ARTICLE

Biomarkers for Huntington's Disease: an update

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1.   Introduction

Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative condition caused by a CAG repeat expansion in the gene encoding huntingtin which is characterized by progressive motor impairment, cognitive decline and neuropsychiatric disturbances. There are currently no disease-modifying treatments available to patients, but a number of therapeutic strategies are currently being investigated, chief among them nucleotide-based 'gene silencing' approaches, modulation of huntingtin post-translation modification and enhancing clearance of the mutant protein [1].

In 2008 our previous review highlighted the need to develop and validate biomarkers and provide a systematic head-to-head comparison of such measures. We searched the PubMed database for publications since 2008 which covered each of the subheadings below. We identified from these lists studies which had relevance to biomarker development, as defined in our previous review. Building on a tradition of collaborative research in HD, great advances have been made in the field since that time and we are now in a position to recommend a range of outcome measures with potential to assess efficacy in future therapeutic trials.

2.   Biomarkers for Clinical Trials

2.1 Neuroimaging

It has long been established that neuroimaging can demonstrate changes within the brain in both early HD subjects and premanifest gene carriers, with both regional and global measures sensitive to change from the very early stages. There is now increasing recognition of the role of white matter in the degenerative process, with widespread change detectable more than 15 years before expected disease onset [2;3]. Thanks to large-scale studies such as TRACK-HD [2] and PREDICT-HD [3], we now have evidence that these imaging metrics are able to show reproducible group differences across multiple sites and that change over time can be demonstrated robustly in this slowly progressive disease over periods as short as 12 months, a timeframe which is realistic for clinical trials [4]. The greatest effect sizes have been reported using rates of caudate loss, with global measures also demonstrating great power to detect disease-related change, providing sample sizes which could feasibly be recruited for therapeutic trials [5]. TRACK-HD has also demonstrated the primacy of cross-site standardization and robust quality control measures to ensure consistency of this data.

More recently, imaging modalities other than structural MRI have been proposed as suitable candidates for clinical trials. Diffusion weighted imaging (DWI), which specifically investigates the structural integrity of white matter, has demonstrated change over time in early HD subjects [6], although this finding was not reproduced by a larger study [7]. Functional MRI (fMRI) has the potential to detect disease-related variation in brain activity, showing cross-sectional differences prior to disease onset [8]. However, a recent study failed to show change over a two year period [9]. Whilst fMRI and DWI remain highly promising candidates for probing dysfunctions in neural networks that are the hallmark of premanifest HD, further work is required to investigate their sensitivity to longitudinal change as well as validate measures across multiple sites before the clinical utility of these modalities are established.

Other recent work has shown cross-sectional disease-related differences in motor cortex excitability using transcranial magnetic stimulation [10]. Altered metabolite profiles, suggested of neuronal dysfunction have also been reported in early HD and treatments aiming to improve neuronal health might effectively be assessed using such a metric [11]. Again, further cross-site validation and follow-up data is required to assess the utility of these modalities.

2.2 Quantitative clinical measures

Clinical ratings scales remain the most widely used measures of progression in Huntington's disease. However, due to their large inter-rater variability and floor and ceiling effects, recent work has focused on more objective techniques which may better capture the range of deficits in both early and premanifest stages of disease.

Quantitative motor assessments such as finger tapping, grip force and tongue protrusion tasks, have shown significant cross-sectional effects across the disease spectrum [2]. These measures have also demonstrated robust change over time in early HD subjects [4;12]; effect sizes suggest such metrics may prove useful as outcome measures in clinical trials [5]. However, they are relatively insensitive in the premanifest stage showing small effect sizes longitudinally [12]. Similarly oculomotor and neuropsychiatric assessments which show cross-sectional disease-related deficits [2], appear to be relatively insensitive to longitudinal change over periods of time which would be feasible for clinical trials [5].

Numerous studies have shown cross-sectional cognitive deficits, even many years before disease onset [2;3]. Longitudinal studies have shown change over time in early HD, although effect sizes vary considerably depending on the specific cognitive test employed [5;13]. Sensitivity to change in the premanifest stages is again very limited [5].

2.3 Biofluids

Robust, pathologically relevant changes in accessible biofluids such as blood remain highly desirable. The pathways previously highlighted as suggesting potential biomarkers remain of interest, particularly oxidative, metabolic and inflammatory markers. These require testing in samples derived from multiple populations and over time, to establish their potential utility for future clinical trials. Newly identified biomarker candidates that require further investigation include cholesterol metabolites [14] and indirect markers of transcriptional dysregulation such as the histone component H2AFY [15]. Meanwhile clusterin, which our own work revealed as a possible HD plasma biomarker [16], has since been highlighted as a major genetic modifier and possible blood biomarker of Alzheimer's disease [17].

Accurately measuring levels of mutant (mHtt) and wild-type huntingtin in biosamples will be essential for testing therapeutics aimed at reducing mHtt levels – including promising nucleotide-based 'gene silencing' treatments expected to enter clinical trials in the near future, as well as drugs aimed at altering the handling and removal of mHtt through post-translational modification and proteolysis. Existing Htt assays have suffered from low sensitivity and been restricted to aggregated mHtt. A recently described agarose gel electrophoresis assay ('AGERA') has lowered the threshold for aggregate detection and quantification [18], but the role of aggregates is debated and it is likely that soluble oligomeric mHtt is most toxic. Until recently, the detection of soluble Htt was limited by the intrinsic insensitivity of assays, and a lack of suitable antibodies. Novel antibodies and a fluorescence-enhanced antibody assay using time-resolved Förster resonence energy transfer (TR-FRET) has now yielded a reliable, highly sensitive method of quantifying both mutant and total huntingtin in blood samples [19]. This assay is likely to empower future clinical trials. Meanwhile, attempts are underway to develop assays that are even more sensitive and scalable.

