

Research Round-Up: Insights of the Year 2013-2014

In our final issue for 2014, the HD Insights team wanted to recognize the most influential papers in HD research in the 2013–2014 year. Our staff, Editorial Board, and leading HD researchers nominated the eleven papers below in three categories: lab research, clinical research, and imaging and biomarkers. The HD Insights Editorial Board then voted to select the three most influential papers, one in each category. The authors of the winning papers will present their research in a panel discussion at the Huntington Study Group meeting on November 7, 2014. Congratulations to all the nominees and winners!

In the lab...

Most influential paper

Neuronal targets for reducing mutant huntingtin expression to ameliorate disease in a mouse model of HD

[*Nat Med.* 2014 May;20\(5\):536-41. doi: 10.1038/nm.3514. Epub 2014 Apr 28.](#)

By: Wang N, Gray M, Lu XH, Cantle JP, Holley SM, Greiner E, Gu X, Shirasaki D, Cepeda C, Li Y, Dong H, Levine MS, Yang XW
(Summary by X. William Yang, MD, PhD)

We developed a conditional transgenic mouse model of HD (BACHD) to address the question of how ubiquitously expressed mutant huntingtin (mHTT) may selectively target striatal and cortical neurons for degeneration. The model expresses full-length human mHTT from a genomic transgene that confers endogenous-like mHTT expression patterns. The expression of mHTT in BACHD mice can be genetically shut off in cells that express Cre recombinase, allowing researchers to precisely assess the role of mHTT that is synthesized in one cell type or a combination of cell types in disease pathogenesis. Our study showed that genetically reducing mHTT in cortical neurons significantly ameliorates psychiatric-like behavioral deficits, modestly improves motor impairment, but does not improve neurodegeneration.

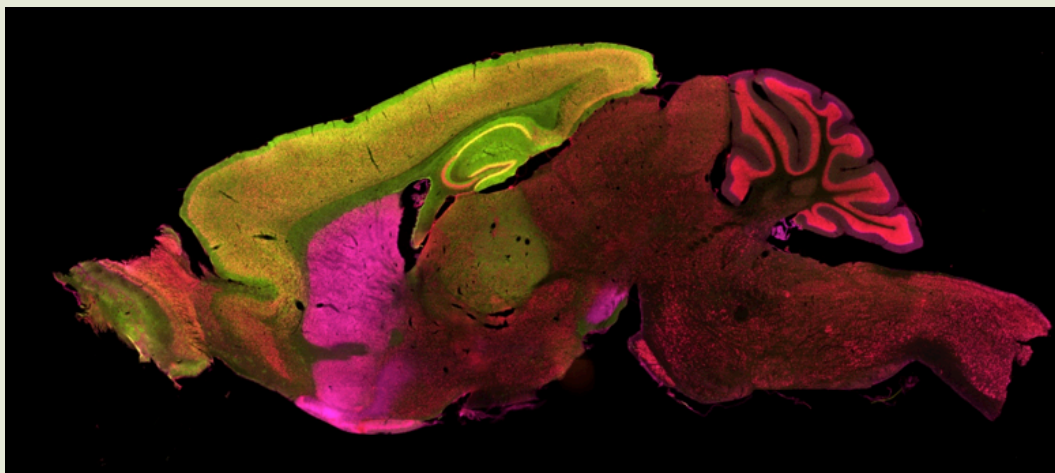


Image: Labeling of mouse cortical pyramidal neurons (green), striatal medium spiny neurons (purple,) and of all other neurons throughout the brain (red).

Importantly, we found that reducing mHTT in both cortical and striatal neurons, but not in either neuronal population alone, consistently improves all the behavioral deficits and selective brain atrophy in this HD mouse model. The study also showed that striatal synaptic dysfunction in BACHD requires both non – cell-autonomous and cell-autonomous toxicities, from cortical and striatal neurons respectively. Together, our study demonstrated distinct but interacting roles of cortical and striatal mHTT in HD pathogenesis, and suggests that optimal HD therapeutics may require targeting mHTT in both cortical and striatal neurons.

Research Round-Up: Insights, cont...

