Huntington disease: natural history, biomarkers and prospects for therapeutics

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Abstract | Huntington disease (HD) can be seen as a model neurodegenerative disorder, in that it is caused by a single genetic mutation and is amenable to predictive genetic testing, with estimation of years to predicted onset, enabling the entire range of disease natural history to be studied. Structural neuroimaging biomarkers show that progressive regional brain atrophy begins many years before the emergence of diagnosable signs and symptoms of HD, and continues steadily during the symptomatic or 'manifest' period. The continued development of functional, neurochemical and other biomarkers raises hopes that these biomarkers might be useful for future trials of disease-modifying therapeutics to delay the onset and slow the progression of HD. Such advances could herald a new era of personalized preventive therapeutics. We describe the natural history of HD, including the timing of emergence of motor, cognitive and emotional impairments, and the techniques that are used to assess these features. Building on this information, we review recent progress in the development of biomarkers for HD, and potential future roles of these biomarkers in clinical trials.

Ross, C. A. *et al. Nat. Rev. Neurol.* **10**, 204–216 (2014), published online 11 March 2014; doi:10.1038/nrneurol.2014.24

Introduction

Huntington disease (HD) is caused by a CAG repeat expansion in the huntingtin (*HTT*) gene on chromosome 4 that codes for polyglutamine in the huntingtin protein. Above a threshold of about 35 or more repeats, the age of HD onset is inversely correlated with the length of the expansion, with variable age-dependent penetrance between 36 and 39 CAG repeats, but full penetrance at 40 or more repeats. In addition, it has been suggested that there may be subtle abnormalities, possibly constituting an endophenotype, in the rare individuals who have repeat lengths in the 27–35 range.[1,2] HD classically manifests with a triad of signs and symptoms, including motor, cognitive and behavioural features.[3,4] According to the current criteria, onset is defined as the point when a person who carries a CAG-expanded *HTT* allele develops "the unequivocal presence of an otherwise unexplained extrapyramidal movement disorder (for example, chorea, dystonia, bradykinesia, rigidity)."[5,6] We add the presence of cognitive disorder as characteristic of HD, and an important contributor to disability. Emotional disorders and personality changes are common and may be a cause of distress, but are not universal, and seem not to progress steadily, as do the motor and cognitive changes.

How we define terms such as 'disease' and 'disability', and how we draw the line between 'normal' and 'abnormal', has long been a point of discussion not only in HD research, but also in the wider fields of medicine, public health, and disability studies. These distinctions have cultural and social as well as biological dimensions. The slow progression of changes initiated by the CAG-expanded *HTT* allele can be usefully considered in the context of recent disability theory. From this perspective, what is currently considered as 'prodromal' and 'early-stage' HD is a period of increasing impairments (biologically based limitations or losses) with environmentally relative disabilities (that is, disadvantage related to the social environment; for example, the inability to drive in a suburban or rural environment, where driving is important for full independence). The prospect of clinical trials for HD increases the need for useful biological benchmarks. Ironically, the closer attention to measurement in the premanifest period also risks enlarging the category of the so-called 'pathological' through more-refined ways of measuring difference, thereby potentially increasing stigmatization and the psychological burden for people at risk. On the other hand, a diagnosis of disease may have some social benefits, conferring legitimacy on symptoms, and opening access to support and services.

In this Review, we begin by outlining the natural history of HD, mapping the emergence of motor, cognitive and emotional disorders. We review the aspects of the disease biology of HD that are relevant to biomarker development. We go on to provide an integrative discussion of the current status of biomarker validation in HD, and the prospects for incorporating these biomarkers into future clinical trials. Biomarkers for HD (Box 1) could aid both cross-sectional assessments and longitudinal monitoring in clinical trials.[7] Cross-sectionally, biomarkers may assist in participant selection and stratification, and

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Competing interests
The authors declare no competing interests.

Key points

- No disease-modifying treatments are currently available for Huntington disease (HD), but clinical trials of potential compounds are imminent; identification of suitable biomarkers to assess therapeutic efficacy is a research priority
- Quantifiable measures of patient function, including motor and cognitive assessments, have shown disease-related change in early HD but still lack sensitivity in premanifest cohorts
- Structural imaging measures such as striatal atrophy show the largest effect sizes both cross-sectionally and longitudinally, and have the potential to track disease progression even in the premanifest period
- Functional MRI and magnetic resonance spectroscopy are also sensitive for detecting change, but have not yet been well-validated longitudinally
- PET imaging is quantitative and shows sensitivity to early premanifest disease, and may be useful longitudinally, but has the disadvantage of being expensive and complex
- Biochemical assays of relevant molecules provide a more direct reflection of disease mechanisms; such measures have not been fully validated, and future work will focus on their development

Box 1 | Biomarker definitions

Biological marker (biomarker)
A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.

