

## **Fluid and Imaging Biomarkers for Huntington's Disease**

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## Introduction

Like many other neurodegenerative disorders, at present there is no disease-modifying treatment available for Huntington's disease. However, the known genetic cause of HD provides a clear therapeutic target: mutant huntingtin protein (mHTT), which is central to HD pathogenesis (Bates, Dorsey et al. 2015). This has been the basis of a number of promising therapeutic approaches specifically targeting Huntingtin DNA and RNA to induce huntingtin-lowering (reviewed in Wild and Tabrizi 2017). Recently, the successful lowering of mutant huntingtin via an anti-sense oligonucleotide (ASO) targeting huntingtin mRNA was demonstrated for the first time in a phase 1/2 trial in early HD patients (Tabrizi 2018), whilst an allele-selective mHTT-lowering ASO is now in clinical trials (clinicaltrials.gov NCT03225846 2018).

This progress underscores the importance and urgent need for biomarkers in HD that can measure target engagement and response to treatment. The term biomarker is sometimes defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" (Biomarkers Definitions Working Group 2001). Biomarkers can serve many different functions, such as *diagnostic*, aiding a more precise definition of onset; *prognostic*, predicting the likely course of the disease in an individual; *monitoring*, measured serially to more accurately identify disease stage and *pharmacodynamic/response*, identifying whether a biological response has occurred in an individual exposed to a medicinal product (FDA 2016).

The conventional diagnosis of manifest HD rests on the presence of unequivocal motor signs of the disease which includes chorea, dystonia, motor impersistence and bradykinesia. Subtle cognitive impairment occurs in mostly sub-cortical domains, such as executive function and visuospatial ability, and is detectable up to 10 years prior to predicted diagnosis (Stout, Paulsen et al. 2011). Psychiatric disturbance is also a feature of HD, with apathy, anxiety, depression, irritability and obsessive compulsive behaviours being the most common neuropsychiatric features (Craufurd, Thompson et al. 2001).

The slow progression of HD poses a challenge for clinical trial design, since changes in clinical outcome measures such as the unified Huntington's disease rating scale (UHDRS) (Huntington Study Group 1996) over the typical time course of a drug trial may be limited. In addition, clinical measures are also influenced by the placebo effect and clinical rater variability, and cannot distinguish between symptom relief and amelioration of the underlying disease process (Scahill, Wild et al. 2012). Biomarkers that track with clinical progression and alter quickly and predictably in response to a therapeutic intervention could greatly facilitate future clinical trials by reducing the duration and numbers required for such studies. This is especially true in younger premanifest HD mutation carriers, who may remain free from all clinical manifestations for decades, effectively precluding the use of clinical endpoints in therapeutic trials. In addition, pharmacodynamic biomarkers can be utilised in preclinical models and early phase clinical trials to instil confidence that the drug is having its intended effect on its target, to assist with "go/no-go" decisions.

To date, biomarker research has included focused small-scale studies as well as large natural history studies, including TRACK-HD, (Tabrizi, Scahill et al. 2011) and PREDICT-HD (Paulsen, Hayden et al. 2006) which has afforded the opportunity to study many potential biomarkers for HD in well-defined cohorts. Studies of premanifest populations have made use of models that utilise the positive association of CAG repeat length and age of disease onset to categorise groups in terms of proximity to disease onset (Penney, Vonsattel et al. 1997, Langbehn, Brinkman et al. 2004). For example, the disease burden score is a function of age and CAG repeat length that provides a proxy measure to lifetime toxic mHTT load (Penney, Vonsattel et al. 1997). Such models are supported by longitudinal cohort studies, although they cannot account for modifying genetic and environmental factors which are yet to be fully characterised.

This review focuses on the most promising imaging and biofluid biomarkers published to date and discusses the likely future direction of HD biomarker research in the huntingtin-lowering era.

## **Neurobiology of HD**

Ideally a biomarker should be closely related to the pathophysiology and a comprehensive understanding of the many pathophysiologic pathways and their contribution to disease phenotype can aid the identification of new biomarkers. The causative mutation in HD is an expanded CAG repeat in the huntingtin gene (HTT). This is translated into full length mHTT protein with an expanded polyglutamine stretch. An amino-terminal HTT exon1 truncated protein formed by aberrant splicing appears to be formed under some circumstances but its pathogenic contribution is uncertain (Bates, Dorsey et al. 2015). Full-length huntingtin is cleaved through proteolysis to generate additional protein fragments, some of which can enter the nucleus. These fragments can be retained in the nucleus forming inclusions, and causing transcriptional dysregulation. Huntingtin fragments also oligomerise and aggregate in the cytoplasm. The presence of mutant huntingtin and its fragments leads to a diversity of cellular impairments including synaptic dysfunction (Reddy and Shirendeb 2012, Nithianantharajah and Hannan 2013) mitochondrial toxicity (Johri, Chandra et al. 2013), immune dysfunction (Ellrichmann, Reick et al. 2013) and decreased axonal transport (Reddy and Shirendeb 2012). Together, these dysfunctions result in progressive neuronal impairment, damage and death (Bates, Dorsey et al. 2015). HD ultimately affects the whole brain (Rub, Seidel et al. 2016) but early in the disease, the striatum is a major site of pathology in HD before more widespread basal ganglia, cortical and white matter changes (Vonsattel, Myers et al. 1985).

The complexity of its pathogenesis, despite its monogenic aetiology, creates challenges for effective treatments but opportunities for understanding HD through multiple biomarker approaches, from huntingtin protein in its various forms, to markers of individual pathogenic pathways, to their convergence upon the final common pathway of neuronal damage and death. Some of these biomarkers may have utility across multiple therapeutic programs or even other diseases.

## **Imaging biomarkers**

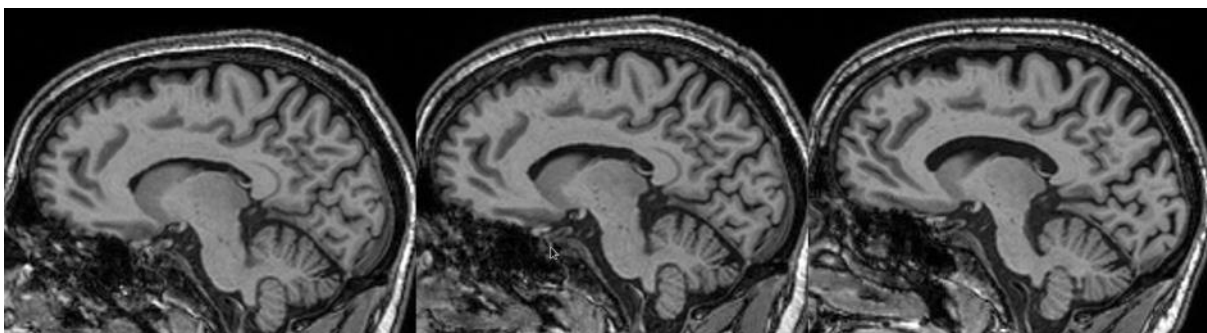
Neuroimaging techniques have been widely studied in HD and have helped shape our understanding of the natural history of the disease. Imaging is appealing as a source of

biomarkers since it is generally non-invasive; data acquisition, processing and quality control can be standardised; and data can be transferred over long distances easily which is beneficial for multi-site studies. The ideal imaging biomarker would be readily available, relatively inexpensive, reproducible across multiple sites with different scanner manufacturers and field strengths and have a feasible acquisition time - particularly since HD patients may not tolerate longer scanning protocols and movement significantly reduces image quality.

There are a number of different imaging modalities including structural MRI, diffusion imaging, functional MRI and PET. For each modality there are numerous image processing techniques and the method chosen can have a significant impact on output metrics being considered as biomarkers. For example, some automated techniques can introduce error and systematic bias, particularly in atrophic brains (Johnson, Gregory et al. 2017). Before such measures can be effectively utilised as a biomarker, rigorous validation of the acquisition and analysis technique is required to minimise such problems and has been lacking in many imaging studies to date.

## Structural Imaging

The most widely-studied imaging acquisition in HD is the structural volumetric MRI scan. Typically a T1-weighted image is used as it presents the best contrast between grey and white matter which makes delineation of structures of interest more accurate. A number of different brain regions have potential as biomarkers of disease progression in HD.