Glutathione peroxidase activity is neuroprotective in models of HD

Nat Genet. 2013 Oct;45(10):1249-54. doi: 10.1038/ng.2732. Epub 2013 Aug 25.

By: Mason RP, Casu M, Butler N, Breda C, Campesan S, Clapp J, Green EW, Dhulkhed D, Kyriacou CP, Giorgini F
(Summary by Robert Mason, PhD and Flaviano Giorgini, PhD)

Genetic modifiers of HD are a valuable source of potential therapeutic targets for this devastating disorder¹. To uncover such modifiers we performed genetic screens in baker's yeast, and identified 317 genes whose increased expression led to reduced mHTT toxicity². These modifiers are involved in a wide variety of cellular processes and include members of the glutathione peroxidase (GPx) family of antioxidant enzymes, which may help protect against the increase in oxidative stress observed in HD. Glutathione peroxidases are an exciting therapeutic target due to the availability of compounds that mimic their activity. These enzymes have been tested in humans for the treatment of stroke and noise-induced hearing loss,^{3,4} possibly expediting translation into the clinic for HD patients. Notably, we found that increased levels of mouse GPX1, the most abundant mammalian glutathione peroxidase, or treatment with the GPx-mimicking compound ebselen, improved disease phenotypes in fruit fly and mammalian cell models of HD. Interestingly, increasing GPx activity was more protective than other antioxidant strategies. Unlike other antioxidant strategies, increasing GPx activity does not inhibit autophagy, an important process for the clearance of mHTT from the cell, which may contribute to the differences in protection observed. Future studies seek to evaluate the efficacy of increasing GPx activity in animal models of HD, providing critical validation before pursuing this novel candidate therapeutic approach in patients.

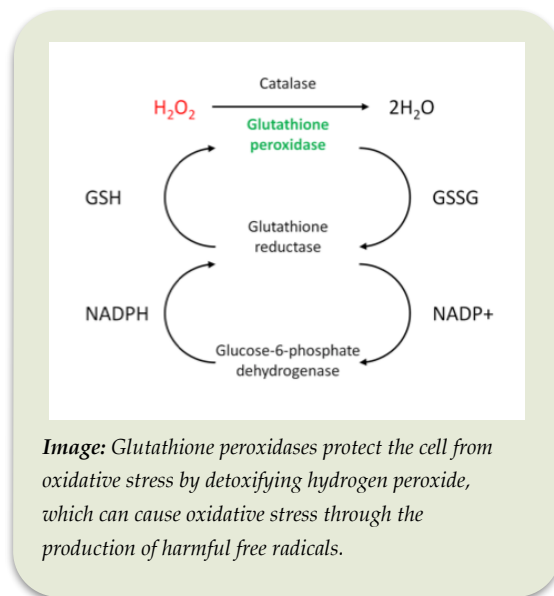


Image: Glutathione peroxidases protect the cell from oxidative stress by detoxifying hydrogen peroxide, which can cause oxidative stress through the production of harmful free radicals.

¹ Gusella JF, MacDonald ME, Lee JM. Genetic modifiers of Huntington's disease. *Mov Disord.* 2014 Sep 15;29(11):1359-65. doi: 10.1002/mds.26001. Epub 2014 Aug 25.

² Mason RP, Casu M, Butler N, Breda C, et al. Glutathione peroxidase activity is neuroprotective in models of Huntington's disease. *Nat Genet.* 2013 Oct;45(10):1249-54. doi: 10.1038/ng.2732. Epub 2013 Aug 25.

³ Day, B.J. Catalase and glutathione peroxidase mimics. *Biochem Pharmacol.* 2009 Feb 1;77(3): 285-296.

⁴ Lynch ED, Kil J. Compounds for the prevention and treatment of noise-induced hearing loss. *Drug Discov Today.* 2005 Oct 1;10(19):1291-8.

Transneuronal propagation of mutant huntingtin contributes to non-cell autonomous pathology in neurons

Nat Neurosci. 2014 Aug;17(8):1064-72. doi: 10.1038/nn.3761. Epub 2014 Jul 13.

