenesis of HD, but also to ensure that the levels of all soluble and aggregated

isoforms of the HTT protein can be measured. This would also help with tracking how HTT isoforms change in relation to disease onset and progression and assessing the impact of potential therapeutic interventions in preclinical studies and clinical trials. "A plethora of novel assays have recently been established and developed to address this need, which when used in combination with those already in existence, provide a toolkit to track total soluble mutant HTT protein, soluble exon 1 HTT protein, soluble mutant HTT protein (excluding the exon 1 HTT protein), and total soluble full- length HTT protein (mutant and wild type)," said Dr. Landles. In addition, several novel aggregation assays have been developed that track with disease progression. "Alongside other HD biomarkers, these selected assays can now be used to compare the relative levels of HTT protein isoforms in a wide variety of preclinical studies of HD and to determine how these change in response to genetic or therapeutic manipulations."[7]

## Collaborating for success

The progress and momentum seen in HD research in recent years has been facilitated in part by advances in technology, automation, and artificial intelligence as well as increased collaboration from an amalgamation of research disciplines. For example, CHDI Foundation annually hosts a conference that brings together neurobiologists, clinicians, medicinal chemists, and translational scientists.[8] Furthermore, data repositories such as "HDinHD" encourage the sharing of HD-related primary scientific data, analyses, and computational models, providing a vast amount of freely available data for the community to analyze, compare, and explore.[9] Dr. Landles says he has observed progressively more and more optimistic data coming out from the HD research community over the past decade. "Our combined approaches seem to be culminating in the right direction for the eventual successful therapeutic treatment of HD in patients."

Not only has the community become more proficient at data sharing, observational studies such as Enroll-HD,[10] which is the world's largest prospective registry for HD, have augmented researchers' capabilities to objectively study HD. Enroll-HD also shares all of its clinical phenotypic data gathered within the longitudinal observational study to any legitimate researcher, which makes the associated biosamples - such as CSF, plasma, PBMCs, and others - invaluable for research. Any invested scientist can access this data at www.enroll-hd.org/ for-researchers.

Currently, the study has enrolled over 21,000 participants spread across 22 nations. "The aspiration is to know where every gene expansion carrier is and to have them well characterized," explained Dr. Pacifici. "It has been incredibly powerful to collect biological samples from individuals who are incredibly well phenotyped. We then have matched plasma, CSF, and PBMCs and can use those reagents for drug discovery." The Enroll-HD cohort also supports other studies, such as Origin-HD,[11] which is a proposed nested cross-sectional observational study to investigate differences in germline and somatic HTT CAG-repeat instability and identify genetic modifiers of intergenerational CAG instability. "A whole new category of investigation has been opened up thanks to the participation of these individuals and their families," said Dr. Pacifici.

## Driving research forward

To help bridge the gap between academic and industrial research pursuits, organizations like CHDI Foundation have become critical players in the HD drug discovery and development landscape by encouraging scientific collaboration and providing funding to researchers around the world. "We all know drug discovery is difficult, time consuming, and expensive, and CHDI tries to fill the gaps when resources are needed to make sure research continuously moves forward," said Dr. Pacifici. "We try to bring the money, the tools, and the people together." By providing high-quality tools and technologies to the research community, as well as critical funding behind the research, CHDI Foundation also aims to lower barriers to entry. "Our policy is that tools, reagents, cell lines, etc., are considered precompetitive and should be readily shared across the community in a way that allows everyone to go as far down the drug discovery pipeline as their research will take them."

## Outlook on HD

In recent years, various approaches to determine the functions of HTT and its pathological effects have been explored. However, it is becoming apparent that the role of HTT is complex and operates at many different cellular levels. Advances in technology, automation, and artificial intelligence have enabled discoveries and setbacks to happen at a more rapid pace than ever before, and organizations such as CHDI Foundation have acted as collaborative enablers for the HD research community. "Only time will tell what remains

to be discovered and understood regarding the biology of HD, but with greater advancement I am confident that better technology will play an important role in providing us with these answers," said Dr. Landles. "As we strive ever forward in our understandings of HD, I would hope that advances in technology will enlighten us further and continue to provide answers much faster so that this devastating disease might one day be perceived as being completely curable."