**Figure 1. Longitudinal grey matter changes in HD.** Magnetic Resonance Imaging scans of a gene carrier aged (left to right) 51, 54 (premanifest) and 57 (early HD) showing progressive caudate volume loss later accompanied by cortical atrophy. Reproduced from Scahill et al, 2017 with permission from Elsevier

## The striatum

Cross-sectional and longitudinal studies have shown that atrophy of the caudate and putamen can be observed from 15-20 years prior to predicted disease onset, and this atrophy generally increases from one stage to the next (Harris, Pearlson et al. 1992, Paulsen, Langbehn et al. 2008, Tabrizi, Langbehn et al. 2009, Aylward, Nopoulos et al. 2011, Tabrizi, Scahill et al. 2013). Estimates of the annual rate of change vary between studies but suggest up to 4% per year in the caudate and 3% a year in putamen, with higher rates seen in manifest disease (Georgiou-Karistianis, Scahill et al. 2013). Based on effect sizes, caudate volumes have outperformed putamen volume across all disease stages (1.5-3.0 vs. 1.1-1.5 respectively in TRACK-HD) in 3 separate cohorts (Aylward, Nopoulos et al. 2011, Tabrizi, Scahill et al. 2013, Hobbs, Farmer et al. 2015), although total striatum volume may prove a more robust measure of change (Aylward, Nopoulos et al. 2011). The caudate is easier to delineate than the putamen due to its boundary with lateral ventricular CSF, and this may contribute to measurements being more sensitive and less variable in the caudate (Tabrizi, Scahill et al. 2013, Scahill, Andre et al. 2017).

When utilised as an endpoint for a clinical trial, the rate of change of a proposed biomarker can influence the trial duration and numbers needed to detect a significant change. There is a lack of consensus over whether rate of change in striatal atrophy varies with disease stage. TRACK-HD reported step-wise accelerated rates of change from the earliest premanifest stage through to early stage disease, with some suggestion that the acceleration slows down after the onset of symptoms (Tabrizi, Scahill et al. 2011, Tabrizi, Reilmann et al. 2012). TRACK-HD also reported highly significant correlations between rate of change and disease burden scores for both caudate and putamen, after accounting for age. However, the PREDICT-HD study did not find that rates accelerated across their premanifest cohort, although this may be due to differences in methodology for assessing longitudinal change (Aylward, Nopoulos et al. 2011). Baseline striatal volume (Aylward, Liu et al. 2012) and atrophy rates (Tabrizi, Scahill et al. 2013, Paulsen, Long et al. 2014) emerged as predictors of conversion to manifest HD in both studies, suggesting that striatal measures may assist in enriching clinical trials where delay of onset of diagnosable motor symptoms is a primary outcome measure.

To be a biomarker, a parameter must demonstrate correlations with clinical measures of disease progression to signify its relevance to clinical outcomes. The striatum is also known to be central to many 'subcortical' cognitive functions that are commonly impaired in HD (Papoutsis, Labuschagne et al. 2014). Striatal atrophy shows significant correlations with UHDRS total motor score (TMS) (Jurgens, van de Wiel et al. 2008, Paulsen, Nopoulos et al. 2010, Aylward, Liu et al. 2012), whilst paced finger tapping and tongue force, more sensitive motor measures in premanifest HD, also correlate with striatal volume (Tabrizi, Langbehn et al. 2009). Caudate volume loss is associated with deficits in verbal learning, working memory and emotion recognition (Aylward, Harrington et al. 2013) whilst putaminal atrophy is related to executive dysfunction and emotion recognition (Jurgens, van de Wiel et al. 2008, Aylward, Harrington et al. 2013, Harrington, Liu et al. 2014).

### **Other subcortical structures**

Volume reduction has been reported in the nucleus accumbens, pallidum and thalamus from the premanifest stage (van den Bogaard, Dumas et al 2011) and longitudinal studies have also highlighted thalamic atrophy in premanifest (Aylward, Nopoulos et al. 2011, Majid, Aron et al. 2011) and manifest cohorts (Hobbs, Barnes et al. 2010). However effect sizes were small compared with the caudate and putamen. Their rates of atrophy appear to be inversely related to CAG length (Hobbs, Barnes et al. 2010), and thalamic volume has also been shown to correlate with TMS (van den Bogaard, Dumas et al. 2011) and cognitive dysfunction (Kassubek, Juengling et al. 2005). There is a relative lack of longitudinal studies of non-striatal subcortical structures, and no differences were found either cross-sectionally or longitudinally in any of these structures in one such study using an automated segmentation technique (Majid, Aron et al. 2011). The apparent lack of sensitivity of these structures to HD pathology compared with the caudate and putamen may be a real biological phenomenon or may just reflect the paucity of well-powered studies and/or the fact that these small structures have relatively poorly defined boundaries. Consequently they are not currently viable biomarkers for HD although the increasing availability of high field strength MRI and more advanced automated segmentation techniques may improve sensitivity to change in these structures in the future.

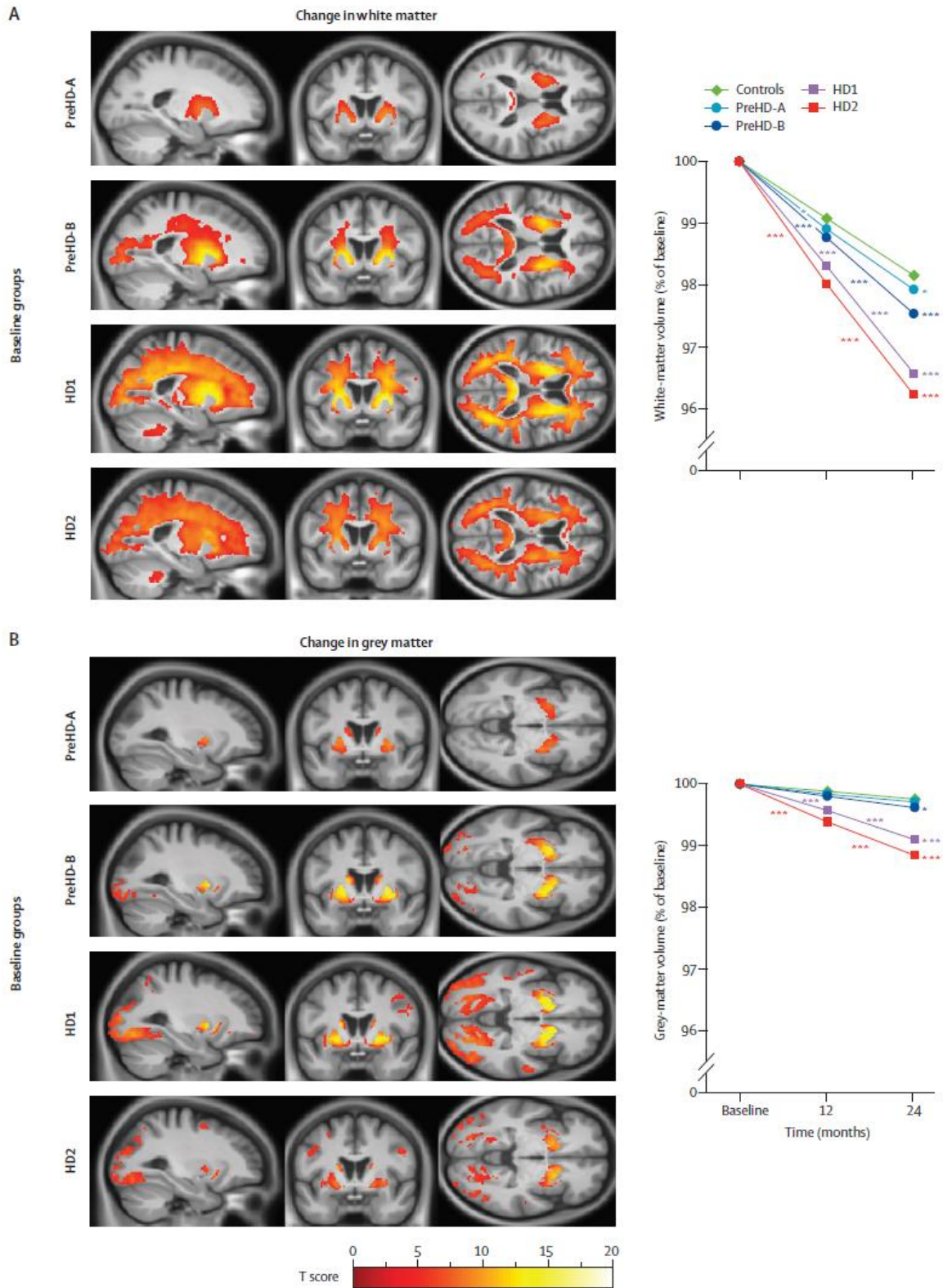
### **Cortical structures**

Cortical volumes can be assessed using a number of manual (Aylward, Anderson et al. 1998), semi-automated (Henley, Frost et al. 2006) and automated segmentation techniques (e.g. Statistical Parametric Mapping (<https://www.fil.ion.ucl.ac.uk/spm/>); Freesurfer (<https://surfer.nmr.mgh.harvard.edu/>); FSL (<https://fsl.fmrib.ox.ac.uk/fsl/>)), although manual delineation of the cortex is generally more challenging than subcortical segmentation due to its convoluted structure.