By: Pecho-Vrieseling E, Rieker C, Fuchs S, Bleckmann D, Esposito MS, Botta P, Goldstein C, Bernhard M, Galimberti I, Müller M, Lüthi A, Arber S, Bouwmeester T, van der Putten H, Di Giorgio FP
(Summary by Lise Munsie, PhD)

This thorough study demonstrates the transneuronal spreading of mHTT that may contribute to non-cell autonomous neuropathology. The authors cultured human neurons from embryonic stem cells and seeded them in organotypic brain slices from the R6/2 mouse model. They subsequently found that mHTT aggregates accumulate in human neurons, leading to pathological consequences for these neurons. These results were recapitulated in vivo in the mouse model. The cortico-striatal pathway was examined using mixed-genotype cortico-striatal brain slice cultures from R6/2 mice and their wild-type counterparts. The authors found that mHTT can spread in a pre- to post-synaptic manner, as they found aggregates in wild-type medium spiny neurons after culturing with R6/2 cortex, but not the other way around. Finally, the authors used endoproteases that cleave part of the synaptic vesicle docking fusion SNARE complex, to show that this spread is mediated through synaptic vesicle recycling. The endoproteases used cleave SNAP25 and VAMP. Both treatments significantly reduced the spread of mHTT in their model.

Research Round-Up: Insights, cont...

Inhibition of mitochondrial protein import by mutant huntingtin

[Nat Neurosci. 2014 Jun;17\(6\):822-31. doi: 10.1038/nn.3721. Epub 2014 May 18.](#)

By: Yano H, Baranov SV, Baranova OV, Kim J, Pan Y, Yablonska S, Carlisle DL, Ferrante RJ, Kim AH, Friedlander RM.
(Summary by Lise Munsie, PhD)

Mitochondrial dysfunction is intimately involved in the progression of HD; however, the mechanism of this dysfunction is unknown. Robert Friedlander's group explored this in a recent *Nature Neuroscience* paper. Initially, the authors found that mHTT is specifically localized to mitochondria in brains from HD patients and also mouse models. Unbiased protein identification from immunoprecipitation shows that mHTT binds members of the TIM23 complex, a complex that imports matrix proteins into the inner mitochondrial membrane. Using *in vitro* mitochondrial protein assays, the group demonstrated that the N-terminus of mHTT is involved in this aberrant binding, leading to decreased mitochondrial protein import. This defect is enhanced in mitochondria purified from synaptosomes, compared to mitochondria purified from other parts of the cell or other cell types. The group showed that the mitochondrial protein import dysfunction is an mHTT-specific function and not mediated through the polyglutamine expansion alone, and thus is a mechanism specific to HD. They additionally showed that altering protein import to the mitochondria is neurotoxic and that overexpressing major subunits of the TIM23 complex can rescue mHTT-induced neurotoxicity.

In the clinic...

Most influential paper

Mutant huntingtin is present in neuronal grafts in Huntington's disease patients

[Ann Neurol. 2014 Jul;76\(1\):31-42. doi: 10.1002/ana.24174. Epub 2014 Jun 6.](#)

By: Cicchetti F, Lacroix S, Cisbani G, Vallières N, Saint-Pierre M, St-Amour I, Tolouei R, Skepper JN, Hauser RA, Mantovani D, Barker RA, Freeman TB
(Summary by Francesca Cicchetti, PhD)

Mutant huntingtin (mHTT), which drives HD pathology, has long been thought to exert its effects in a cell-autonomous manner, where degeneration occurs within individual cells that carry the mutant gene. We investigated the hypothesis that mHTT is capable of spreading within cerebral tissue.

The brains of four HD patients who received genetically unrelated fetal neural allografts at least a decade earlier were examined postmortem. We found a number of mHTT protein aggregates located within intracerebral allografts of striatal tissue in three of these HD patients. No grafts survived in the fourth transplant recipient. The mHTT aggregates were observed in the extracellular matrix of the genetically unrelated transplanted tissue, while in the host brain they were localized in neurons, neuropil, extracellular matrix, and blood vessels. In addition, peripheral immune cells in separate HD patients contained mHTT. There are thus a number of non-cell autonomous mechanisms that could explain these observations, including trans-synaptic propagation and hematogenous transport of mHTT, among others.