## Biographies

**Robert Pacifici, Ph.D.**

Dr. Robert Pacifici is the CSO of CHDI Foundation, a private, nonprofit research organization that accelerates therapeutics development for HD. Previously he was at Eli Lilly, Xencor, and Amgen. Dr Pacifici received a BS in Biochemistry from the UMass, Amherst, and a PhD in Biochemistry from University of Southern California. Dr. Pacifici participates as a lecturer at the USC and as a member of the Board of Directors for UCLA Technology Development Group.

**Christian Landles, Ph.D.**

Dr. Christian Landles is a Senior Research Fellow at the Bates lab, part of the Department of Neurodegenerative Disease, Huntington's Disease Centre, UK Dementia Research Institute at UCL, Queen Square Institute of Neurology, University College London, London, UK. He obtained his PhD from Cancer Research UK and has held numerous research positions at various renowned academic institutes, including King's College London, Imperial College London, and UCL. He has more than a decade of research experience resulting in over 45 publication contributions and has worked on studies funded by CHDI Foundation and UK Dementia Research Institute. His area of expertise includes molecular pathogenesis, pre-clinical mouse models, and protein biology and aggregation in HD. His research is not only limited to HD, and he has extensive experience in technology and molecular assay development with translational applicability for biomarker discovery and therapeutics.

### CHDI foundation

CHDI Foundation is a privately-funded, not-for-profit biomedical research organization devoted to a single disease – Huntington's disease. The mission of CHDI is to develop drugs that will slow the progression of Huntington's disease and provide meaningful clinical benefit to patients as quickly as possible. To achieve this CHDI manages a diverse portfolio of research projects through a novel virtual model that encourages scientific collaboration to more directly connect academic research, drug discovery and clinical development. This helps bridge the translational gap that often exists between academic and industrial research pursuits, and which adds costly delays to therapeutic development. CHDI's activities extend from exploratory biology to the identification and validation of therapeutic targets, and from drug discovery and development to clinical studies and trials. chdifoundation.org

## References


1. Gusella J, Wexler N, Conneally P, Naylor S, Anderson M, Tanzi R et al. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature.* 1983;306(5940):234-238.

2. Macdonald M. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell.* 1993;72(6):971-983.

3. Roche provides update on tominersen programme in manifest Huntington's disease [Internet]. [cited 26 May 2022]. Available from: https://www.roche.com/media/releases/med- cor-2021-03-22b.

4. Wave Life Sciences Provides Update on Phase 1b/2a PRECISION-HD Trials [Internet]. 2022 [cited 26 May 2022]. Available from: https://ir.wavelifesciences.com/news- releases/news-release-details/wave-life-sciences-provides- update-phase-1b2a-precision-hd.

5. Novartis receives FDA Fast Track designation for branaplam (LMI070) for the treatment of Huntington's Disease [Internet]. Novartis. 2022 [cited 26 May 2022]. Available from: https://www.novartis.com/news/novartis-receives-fda-fast-track-designation-branaplam-lmi070-treatment- huntingtons-disease.

6. Byrne L, Rodrigues F, Blennow K, Durr A, Leavitt B, Roos R et al. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. *The Lancet Neurology*. 2017;16(8):601-609.

7. Landles C, Milton R, Jean A, McLarnon S, McAteer S, Taxy B et al. Development of novel bioassays to detect soluble and aggregated Huntingtin proteins on three technology platforms. *Brain Communications*. 2021;3(1).

8. 2022 Conference | CHDI Foundation [Internet]. Chdifoundation.org. 2022 [cited 26 May 2022]. Available from: https://chdifoundation.org/2022-conference/.

9. HDinHD – Huntington's Disease in High Definition [Internet]. Hdinhd.org. 2022 [cited 26 May 2022]. Available from: https://www.hdinhd.org/.

10. Enroll – Clinical Research Platform [Internet]. Enroll-hd.org. 2022 [cited 26 May 2022]. Available from: https://www.enroll-hd.org/.

11. Origin-HD [Internet]. Health Research Authority. 2022 [cited 26 May 2022]. Available from: https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/origin-hd/.

**Revvity, Inc.**
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com