Whole brain and grey matter volume has been shown to be reduced cross-sectionally (Tabrizi, Langbehn et al. 2009, Paulsen, Nopoulos et al. 2010) and longitudinally, including in the premanifest stages (Figure 2) (Kipps, Duggins et al. 2005, Tabrizi, Scahill et al. 2011, Tabrizi, Reilmann et al. 2012). Effect sizes are smaller than for striatal or white matter measures, particularly in the early premanifest stage of the disease (Tabrizi, Reilmann et al. 2012, Tabrizi, Scahill et al. 2013, Hobbs, Farmer et al. 2015). Although it is possible that the higher complexity of measuring the convoluted structure of the cortex is contributing to reduced sensitivity of global atrophy, previous work suggests that striatal and white matter loss does indeed occur early in the disease prior to symptom manifestation and that there is an acceleration of grey matter loss around the time of clinical conversion (Tabrizi, Scahill et al. 2013).

Reductions in cortical thickness have been demonstrated in manifest HD (Rosas, Liu et al. 2002, Tabrizi, Langbehn et al. 2009) and premanifest disease (Tabrizi, Langbehn et al. 2009). However, this fully automated technique can introduce errors and in particular misclassification around the mid-sagittal plane has led to spurious results (Rosas, Salat et al. 2008, Hobbs, Pedrick et al. 2011). Longitudinal measures of cortical thinning appear to be less sensitive to change over time in symptomatic HD than other structural measures (Hobbs, Farmer et al. 2015).





**Figure 2. Longitudinal changes in grey and white matter.** Parametric maps from the TRACK-HD study showing regions with statistically significant atrophy in (A) white matter and (B) grey matter over 24 months, relative to controls. Corresponding longitudinal plots show mean values at baseline, 12 months, and 24 months. Significant change differences relative to controls over 0-12, 12-24, and 0-24 months are represent by \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ . Reprinted from Tabrizi et al, 2012 with permission from Elsevier.

In early manifest HD, total grey matter volumes were significant baseline predictors of decline in total functional capacity (Tabrizi, Scahill et al. 2013). CAG repeat length and age predicted longitudinal changes in premanifest HD in all images measures except grey matter, suggesting that grey matter loss is a key marker of disease onset with significant prognostic significance above that provided by age and CAG repeat length (Tabrizi, Scahill et al. 2013).

### **White matter**

The importance of white matter degeneration and resulting loss of brain connectivity has been increasingly recognised in both premanifest and manifest stages of the disease. Like grey matter, white matter volumes can be analysed using manual or automated techniques, either in specific regions of interest or looking at total white matter volume.

Reduction of global white matter volume has been reported cross-sectionally (Thieben, Duggins et al. 2002, Paulsen, Magnotta et al. 2006, Tabrizi, Langbehn et al. 2009, Paulsen, Nopoulos et al. 2010) whilst region of interest studies have reported volume reductions in the corpus callosum (Crawford, Hobbs et al. 2013) and frontal white matter (Aylward, Nopoulos et al. 2011). Both TRACK-HD (Figure 2) and PREDICT-HD demonstrated progressive white matter atrophy in premanifest HD, including in the groups furthest from estimated onset (Aylward, Nopoulos et al. 2011, Tabrizi, Scahill et al. 2011, Tabrizi, Reilmann et al. 2012). A similar picture has been observed in manifest disease, with cross-sectional reductions in white matter volume compared to controls (Aylward, Anderson et al. 1998, Halliday, McRitchie et al. 1998, Rosas, Koroshetz et al. 2003, Beglinger, Nopoulos et al. 2005, Tabrizi, Langbehn et al. 2009) and elevated atrophy rates in longitudinal studies (Hobbs, Barnes et al. 2010, Tabrizi, Scahill et al. 2011, Tabrizi, Reilmann et al. 2012) with the most prominent changes around the striatum and corpus callosum (Tabrizi, Scahill et al. 2011, Crawford, Hobbs et al. 2013). White matter atrophy has been shown to correlate with motor function (Jech, Klempir et al. 2007, Paulsen, Nopoulos et al. 2010, Aylward, Nopoulos et al. 2011, Scahill, Hobbs et al. 2013), cognitive function (Beglinger, Nopoulos et al. 2005, Paulsen, Nopoulos et al. 2010, Scahill, Hobbs et al. 2013) and total functional capacity (TFC) (Della Nave, Ginestroni et al. 2010, Rosas, Reuter et al. 2011). The predictive power of white matter volume loss for conversion to manifest HD is less clear, with contradictory

findings in two large observational studies (Aylward, Nopoulos et al. 2011, Tabrizi, Scahill et al. 2013). Nevertheless white matter atrophy does track disease progression and is ongoing from the very earliest premanifest phase through to established disease.

### **Diffusion MRI**

Diffusion MRI provides complementary information to volumetric MRI by assessing the microstructural integrity of white matter fibres. This technique measures the diffusion of water in different directions within the brain. Healthy white matter fibres typically show water diffusion in one direction i.e. are anisotropic. When white matter breaks down due to axonal damage or demyelination for example, there is increased diffusion in directions other than along the axons. In theory, diffusion MRI could provide information about neuronal damage or dysfunction that precedes volumetric loss.

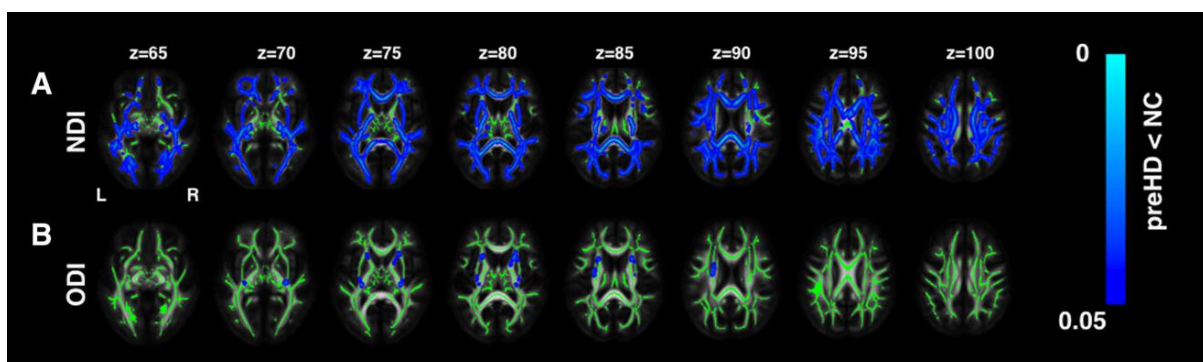
The most widely-studied diffusion technique in HD is diffusion tensor imaging (DTI). Typically reductions in DTI metrics of fractional anisotropy (FA) and increases in mean diffusivity (MD) are seen across various neurodegenerative diseases (Zhang, Schuff et al. 2009, Atkinson-Clement, Pinto et al. 2017, Slattery, Zhang et al. 2017), attesting to their sensitivity but relative lack of specificity to the underlying neurodegenerative process. These changes are generally considered to reflect axonal loss, demyelination and less coherent white matter tracts which would be expected to occur in advance of volume loss.

In cross-sectional studies, diffusion metric change has been demonstrated in premanifest HD, particularly in the corpus callosum, internal capsule and thalamic radiations (Rosas, Lee et al. 2010, Stoffers, Sheldon et al. 2010, Poudel, Stout et al. 2014, Harrington, Long et al. 2016). In manifest HD, these changes become more marked and widespread in the white matter including the frontal, parietal and occipital white matter (Rosas, Tuch et al. 2006, Bohanna, Georgiou-Karistianis et al. 2011, Hobbs, Cole et al. 2013). Longitudinal study findings using diffusion metrics have been inconsistent. In premanifest HD, two studies have failed to find 12-30 month changes (Poudel, Stout et al. 2014, Odish, Leemans et al. 2015) whereas two larger studies demonstrated progressive changes over 1-5 years in premanifest HD cohorts including those up to 10 years away from onset (Harrington, Long et al. 2016,

Shaffer, Ghayoor et al. 2017). Longitudinal changes in DTI metrics have also been demonstrated in manifest-HD (Gregory, Cole et al. 2015).

Changes in regional DTI metrics have correlated with a number of clinical measures including TMS , paced finger tapping, executive function (Poudel, Stout et al. 2014), apathy (Delmaire, Dumas et al. 2013) and depression (Sprengelmeyer, Orth et al. 2014). However no studies have examined the utility of DTI measures in predicting clinical progression. Furthermore, in a comparative study, DTI metrics typically had smaller effect sizes than volumetric measures over periods between 6-15 months (Hobbs, Farmer et al. 2015) which would limit the use of DTI as a biomarker of HD progression.