This is the first *in vivo* demonstration of mHTT spread in patients with a monogenic neurodegenerative disorder of the CNS. These observations raise questions about the importance of non-cell-autonomous mechanisms of pathological protein spread, and provide new targets for the development of therapeutic strategies.

Image: A. Double immunohistochemical staining for the neuronal marker NeuN (revealed with the chromogen DAB - brown color) and EM48 (that stains for mHTT aggregates, revealed with nickel intensified DAB - black color) in the cortex of an HD grafted case. mHTT aggregates were often observed aligning in a linear array, seemingly following the pattern of a dendritic segment.

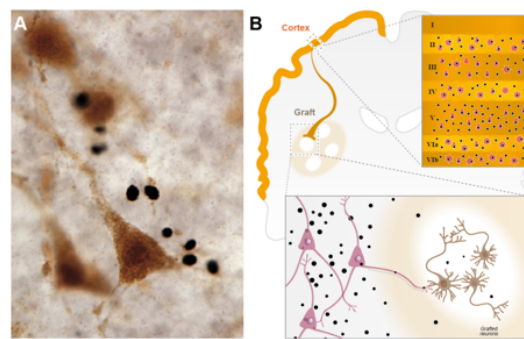


Image: B. Schematic of how mHTT in layer V of the host cortex may spread trans-synaptically to the striatum and graft.

Research Round-Up: Insights, cont...

Deep brain stimulation for Huntington's disease: long-term results of a prospective open-label study

J Neurosurg. 2014 Jul;121(1):114-22. doi: 10.3171/2014.2.JNS131722. Epub 2014 Apr 4.

By: Gonzalez V, Cif L, Biolsi B, Garcia-Ptacek S, Seychelles A, Sanrey E, Descours I, Coubes C, de Moura AM, Corlobe A, James S, Roujeau T, Coubes P

(Summary by Victoria Gonzalez, MD, PhD)

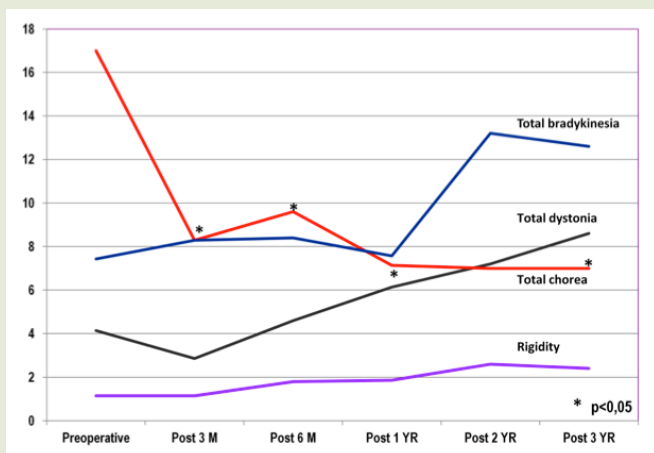


Image: Evolution of mean UHDRS motor subscores over the course of the study.

The role of deep brain stimulation (DBS) in the clinical management of HD has not yet been validated, although promising case reports have shown its efficacy in the treatment of severe chorea. This study aimed to analyze long-term motor outcome of a cohort of HD patients treated with globus pallidus internus (GPi) DBS. Seven patients with pharmacologically resistant chorea were included in a prospective open-label study with a median followup of three years. The Unified HD Rating Scale motor section was the main outcome measure. GPi DBS led to significant reduction of chorea for all patients with a mean improvement of 58.3% at one-year visit and 59.8% at three-year visit ($p < 0.05$). Switching OFF stimulation tests confirmed sustained therapeutic effect for chorea throughout the study period. Bradykinesia and dystonia showed a non-significant trend towards progressive worsening. Increased bradykinesia was partly induced by DBS settings, and improved after adjustment of stimulation parameters.

GPi DBS may provide sustained reduction of chorea in selected HD patients, with transient benefit in physical aspects of quality of life before progression of behavioral and cognitive disorders. Further studies are needed to assess the impact of GPi DBS on quality of life and cognitive measures in HD.

PRECREST: a phase II prevention and biomarker trial of creatine in at-risk Huntington disease

Neurology 2014 Mar 11;82(10):850-7. doi: 10.1212/WNL.0000000000000187. Epub 2014 Feb 7.