Given its close biochemical and physical relationship with the brain, and utility in other neurodegenerative diseases as a source of biomarkers, cerebrospinal fluid (CSF) has natural appeal, despite being time-consuming and more invasive than phlebotomy. Efforts to identify CSF biomarkers for HD have been hampered by a shortage of samples and lack of consistency between existing small collections. In one major study, proteomic data generated from one sample set by five laboratories were integrated, revealing changes (mainly reductions) in many brain-specific proteins, but raising concerns about consistency between laboratories [20]. Pursuing a hypothesis-driven approach, another group has identified neurofilament light chain and tau as potential neuron-specific CSF markers [21]; but neither provides complete separation from controls or has been studied longitudinally. Multi-site longitudinal CSF collection studies are in the planning stage.

3. Future directions

3.1 Neuroimaging

It is clear from studies such as TRACK-HD and PREDICT-HD that there are striking neuropathological changes up to 15 years prior to the onset of manifest disease [2,3]. Nevertheless, these subjects maintain their performance on functional measures. It would appear that there is a compensatory mechanism within the brain which finally breaks down at disease onset [8]; imaging modalities other than structural MRI will be required to elucidate this process. TrackOn-HD seeks to apply novel imaging techniques across multiple sites, following the premanifest cohort from the TRACK-HD study. Whether these imaging modalities are able to produce metrics which are robust and sensitive enough to be used in clinical trials is yet to be determined.

A means of quantifying wild-type and mutant huntingtin in the brain would be a valuable tool for demonstrating CNS target engagement of huntington-lowering therapies in the main organ of interest, especially since early gene silencing therapeutics are likely to be delivered directly into the CNS rather than systemically. Work is underway to develop and test PET ligands that will enable visualisation of huntington load, as a predictive tool and a means of assessing response to therapy.

3.2 Quantitative clinical measures

Further refinement and validation is required if objective quantitative measures of motor and cognitive function are to demonstrate sensitivity to premanifest disease, or be accepted as outcome measures in clinical trials. A number of cognitive tasks show promise as biomarkers, particularly measures of visuospatial integration [4]. A cognitive 'toolkit' for premanifest and manifest HD, which will build on the existing evidence, is nearing completion [22]. Neuropsychiatric measures are likely to be included in future clinical trials since assessments of patient well-being are of paramount importance, but these too require the development of more reproducible and sensitive methodology. In addition to these initiatives, ongoing longitudinal studies such as PREDICT-HD and TrackOn-HD seek to optimize measures in these different functional modalities.

3.3 Biofluids

Novel assays to measure soluble mutant huntingtin, and their refinement for high-throughput use, is likely to emerge as one of the most important developments in the field and will become a standard measure for clinical trials. The use of CSF as the biofluid of choice for interrogating the CNS milieu is likely to occur in HD as it has in other neurodegenerative diseases, but standardised, multisite, longitudinal CSF natural history studies are needed. Finally, the most promising biomarker candidates need to be studied 'head-to-head' using the large longitudinal blood sample collections that studies such as TRACK-HD have generated.

Though a decade of research has not yielded robust biochemical biomarkers of HD, progress has been made. A consensus is emerging that the detection of neuron-specific proteins in blood may be an unrealistic aim, but since the mutant huntingtin protein is expressed ubiquitously, that does not mean that tracking change in peripheral tissues lacks merit. What is needed is scrupulous study of how any measure is involved in HD pathogenesis, and judicious interpretation of changes seen in the context of therapeutic trials.

Expert Opinion
Considerable progress has been made towards identifying biomarkers which show promise as outcome measures in clinical trials. Multi-national studies such as PREDICT-HD and TRACK-HD have proved invaluable in validating measures which are robust and reproducible across different sites and cultures. The most recent publication from the TRACK-HD study provides a direct comparison of the effect sizes for a range of modalities, and outlines a battery suitable for use in clinical trials in early HD [5]; this includes structural neuroimaging, quantitative motor, cognitive and neuropsychiatric assessments. However, as yet such a battery does not exist for a premanifest cohort as current functional measures appear to be relatively insensitive at this disease stage. Neuroimaging demonstrates the largest effect sizes, but further investigation of the relationship between such metrics and functional performance is required. There is clearly a need for robust functional assessments to be included in any trial and there are now a number of candidate biomarkers from different modalities which can feasibly be implemented. The individual elements of this battery selected for a particular trial will of course depend on the proposed mode of action of therapy, timeframe and cohort. We previously predicted that ultimate evaluation of biomarker candidates would need to take place within the framework of clinical trials of disease-modifying treatments. This model for biomarker development is now deployed in HD clinical trials (e.g. mHtt and interleukins are being measured as part of the PADDINGTON study of sirtuin-1 inhibition) and, though challenging, this approach is essential, since it is only when disease, biomarker and drug are studied together that the true interplay between them can emerge.

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