Recent advances in diffusion acquisition and modelling techniques including the use of neurite orientation dispersion and density imaging (NODDI) methods (Figure 3) (Zhang, Schneider et al. 2012, Kaden, Kelm et al. 2016, Zhang, Gregory et al. 2018) provide the potential to improve the sensitivity of diffusion MRI measures in HD. However there is still a lack of consensus on acquisition parameters, processing and analysis techniques for diffusion imaging and this variability likely accounts for some of the inconsistency of findings to date.



**Figure 3. White matter abnormalities: neurite orientation dispersion and density imaging (NODDI) analysis.** The regional distribution of differences in NODDI parameters in premanifest HD gene carriers compared to controls is shown. There were reductions in neurite density (neurite density index [NDI]) across the whole brain indicating a reduction in axonal density (A). Localised reductions in the dispersion of fibres (orientation dispersion index [ODI]) in the corpus callosum and the internal and external capsule (B), indicates select pruning of white matter fibers. Threshold-free cluster enhancement  $p < 0.05$ . Group differences in NODDI metrics are overlaid on a white matter skeleton. Z= slice location in the Z plane.. Reprinted from Zhang J et al. 2018 under creative commons license.

Future studies will require optimisation and standardisation in these domains, further focus on test-retest reliability and replication across different cohorts before these metrics can be accepted as potential biomarkers in HD.

### **Other imaging modalities**

Functional MRI studies use regional cerebral blood flow to infer brain activity and connectivity at rest or during tasks and have provided useful insights into HD physiology. However as a potential biomarker this technique suffers from poor within-subject reproducibility, a large variability in analytical methods used and lack clear patterns in disease progression, a challenge across the field of neurodegeneration (Hohenfeld, Werner et al. 2018). Measures of structural connectivity derived from diffusion MRI suffer from similar shortfalls (Farquharson, Tournier et al. 2013).

Positron emission tomography (PET) has been utilised in HD to study metabolic markers of hypometabolism, dopaminergic function, microglial activation and PDE10A enzyme expression (Wilson, De Micco et al. 2017). However such studies have all been in relatively small numbers, with some inconsistent findings. Furthermore PET scanning is more costly than volumetric or diffusion MRI, less widely available for large multicentre studies and is more invasive, involving ionising radiation. Nevertheless PET has the advantage that it may provide more specific information about pathological processes and a potential future role for PET could be as a biomarker for target engagement in smaller proof of concept or phase 1 studies. For example, PET was recently utilised to demonstrate effective target engagement of a novel PDE10A inhibitor following a single dose which supported further clinical development into a phase 2 trial (Delnomdedieu, Forsberg et al. 2017). Amyloid PET has found a useful role in Alzheimer's disease in both experimental and clinical use (Laforce, Soucy et al. 2018), and a ligand capable of binding some pathogenic form of mutant huntingtin protein could provide a valuable PET biomarker for relevant pathology and regional brain tissue target engagement in huntingtin lowering studies (MacDonald, Borrowsky et al. 2015).

Other imaging techniques such as magnetic resonance spectroscopy (Sturrock, Laule et al. 2015) are helping construct a picture of many of the processes involved in HD neurodegeneration but have been far less extensively studied to date and will require further study in large longitudinal cohorts.

## **Biofluid biomarkers**

Biofluid markers that reflect neuropathology have great potential to track disease change and show measurable responses to therapeutic intervention. Biofluids are also capable of generating precise, reliable quantifications that can be processed in bulk, performed retrospectively and a single sample can produce results for multiple analytes of interest.

Cerebrospinal fluid (CSF), which is enriched with brain-derived substances, has been a focus of particular interest, but other biofluids have the potential to yield relevant biomarkers if their composition reflects that of the CNS. All biofluids, including CSF, may reflect peripheral as well as central disease-related changes, especially since mHTT is ubiquitously expressed.

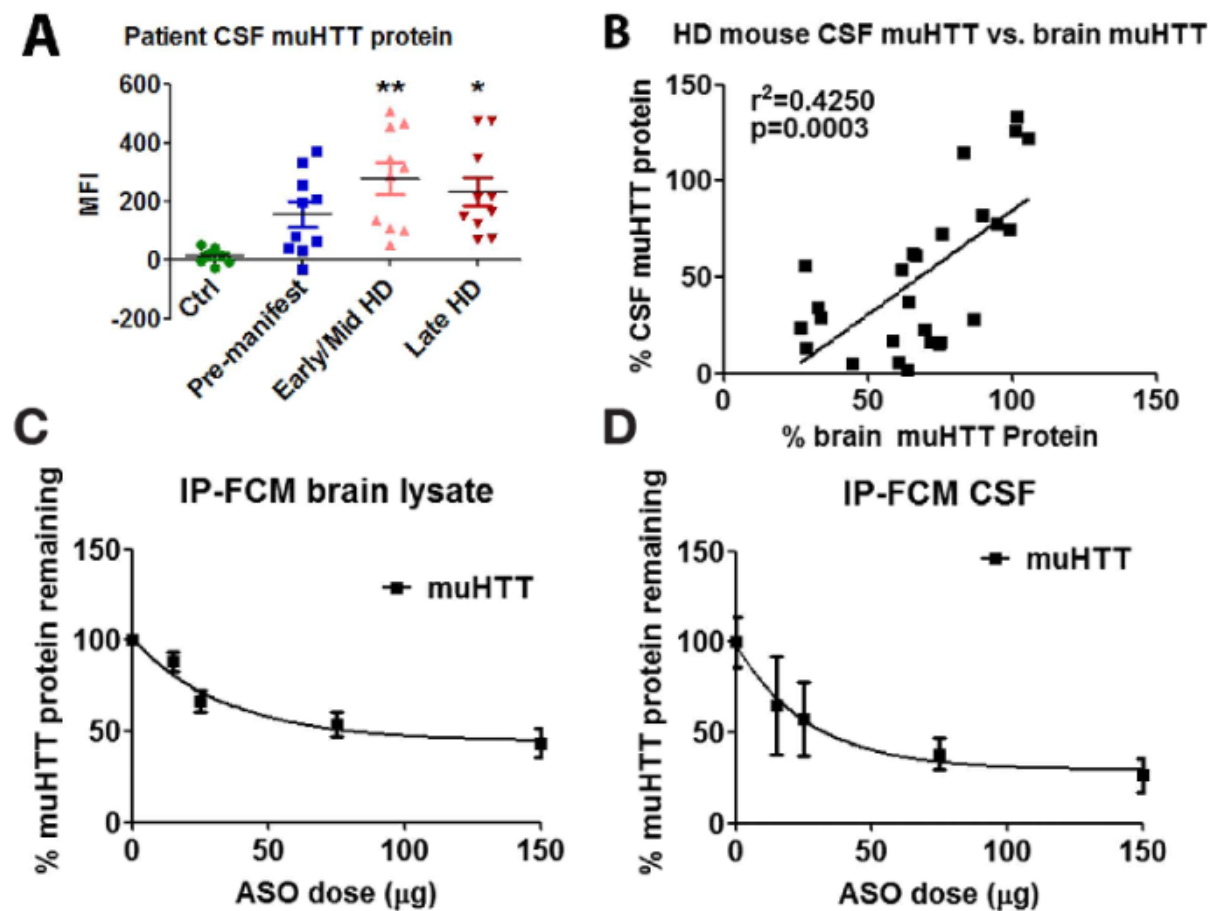
New ultra-sensitive assays nonetheless offer the prospect of accurate quantification of CNS-derived substances in peripheral biofluids.

## **Huntingtin**

The central role of mHTT in HD pathogenesis makes it a key potential biomarker of interest. It is the pathogenic agent itself, while for huntingtin-lowering it is an important measure of pharmacodynamics, i.e. whether the drug has successfully engaged with its target and exerted the desired immediate biological effect (Rodrigues and Wild 2018). CSF mHTT can be quantified with a femtomolar-sensitive “single molecule counting” (SMC) immunoassay (Wild, Boggio et al. 2015). Mutant HTT levels also correlate with clinical scores cross-sectionally as well as with CSF tau and neurofilament light chain (NfL) (Wild, Boggio et al. 2015, Byrne, Rodrigues et al. 2018), both measures of neuronal damage, suggesting that mHTT is released from damaged or dying neurons. CSF mHTT was recently shown to be highly stable within individuals over short intervals (Byrne, Rodrigues et al. 2018). This assay uses the 2B7 antibody, which binds the very N-terminus of HTT, for capture, and the MW1 antibody, which binds to polyglutamine tracts, for detection. It has since been validated to a

high standard along the guidelines for regulatory approval (Fodale, Boggio et al. 2017) and is in use in multiple clinical trials.

Using a microbead-based immunoprecipitation and flow cytometry method (IP-FCM), Southwell *et al.* (Figure 4) demonstrated increased CSF mHTT after striatal quinolinic acid lesioning and reduced mHTT following administration of a HTT-lowering antisense oligonucleotide (ASO) (Southwell, Smith et al. 2015), affirming that CSF mHTT level reflects that in the brain and responds to neuronal damage and HTT lowering.