By: Rosas HD, Doros G, Gevorkian S, Malarick K, Reuter M, Coutu JP, Triggs TD, Wilkens PJ, Matson W, Salat DH, Hersch SM

(Summary by H. Diana Rosas, MD)

PRECREST, the first clinical trial of a drug intended to delay the onset of symptoms in individuals at risk for HD, enrolled sixty-four participants, of whom nineteen knew their gene status. Participants were randomized into two groups, regardless of gene status: one group to twice-daily oral doses of creatine, up to thirty grams per day, the other, placebo for six months. Participants were followed for an additional twelve months on open-label creatine and assessed at regular study visits for adverse effects, and dosage levels were adjusted, if necessary, to reduce unpleasant side effects.

Cognitive assessments, measurement of blood markers and MRI brain scans were conducted at the trial's outset, at six months, and at the end of the study. Fifteen participants, including several who knew that they carried the HD mutant gene, discontinued taking creatine because of gastrointestinal discomfort, taste of the drug, inconvenience, or the stress of being constantly reminded of their HD risk. MRI scans at six months showed a slower rate of cortical and basal ganglia atrophy in gene-positive carriers who took creatine, compared to placebo. After twelve months, atrophy rates in those who crossed over to treatment were also slower than during the period of taking placebo. The results of the trial suggest that at-risk individuals can participate in clinical trials even if they do not want to learn their genetic status, and that useful biomarkers can be developed to help assess therapeutic benefits.

Research Round-Up: Insights, cont...

In imaging and biomarkers...

Most influential paper

Metabolic network as a progression biomarker of premanifest Huntington's disease

[J Clin Invest. 2013 Sep 3;123\(9\):4076-88. doi: 10.1172/JCI69411. Epub 2013 Aug 29.](#)

By: Tang CC, Feigin A, Ma Y, Habeck C, Paulsen JS, Leenders KL, Teune LK, van Oostrom JC, Guttman M, Dhawan V, Eidelberg D
(Summary by Chris C. Tang, MD, PhD)

The need for sensitive and accurate measurements of preclinical disease progression in at-risk individuals has been a major roadblock in the development of effective treatment for neurodegenerative disorders. In this fluorodeoxyglucose positron emission tomography (FDG-PET) study, we used a novel computational approach to identify and validate a brain network biomarker of disease progression in premanifest HD carriers. The subjects, who underwent longitudinal metabolic imaging in the rest state, exhibited a significant linear increase in network activity over seven years, continuing even as clinical manifestations emerged. The progression rate of network activity in this cohort was nearly identical to that measured prospectively in an independent cohort of premanifest HD carriers scanned longitudinally over a 2.3 ± 0.3 – year period. Moreover, this rate was found to be faster than the corresponding rates of conventional single-region measurements (i.e. caudate D₂ binding and tissue volume) acquired in the same subjects. Thus, the metabolic network can provide a sensitive and reliable means of assessing systems-level changes in the progression of premanifest HD. This network biomarker is currently undergoing further validation in a multicenter longitudinal trial as part of the PREDICT-HD study. The results will help determine its utility in future clinical trials on new treatments targeted at slowing the progression of HD in the premanifest period.

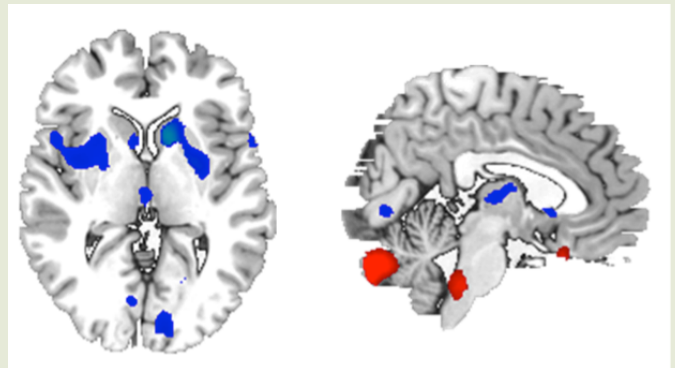


Image: An example of the FDG-PET imaging – based brain metabolic network biomarker identified in premanifest HD gene carriers.