**Figure 4. mHTT quantification in HD mutation carriers and in a mouse model with ASO administration.** CSF mHTT positively correlates with brain levels after treatment with HTT-lowering anti-sense oligonucleotide (ASO). The IP-FCM mHTT assay confirmed (A) mHTT can distinguish between controls and gene carriers; (B) CSF and brain mHTT levels are strongly correlated in a mouse model and a dose-dependent reduction in brain (C) and CSF (D) mHTT levels with increasing ASO dose. Adapted from Southwell et al. 2015 under creative commons license.

CSF mHTT concentration using the 2B7-MW1 assay was recently used to demonstrate successful target engagement and dose-dependent CSF mHTT lowering of as much as 60% in a phase 1/2a study of the huntingtin ASO HTT<sub>Rx</sub>/RG6042 (clinicaltrials.gov NCT02519036

2015, Rodrigues and Wild 2018, Tabrizi 2018). On the basis of this pharmacodynamic biomarker success, a phase 3 pivotal trial of RG6042 was recently announced (Schobel, Palermo et al. 2018).

The performance of CSF mHTT alongside other biofluid biomarkers was recently evaluated in the 80-participant HD-CSF study. CSF mHTT levels were significantly higher in manifest than premanifest individuals and were able to completely discriminate between healthy controls and HD mutation carriers. Age-adjusted mHTT levels correlated with motor and cognitive scores. However, these associations did not survive additional adjustment for CAG repeat length, and mHTT did not correlate significantly with brain volumes. It seems that notwithstanding its undeniable importance as the cause of HD, CSF mHTT alone may not be the most useful single biomarker of clinical state or progression (Byrne, Rodrigues et al. 2018).

Replication of these results in longitudinal studies will be required to examine the clinical predictive power of CSF mHTT. Further work to enable quantification of wild-type huntingtin will be important to evaluate the precision of allele-selective huntingtin lowering therapies currently in clinical trials (clinicaltrials.gov NCT03225846 2018). Different antibody combinations could also identify more pathogenic forms of mHTT.

### **Neurofilament light protein**

Neuronal injury and death is the final common pathway of the many strands of HD pathogenesis. Biofluid biomarkers of neuronal damage may prove more sensitive to disease progression than more upstream biomarkers.

Neurofilament light (NfL), a protein of the axonal cytoskeleton, is a non-specific marker of neuronal damage with elevated NfL levels reported across a spectrum of neurological conditions (Khalil, Teunissen et al. 2018). Detectable using enzyme-linked immunosorbent assay (ELISA), CSF levels are elevated in both premanifest and manifest HD (Constantinescu, Romer et al. 2009, Niemela, Landtblom et al. 2017, Byrne, Rodrigues et al. 2018) and also closely associated with CSF mHTT, suggesting both proteins are released together from

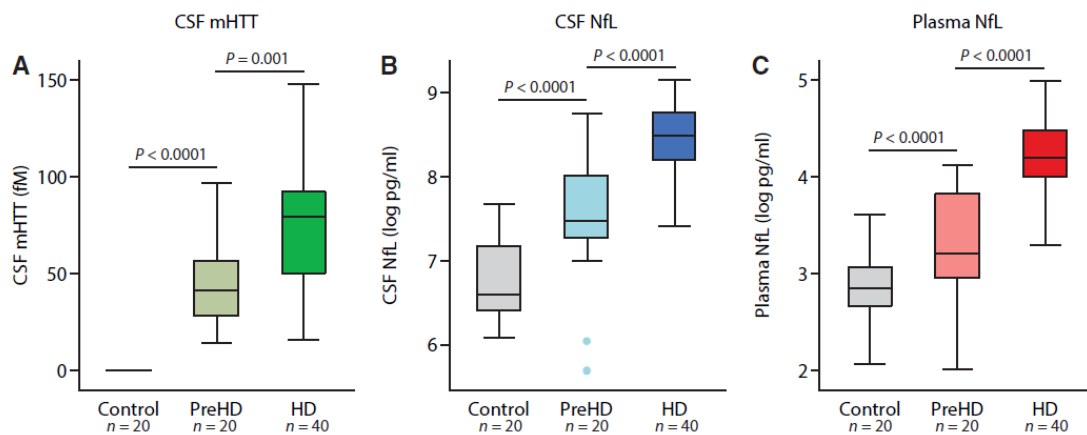


damaged neurons (Wild, Boggio et al. 2015, Byrne, Rodrigues et al. 2018). Cross-sectionally, CSF NfL levels correlate with disease stage, motor and cognitive impairment and functional impairment in HD (Vinther-Jensen, Bornsen et al. 2016, Niemela, Landtblom et al. 2017, Byrne, Rodrigues et al. 2018). CSF NfL has also been shown to correlate with whole brain, white matter, grey matter and caudate volumes (Byrne, Rodrigues et al. 2018).

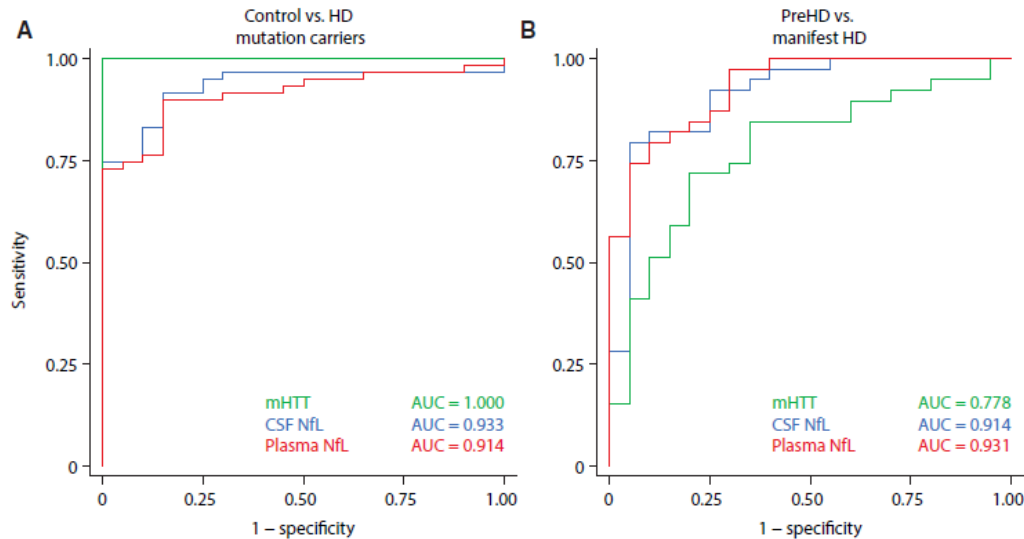
NfL is also detectable at lower levels in blood using a single-molecule 'Simoa' assay and has been shown to closely correlate to CSF NfL, implying a CNS origin of NfL detected in plasma (Byrne, Rodrigues et al. 2017, Byrne, Rodrigues et al. 2018). In the TRACK-HD cohort, plasma NfL levels rose with every subsequent disease stage compared to the control group and were closely associated with CAG repeat length, with higher CAG lengths associated with earlier and steeper increases in plasma NfL. Plasma NfL rose significantly during the 3-year follow period in mutation carriers. Baseline plasma NfL predicted disease onset within 3 years in premanifest subjects, the first time a biofluid marker has shown such predictive value. Baseline values also predicted subsequent change in cognitive and functional measures and brain atrophy, beyond the known predictive power of age and CAG repeat length (Byrne, Rodrigues et al. 2017). Using voxel-based morphometry to further characterise associations with regional brain volumes, plasma NfL predicted occipital grey matter atrophy and widespread white matter reduction, independently of age and CAG length (Johnson, Byrne et al. 2018).

In the HD-CSF head-to-head comparison with mHTT, NfL in both plasma and CSF was significantly better at discriminating between premanifest and manifest HD than CSF mHTT (Figure 6.). After adjustment for age and CAG repeat, NfL measures showed stronger independent predictive ability than mHTT for clinical measures and unlike mHTT, CSF NfL was strongly associated with all brain volume measures. All clinical and imaging correlations were stronger for CSF and plasma NfL than for mHTT. Like mHTT, both CSF and plasma NfL were highly stable within individuals, in keeping with the notion that neuronal damage, rather than the presence of mHTT per se, is the direct determinant of clinical progression and brain atrophy (Byrne, Rodrigues et al. 2018). Though cross-sectional, these results support NfL as a robust biomarker of neuronal damage and HD progression, but longitudinal

study across different biofluids will be required to determine the relative value of plasma and CSF NfL as well as their response to disease-modifying treatments.



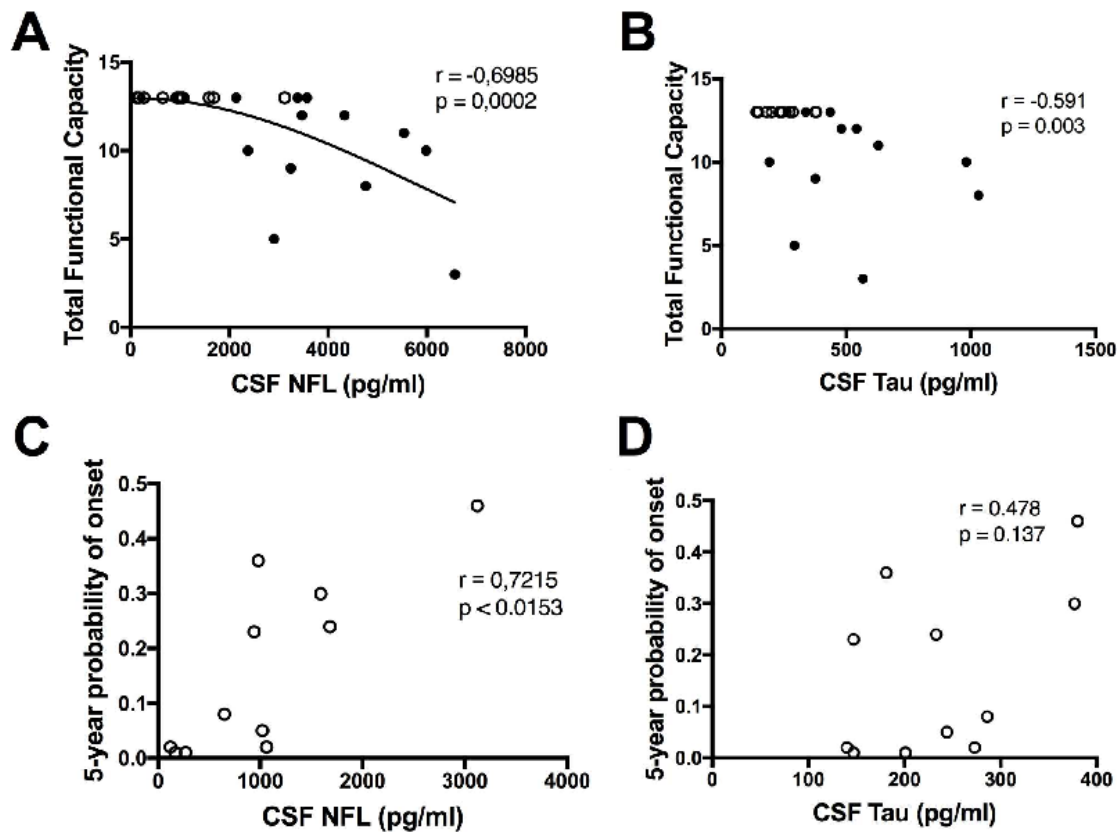
**Figure 5. Comparison of NfL and mHTT concentrations across disease stage.** Concentration of (A) CSF mHTT, (B) CSF NfL, and (C) plasma NfL in healthy controls, premanifest and manifest HD patients. NfL values are natural log-transformed. P values generated from multiple linear regressions and are Bonferroni-corrected. Reprinted from Byrne et al. 2018 with permission from Elsevier.



**Figure 6. Parallel assessment of CSF mHTT, CSF and plasma NfL on diagnostic ability.** Receiver operating characteristic (ROC) curves for (A) discrimination between controls and HD mutation carriers (95% CIs for area under the curves (AUCs): CSF mHTT, 1.000 to 1.000; CSF NfL, 0.876 to 0.989; plasma NfL, 0.852 to 0.976) and (B) discrimination between premanifest and manifest HD (95% CIs for AUCs: CSF mHTT, 0.650 to 0.900; CSF NfL, 0.831 to 0.996; plasma NfL, 0.869 to 0.993). This ROC analysis shows no significant difference between CSF and plasma NfL in discriminating between disease groups. Whilst CSF mHTT was superior to both CSF and plasma NfL in distinguishing between controls and mutation carriers, NfL in either CSF or plasma surpassed mHTT in discriminating between premanifest and manifest HD. Reprinted from Byrne et al. 2018 with permission from Elsevier.

## Tau

Tau is an axonal protein that promotes microtubule assembly and stability (Zetterberg 2017). It is normally secreted from neurons into the brain interstitial fluid and subsequently the CSF. Total tau levels increase in response to acute brain injury (Hesse, Rosengren et al. 2001, Zetterberg, Hietala et al. 2006) suggesting that tau levels may reflect non-specific neuronal damage. Abnormal phosphorylation and truncation of tau may also promote neuronal damage by causing disassembly of microtubules and impaired axonal transport (Mandelkow and Mandelkow 2012). Abnormally phosphorylated tau inclusions are found in the HD brain, indicating that tau may also directly contribute to pathogenesis (Vuono, Winder-Rhodes et al. 2015).



**Figure 7. Correlations of NfL and Tau with disease progression.** CSF NfL shows stronger correlation with clinical measures such as total functional capacity (A) and 5-year probability of disease onset (C) than CSF tau (B and D). Adapted from Niemela et al. 2017, under creative commons license.

By comparison with NfL, tau studies have produced less consistent results in HD. CSF tau levels have been reported to be significantly elevated in manifest HD carriers (Constantinescu, Romer et al. 2011, Rodrigues, Byrne et al. 2016) and correlated with

motor, cognitive and functional measures after adjustment for age and disease burden (Rodrigues, Byrne et al. 2016). However, others have failed to find significant differences in CSF tau between manifest HD groups against controls after age adjustment (Vinther-Jensen, Bornsen et al. 2016). Phosphorylated tau has been reported to be lower in premanifest HD compared to controls after adjusting for age before rising in manifest disease but there was no significant difference after adjusting for age (Niemelä, Burman et al. 2018). A head to head comparison of NfL with tau confirmed that NfL is more strongly correlated with disease phenotype (Figure 7) (Niemela, Landtblom et al. 2017).

### **Inflammatory markers**

Central and peripheral immune system hyperactivity driven by the effects of mHTT in monocytes and microglia has been implicated in HD pathogenesis (Björkqvist, Wild et al. 2009) and identified as a potential therapeutic target (Wild and Tabrizi 2014).

Elevated levels of proinflammatory cytokine IL-6 have been reported in HD (Dalrymple, Wild et al. 2007, Bjorkqvist, Wild et al. 2008, Chang, Wu et al. 2015). In one study of 194 premanifest and manifest HD subjects, elevated plasma levels were found, on average, 16 years from predicted onset of clinical symptoms (Bjorkqvist, Wild et al. 2008). Inverse correlations to the UHDRS TFC (Chang, Wu et al. 2015) and cognitive tests (Bouwens, van Duijn et al. 2016) have also been reported. Since IL-6 has been noted to cross the blood brain barrier (Banks, Plotkin et al. 1995) and CSF levels are reported to correlate with serum levels (Bjorkqvist, Wild et al. 2008), it is uncertain whether such increases reflect parallel peripheral and CNS activation or peripheral activation alone.

YKL-40, also called chitinase-3-like 1 (CHI3L1) is a poorly-understood inactive enzyme associated with astrocytes and microglia (Bonneh-Barkay, Bissel et al. 2012) and has been implicated in various neurodegenerative and neuroinflammatory conditions (Baldacci, Lista et al. 2017). CSF levels of YKL-40 have shown mixed results in HD to date. Two studies reported no significant differences between controls and premanifest/manifest HD after adjustment for age (Vinther-Jensen, Bornsen et al. 2016, Niemelä, Burman et al. 2018). However the latter study found that levels correlated with motor and cognitive clinical

measures and predicted 5 year onset (Niemelä, Burman et al. 2018). A separate study found a strong association with motor and functional scores and disease stage independent from disease burden score and other markers of neuronal damage (Rodrigues, Byrne et al. 2016).

Other immune markers involved in inflammatory response pathways such as clusterin, CRP and complement components have also been studied in HD without a clear inflammatory biomarker emerging (Dalrymple, Wild et al. 2007, Silajdzic, Rezeli et al. 2013). This may reflect a lack of proper replication studies or a general limitation in immune markers, in that they are liable to be influenced by infections or other processes unrelated to HD pathology. It also remains uncertain whether these changes represent primary peripheral abnormalities due to the ubiquitous expression of mHTT, transfer of inflammatory molecules across the blood brain barrier or both. These limitations restrict the utility of such immune markers in HD. They could however have a role in evaluating the relative impact of various immune pathways on HD progression at different stages of disease or as a proof of target engagement for an anti-inflammatory drug.

### **Kynurenine pathway metabolites**

The kynurenine pathway (KP) is involved in the oxidative metabolism of tryptophan in the microglia and its metabolites, some of which can be excitotoxic, have been suggested to play a key role in HD pathology (Campesan, Green et al. 2011). Inhibition of kynurenine monooxygenase (KMO) is a longstanding therapeutic target in HD (Giorgini, Guidetti et al. 2005).