Clinical and biomarker changes in premanifest Huntington disease show trial feasibility: A decade of the PREDICT-HD study

[Front Aging Neurosci. 2014; 6: 78. Published online Apr 22, 2014. doi: 10.3389/fnagi.2014.00078](#)

By: Paulsen JS, Long JD, Johnson HJ, Aylward EH, Ross CA, Williams JK, Nance MA, Erwin CJ, Westervelt HJ, Harrington DL, Bockholt HJ, Zhang Y, McCusker EA, Chiu EM, Panegyres PK; PREDICT-HD Investigators and Coordinators of the Huntington Study Group
(Summary by Christina Colletta, BA)

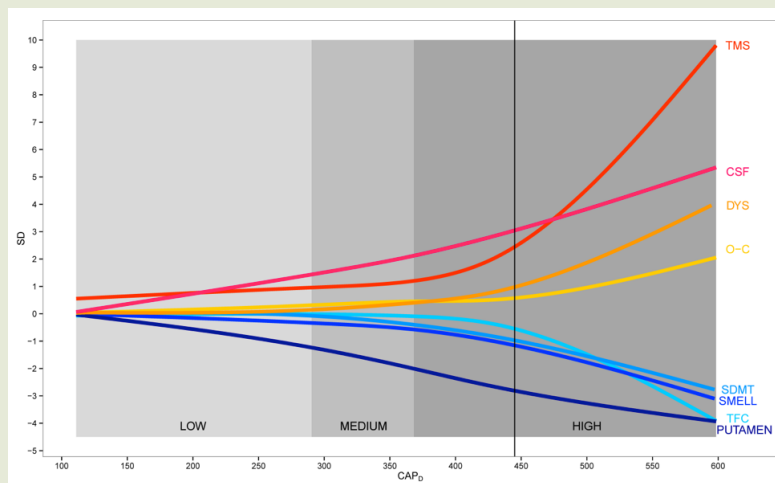
Clinical trials to test novel therapies in individuals with premanifest HD have been limited by the scarcity of proven outcome measures and objective measures of disease progression. PREDICT-HD, a thirteen year study, aims to identify markers of HD-related change in individuals with premanifest and early HD needed to determine whether HD treatments are effective early in the disease process. This year's analysis of the PREDICT-HD data found changes occurring in 36 of 39 potential outcome measures examined over a ten year period in individuals with premanifest HD, which could potentially be used as outcome measures in future therapeutic trials. Specifically, outcome measures of imaging based on regional brain volumes had the largest effect sizes. A motor assessment showed the next highest effect size, followed by a cognitive assessment of working memory, complex scanning and processing speed. Measures of function related to health and disability and measures of psychiatric symptoms such as obsessive-compulsive disorder were also found to show significant change over time. Using these and other outcome measures, clinical trials could be initiated seven to twelve years before motor diagnosis.

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Research Round-Up: Insights, cont...

Clinical and biomarker changes in premanifest Huntington disease show trial feasibility: A decade of the PREDICT-HD study, cont...

Image: Scaled fitted spline curves of key variables plotted over CAP_D (CAG-corrected age). Blue colors indicate variables that decrease and red colors indicate variables that increase. Vertical axis is in standard deviation (SD) units. TMS indicates total motor score; CSF, cerebral spinal fluid; DYS, dysrhythmia; O-C, obsessive-compulsive; SDMT, Symbol Digit Modalities Test; SMELL, University of Pennsylvania Smell Identification Test (UPSIT); TFC, total functional capacity.



Source: Figure and caption as originally published in Paulsen JS, Long JD, Johnson HJ, Aylward EH, et al; PREDICT-HD investigators and coordinators of the Huntington Study Group. Clinical and biomarker changes in premanifest Huntington disease show trial feasibility: A decade of the PREDICT-HD study. *Front Aging Neurosci.* 2014; 6: 78. Published online Apr 22, 2014. doi: 10.3389/fnagi.2014.00078

HTRF analysis of soluble huntingtin in PHAROS PBMCs

Neurology. 2013 Sep 24;81(13):1134-40. doi: 10.1212/WNL.0b013e3182a55ede. Epub 2013 Aug 21.