CSF studies of the KP have shown alterations in tryptophan and metabolites kynurenic acid and quinolinic acid in HD (Beal, Matson et al. 1990, Heyes, Swartz et al. 1991, Heyes, Saito et al. 1992). However all published CSF studies precede the discovery of the HD gene and also used inconsistent methods and sample collection procedures. In blood, increased kynurenine-tryptophan ratio and decreased tryptophan has been observed in manifest but not premanifest HD, suggesting a greater conversion of tryptophan to kynurenine in patients which becomes more prominent in the later stages of HD (Stoy, Mackay et al. 2005, Forrest, Mackay et al. 2010). These findings are also consistent with evidence from post-

mortem and animal models (Pearson and Reynolds 1992, Jauch, Urbanska et al. 1995, Guidetti, Reddy et al. 2000). Complicating matters further, kynurenine metabolites have differential abilities to cross the blood brain barrier. KP metabolites therefore remain biomarkers of interest in HD, but informative study will be required with high-quality CSF and matched blood samples from large well-characterised cohorts of HD and matched control samples for comparison.

### **Oxidative stress**

Evidence from studies in both human and animal models has suggested mitochondrial dysfunction and oxidative stress may play a role in HD pathogenesis (Browne, Ferrante et al. 1999, Johri and Beal 2012). 8OH2'dG is a product of the oxidation of guanine by reactive oxygen species (Beckman and Ames 1997) and has been one of the most widely studied marker of oxidative stress in HD with some conflicting early results (Montine, Beal et al. 1999, Hersch, Gevorkian et al. 2006, Biglan, Dorsey et al. 2012, Long, Matson et al. 2012). However a rigorously conducted large scale study using an improved assay established that 8OH2'dG in blood is not a disease marker of HD (Borowsky, Warner et al. 2013). Elevated levels of F2-isoprostanes, a product of lipid peroxidation, have been reported in the CSF of HD patients compared to healthy controls, (Montine, Beal et al. 1999) but a further investigation from the same group using blood and urine samples could not replicate this finding (Montine, Shinobu et al. 2000).

The disappointing performance of oxidative stress markers may reflect, in part, a paucity of larger studies on well-characterised cohorts, or the non-specific nature of oxidative stress in HD disease pathogenesis.

### **Neuropeptide and neuroendocrine markers**

Measuring neuropeptides specific to neuronal populations known to be prominently affected in HD provides another avenue for biomarker discovery. Neuropeptide Y (NPY) is ubiquitous in the CNS and is expressed by striatal neurons. Increased CSF levels of neuropeptide Y have been reported in early HD (Wagner, Bjorkqvist et al. 2016) compared to controls. CSF orexin (hypocretin) levels are unaltered in HD patients (Gaus, Lin et al. 2005, Meier, Mollenhauer et al. 2005, Bjorkqvist, Petersen et al. 2006, Roos and Aziz 2007) despite

the documented loss of orexin expressing neurons in HD (Gabery, Murphy et al. 2010). Cocaine- and amphetamine-regulated transcript (CART) is produced by several brain regions including the hypothalamus and is involved in regulation of energy homeostasis. Elevated CSF levels of CART have been reported in a small sample of early-mid stage HD (Bjorkqvist, Leavitt et al. 2007). The positive findings for NPY and CART have yet to be replicated in larger studies or studies of premanifest populations.

Brain-derived neurotrophic factor (BDNF) is a pro-survival factor produced by cortical neurons that is necessary for striatal neuron survival (Zuccato, Ciammola et al. 2001). Serum levels have been reported to be decreased in both premanifest (Squitieri, Cannella et al. 2009) and manifest HD and correlated with CAG repeat length, motor and cognitive scores (Ciammola, Sassone et al. 2007). However these findings were not corroborated in a larger study of 398 individuals, which found no difference in serum or plasma BDNF between HD and controls (Zuccato, Marullo et al. 2011). Another moderately sized study has also reported no significant differences in plasma between HD and controls (Wang, Ross et al. 2014). The conflicting results may be partly explained by significant inter-assay variations in serum and the influence of sample collection and preparation on BDNF stability (Zuccato, Marullo et al. 2011). Furthermore BDNF is also produced peripherally by megakaryocytes and platelets which may dilute any CNS changes. No study has yet explored BDNF in CSF.

Given the widespread expression of mHTT (Li, Schilling et al. 1993) (van der Burg, Bjorkqvist et al. 2009), alterations of Neuroendocrine and metabolic markers could reflect the dysfunction of neuronal populations involved in endocrine loops, susceptibility of peripheral tissues to mHTT, or both (Sathasivam, Hobbs et al. 1999). Melatonin is a light-sensitive hormone predominantly secreted by the pineal gland and displays a circadian rhythm with levels peaking at night. It has a key role in the sleep-wake cycle which is disrupted early in the course of HD (Lazar, Panin et al. 2015). One preliminary study of 24-hour secretion profiles in 9 early HD patients found no difference in mean diurnal melatonin levels from controls, although the timing of the evening rise in melatonin was significantly delayed (Aziz, Pijl et al. 2009). A larger study reported mean and peak melatonin levels were significantly reduced in manifest HD subjects compared to controls (Kalliolia, Silajdzic et al. 2014).

The hypothalamus is known to be affected by HD, with significant neuronal loss reported in the lateral tuberal nucleus (LTN) of the hypothalamus (Kremer, Roos et al. 1990, Gabery, Murphy et al. 2010). Although Kremer *et al.* reported up to 90% of neuronal loss in the LTN using semi-quantitative measures, a subsequent report using systematic stereological analysis found a more conservative 32% reduction in LTN neuronal populations (Gabery, Murphy et al. 2010). Given its central role in the hypothalamic-pituitary-adrenal axis, several studies have investigated relevant hormones and neuroendocrine peptides in this axis that can be measured in biofluids. Conflicting results in cortisol measurements have been reported (Shirbin, Chua et al. 2013, Hubers, van der Mast et al. 2015, Kalliolia, Silajdzic et al. 2015). Similarly, some studies have reported significantly altered levels of growth hormone in HD, (Durso, Tamminga et al. 1983, Saleh, Moutereau et al. 2009) but others contradicted this finding (Levy, Carlson et al. 1979, Murri, Iudice et al. 1980, Kalliolia, Silajdzic et al. 2015).

There have been conflicting reports regarding the involvement of the hypothalamic paraventricular nucleus (PVN) (Gabery, Murphy et al. 2010, van Wamelen, Aziz et al. 2012) whose neurons express oxytocin and vasopressin. Intranasal oxytocin administration has been shown to normalise HD brain activity in response to the emotion of disgust, which may implicate oxytocin deficiency in impaired emotion recognition that has been widely described in premanifest and manifest HD (Tabrizi, Langbehn et al. 2009, Labuschagne, Jones et al. 2013). CSF vasopressin levels have been recently implicated in social impairments and autism (Parker, Garner et al. 2018). Vasopressin is a potential therapeutic target for neuropsychiatric symptoms associated with HD and a selective vasopressin receptor antagonist is currently in a phase 2 trial to treat irritability in HD (clinicaltrials.gov NCT02507284). There are no published studies of oxytocin or vasopressin as a HD marker to date, although both may have potential as a biomarker and warrant further study.

The inconsistent results of neuroendocrine markers attest to the complexity of understanding the role of such markers in HD. Since they form part of a larger endocrine loop, they are influenced by multiple other hormones as well as external factors that may relate to the environment, such as stress, or treatment and other medications that are not directly related to HD pathology. Further, they also often follow specific circadian rhythms and so can mandate 24-hour sample collections which are logistically more challenging. Nevertheless, they do represent both central and peripheral processes and could be used as



pharmacodynamic markers rather than of progression. Like many other proposed markers, proper validation studies of the assays and the reporting of disease-related changes in larger well-defined cohorts is currently lacking.

### Leading candidate biomarkers and future directions in biomarker research

Imaging and biofluid biomarkers are complementary, not mutually exclusive. Each potential biomarker illuminates the complexity of HD from a different angle. Among imaging measures, volumetric striatal measures have consistently demonstrated the largest effect sizes for baseline measurements and rate of change across all disease stages. Grey matter volumes, given the accelerated rate of change that occurs near onset and their positive predictive value, may have a particularly important role in trials of premanifest HD subjects who are close to predicted onset where the endpoint is rates of conversion to manifest HD.

Structure	Method	Direction	Effect sizes
<b>Caudate Volume</b>	Manual segmentation	C < PM < M	1.5-3.0
<b>Putamen Volume</b>	Automated segmentation	C < PM < M	1.0-1.5
<b>White Matter Volume</b>	Voxel based morphometry	C < PM < M	0.6-2.8
<b>Grey matter volume</b>	Voxel based morphometry	C < PM < M	0.2-1.5

**Table 1. Leading imaging biomarker candidates for HD.** Effect sizes are from the TRACK-HD study (Tabrizi, Scahill et al. 2013). C, healthy controls. PM, premanifest HD. M, manifest HD.

CSF mHTT levels will remain an important source of target engagement for huntingtin lowering strategies, and future huntingtin assays will likely enable relative quantification of mutant and wild-type huntingtin and offer better predictive power for clinical severity. Meanwhile, NfL levels have clear potential for early and sensitive detection of alterations in HD.

Studies combining multiple imaging and biofluid biomarkers offer valuable insights. The temporal sequence in which measured variables become abnormal can be assessed using data-driven statistical models. In one such example using an event-based model (Figure 8) of the HD-CSF cohort, CSF mHTT was the earliest detectable change, followed by plasma and CSF NfL, caudate volume, TMS and global brain volumes (Byrne, Rodrigues et al. 2018).

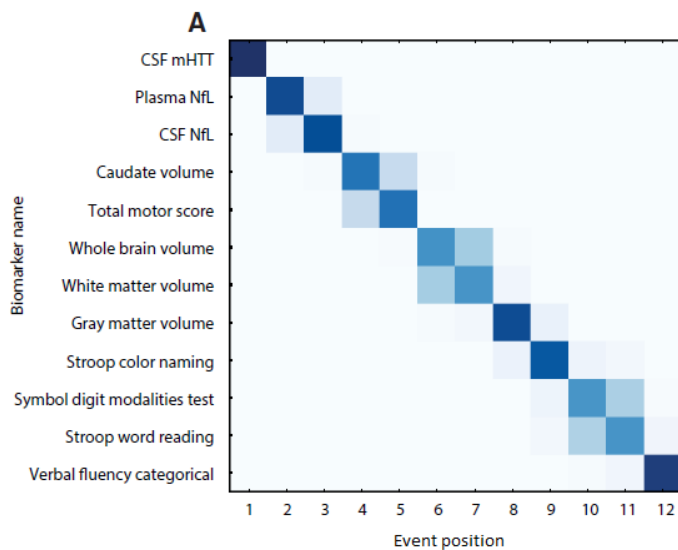
These findings show how such measures might be combined for patient stratification to enrich future preventative clinical trials in HD.

Biofluid	Method	Study	Number	Direction	Correlates with
<b>mHTT</b>	Singulex SMC immunoassay, CSF	(Byrne, Rodrigues et al. 2018)	80	C < PM < M	TFC TMS Cog
<b>NfL</b>	CSF, ELISA	(Byrne, Rodrigues et al. 2018)	80	C < PM < M	TFC TMS Cog Imaging
<b>NfL</b>	Blood, Simoa	(Byrne, Rodrigues et al. 2018)	80	C < PM < M	TFC TMS Cog
<b>Tau</b>	CSF, ELISA	(Rodrigues, Byrne et al. 2016)	67	C < HD	TFC TMS Cog
<b>P-Tau</b>	CSF, ELISA	(Niemelä, Burman et al. 2018)	52	C > PM	DBS TMS
<b>IL-6</b>	CSF, ELISA	(Bjorkqvist, Wild et al. 2008)	194	C < PM < M	
<b>YKL-40</b>	CSF, MSD antibody-based tetraplex array	(Rodrigues, Byrne et al. 2016)	37	C < HD	TFC TMS

**Table 2. Leading biofluid biomarker candidates for HD.** For each biomarker, one exemplar study is detailed for reference. C, healthy controls. PM, premanifest HD. M, manifest HD. mHTT, mutant huntingtin. NfL, neurofilament light. P-tau, phosphorylated tau. IL-6, interleukin-6. SMC, single molecule counting. Simoa, single molecule array immunoassay. MSD, meso scale discovery. TFC, total functional capacity. TMS, total motor score. Cog, cognitive impairment.

Methodologies and disease-related findings both require validation, particularly if they are to be considered as clinical trial endpoints. For biofluid analytes, the European Medicines Agency (EMA) and Food and Drug administration (FDA) provide guidelines on how to validate an assay to ensure it will be robust and methodologically sound (European Medicines Agency 2011, US Department of Health and Human Services 2018). To date, assays for HTT and the protein markers of neuronal damage such as NfL are the closest to validation of all biofluid biomarkers reviewed here and are already being used in clinical trials as exploratory endpoints (clinicaltrials.gov NCT02519036 2015, clinicaltrials.gov NCT03225846 2018). The HD Regulatory Science Consortium (HD-RSC), recently convened

by the Critical Path Institute, aims to consolidate evidence and expertise to facilitate regulatory approval for HD biomarkers and treatments (<https://c-path.org/programs/hdrsc/>).



**Figure 8. Comparison of the temporal order of biofluid analytes, clinical and imaging measures.** This positional variance diagram was produced by event based modelling (EBM) applied to 63 HD-CSF participants (controls, 15; premanifest HD 16; manifest HD, 32) who had data for all biomarkers. The diagram represents the sequence of “events” (individual measures going from normal to abnormal, identified by the EBM). Darker squares represent higher certainty of the biomarker becoming abnormal at the corresponding event. Multiple coloured event boxes indicate more uncertainty about its position. Horizontal axis denotes event position with 1 the earliest event. This suggests that biofluid analytes mHTT and NfL along with caudal volumes are among the earliest detectable changes in HD. Reprinted from Byrne et al. 2018 with permission from Elsevier.

Large multi-site, multi-modality observational studies designed for biomarker evaluation, with tight adherence to methodology across sites will be important to address the shortcomings of existing small, single-site studies. TRACK-HD and PREDICT-HD are successful examples of this and have provided a valuable resource for the investigation of biomarkers in blood and volumetric imaging measures. To date, CSF studies have been hampered by low sample numbers and inconsistent collection procedures. HDClarity, a multisite CSF collection initiative for HD, is collecting CSF and blood samples large numbers of subjects across all disease stages as well as healthy controls, and will provide an important community resource to facilitate the validation of both blood and CSF biomarkers for HD (NCT 02855476). So far, matched CSF and blood samples have been collected from nearly 400 HD patients and controls (TRACK-HD Central Coordination, personal communication), which can be obtained for relevant research by any qualified investigator (<http://hdclarity.net>).

Treating HD mutation carriers as early as possible to prevent accumulating damage is desirable, but the earliest tractable changes in HD and the biomarkers that will be most sensitive to these changes remain unknown. To address this, dedicated studies of younger people are urgently required.

## **Conclusions**

Recent progress in HD biomarker research has seen the identification of imaging, CSF and blood measures that have the potential to monitor and predict disease progression and therapeutic response. The most promising of these appear suitable for use to provide target engagement and efficacy readouts over short intervals or in premanifest HD. In the future as effective treatments come to fruition, such biomarkers may be validated as surrogate endpoints, or even in the clinical setting to guide prognostic discussions and treatment decisions in HD. Work currently underway to standardise methods and replicate findings in large-scale cohorts will help deliver on this promise.

## **Declarations of interest**

PZ is an investigator on the IONIS-HTTRx (RG6042) trial. SJT has been on scientific advisory boards with Hoffmann-La Roche Ltd, Ionis Pharmaceuticals, Shire, Teva Pharmaceuticals, GSK, Takeda Pharmaceuticals, and Heptares Therapeutics and is the global principal investigator on the IONIS-HTTRx (RG6042) trial, for which she receives no personal salary or fees. All honoraria for these advisory boards were paid through University College London (UCL) Consultants Ltd—a wholly owned subsidiary of UCL. EJW has participated in scientific advisory boards with Hoffmann-La Roche Ltd, Ionis Pharmaceuticals, Shire, Novartis, and Wave Life Sciences and is an investigator on the IONIS-HTTRx (RG6042) trial. The authors' host clinical institution, UCL Hospitals NHS Foundation Trust, receives funds as compensation for conducting clinical trials for Ionis Pharmaceuticals, Pfizer, and Teva Pharmaceuticals.

## **Acknowledgements**

PZ and RIS receive funding from a Wellcome Trust collaborative award (200181/Z/15/Z). SJT receives grant funding for her research from the Medical Research Council UK, the Wellcome Trust, the Rosetrees Trust, Takeda Pharmaceuticals, Cantervale Limited, NIHR North Thames Local Clinical Research Network, UK Dementia Research Institute, Wolfson Foundation for Neurodegeneration and the CHDI Foundation. EJW has research funding from the Medical Research Council UK, CHDI Foundation Inc, and European Huntington's Disease Network. The authors are supported, in part, by the National Institute for Health Research UCL Hospitals Biomedical Research Centre, the UK Dementia Research Institute and the UCL Leonard Wolfson Experimental Neurology Centre.

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