By: Moscovitch-Lopatin M, Goodman RE, Eberly S, Ritch JJ, Rosas HD, Matson S, Matson W, Oakes D, Young AB, Shoulson I, Hersch SM; Huntington Study Group PHAROS Investigators

(Summary by Steven M. Hersch, MD, PhD and Miriam Moscovitch-Lopatin, PhD, PMP)

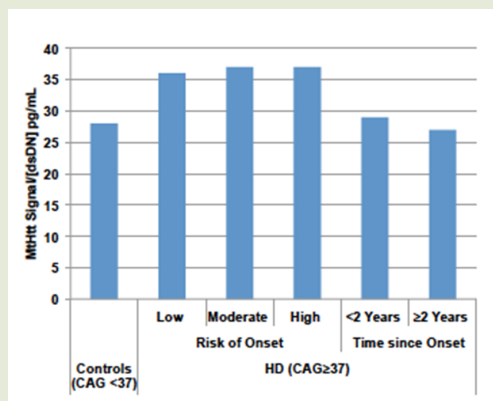


Image: Ratio of mHTT to dsDNA in different groups of HD gene carriers and health controls. Levels of soluble mHTT in white blood cells were elevated in premanifest HD and diminished in individuals with symptomatic HD.

Mutant huntingtin (mHTT) is a target of many treatments currently being developed for HD. Therefore, the ability to measure levels of mHTT in humans will be crucial to future research efforts. Measuring mHTT has been very technically challenging. In our study of white blood cell samples from the PHAROS study, we used a sophisticated fluorescent assay to detect higher relative values of soluble mHTT in gene carriers (CAG_≥37) prior to symptoms, and relatively lower values in symptomatic HD subjects.

The study demonstrated that soluble mHTT can be usefully detected in blood, and that HD may influence its levels. Since then, we have used this same assay in blood from early HD subjects participating in the HSG's Reach2HD trial of PBT2 (PRANA Biotechnology), and found that the treatment can affect soluble mHTT levels. Currently, we are developing an assay that can also measure mHTT aggregates in clinical samples, which we believe will also prove useful for developing treatments that target huntingtin.

Research Round-Up: Insights, cont...

Multi-modal neuroimaging in premanifest and early Huntington's disease: 18 month longitudinal data from the IMAGE-HD study

PLoS ONE. 2013 Sep 16;8(9):e74131. doi: [10.1371/journal.pone.0074131](https://doi.org/10.1371/journal.pone.0074131).

By: Domínguez D JF, Egan GF, Gray MA, Poudel GR, Churchyard A, Chua P, Stout JC, Georgiou-Karistianis N
(Summary by Nellie Georgiou-Karistianis, PhD)

IMAGE-HD is an Australia-based longitudinal multimodal biomarker development study that followed individuals with HD, and healthy controls, over 30 months. Results from IMAGE-HD have made significant contributions to biomarker discovery and to understanding of the impact of HD neuropathology on brain structure, microstructure, connectivity, and function during both the premanifest and early symptomatic stages.

However, much remains to be discovered about the contribution of specific brain regions and/or networks to the phenotypic heterogeneity of HD, and whether these decline at different rates. Such knowledge will be essential for well-targeted disease management strategies and therapeutic interventions. It will be important in the future to investigate the heterogeneity of HD in terms of brain endophenotypes and their relationships with cognitive, psychiatric and motor exophenotypes. Understanding how genetic and/or environmental factors modify these relationships and influence their development will be key to this endeavor. This will require a range of innovative approaches and novel analytical techniques, such as high-throughput connectomics and predictive computational models.

There is also growing evidence for the potential benefit of cognitive and physical training in forestalling further worsening of HD symptoms. Such non-pharmaceutical interventions offer new and exciting possibilities to explore disease-modifying potential and to determine their neural mechanisms of action.

The new CAVE2™ at Monash University will provide state-of-the art capacity for high-throughput neuroimaging data for visualization and sorting of brain images based on pre-defined characteristics, which will enable imaging data to be visualized in new and exciting ways.



Image: The Monash CAVE2™, next-generation immersive hybrid 2D and 3D virtual reality environment that enables the interactive exploration of high-throughput imaging data to develop new hypotheses and discover new biomarkers.