Clinical end point
A characteristic or variable that reflects how a patient feels or functions, or how long a patient survives.

Surrogate end point
A biomarker intended to substitute for a clinical end point. A clinical investigator can use epidemiological, therapeutic, pathophysiological or other scientific evidence to select a surrogate end point that is expected to predict clinical benefit or harm, or lack thereof.

Criteria for biomarkers

- Can be objectively measured
- Predicts clinically meaningful end points
- Associated with known disease mechanisms and pathology
- Predicts response to treatment
- Associated with biologically relevant response to treatment

It is important to evaluate biomarkers critically in the context of the disease mechanism. For instance, some have speculated that striatal volumes could be artefactually increased by oedema or inflammation, or even conceivably by administration of large quantities of substances that add bulk to cytoplasm or cell membranes. In these instances, changes in striatal volumes would not reflect disease status, and such measurements could give unreliable or incorrect information about the disease and potential treatments.

statistical covariance for higher power to detect treatment effects. For biomarkers to be useful longitudinally, they must show consistent changes with progression of the disorder, and should predict some aspects of clinical progression. They must also be responsive to therapeutics. Ideally, a biomarker will be close enough to the disease process and sufficiently predictive of future progression that it can be used as a 'surrogate marker' (Box 1).

Natural history of HD

The course of HD can be divided into 'premanifest' and 'manifest' periods (Figure 1). The premanifest period can be further subdivided. Initially, there is a period when individuals are not distinguishable clinically from controls ('presymptomatic'), usually up to 10–15 years before onset. Individuals may then enter the 'prodromal' period, which is characterized by subtle motor, cognitive and behavioural changes. Once motor and cognitive signs and symptoms begin, they progress inexorably over the course of the illness, which—with the exception of late-onset cases, who may die of other causes—is uniformly fatal.

The Unified HD Rating Scale (UHDRS) is currently the most commonly used clinical and research tool for the assessment of HD. This scale includes motor, cognitive, behavioural, emotional and functional components. The clinical assessment of premanifest individuals currently includes a 'diagnostic confidence score' subscale of the UHDRS, which scores the motor examination according to the clinician's belief that the motor signs represent HD, from 0 (no motor abnormalities suggestive of HD) to 4 (motor abnormalities ≥99% likely to be due to HD).[5,8,9] A patient who receives a score of 4 on this scale for the first time, when assessed by an expert rater, is said to have experienced 'motor onset'. The advantage of this model is that amid the considerable clinical phenotypic heterogeneity of the disease, motor onset emerges as one of the more robust and consistently agreed disease features.[5] However, the diagnostic confidence score involves subjective assessment of ambiguous probabilities, and the concept of motor onset, or 'phenoconversion', especially if interpreted simplistically, may suggest a false dichotomy between sick and well, obscuring the fact that disease onset is really a process that occurs gradually over years or even decades.

The manifest HD period is sometimes divided into five stages.[8,9] However, these stages are purely descriptive characterizations based on continuously changing functional capacity rather than on biology. This situation contrasts with many other diseases, such as cancer, in which staging relates to biological events with specific implications for prognosis and treatment. For instance, staging systems for breast or colon cancer are based on events such as conversion of cells to unchecked growth, penetration of the lamina propria, dissemination to lymph nodes, and metastasis to distant locations. These events critically influence prognosis, choice of treatments, and response to those treatments. Without such biological events to determine staging in HD, we think it simpler to divide HD into three broad phases: 'early' (patients are generally still active in most areas of functioning, and are often still working or driving), 'moderate' (patients become unable to perform complex functions such as work, driving or shopping independently, but still take care of activities of daily living [ADLs] and simple household tasks), and 'late' stages (patients can no longer take care of ADLs without help).

The systematic study of HD, leading to the identification of the *HTT* gene, began with the seminal and continuing study of the condition in a very large pedigree in Venezuela.[10,11] Subsequently, HD research has benefited from several longitudinal single-centre and multicentre studies. PREDICT-HD[12] is a large multicentre study with a total of about 800 premanifest HD cases and 200 control individuals, studied by use of clinical, neuropsychological and imaging measures for up to 10 years. TRACK-HD has studied 360 individuals

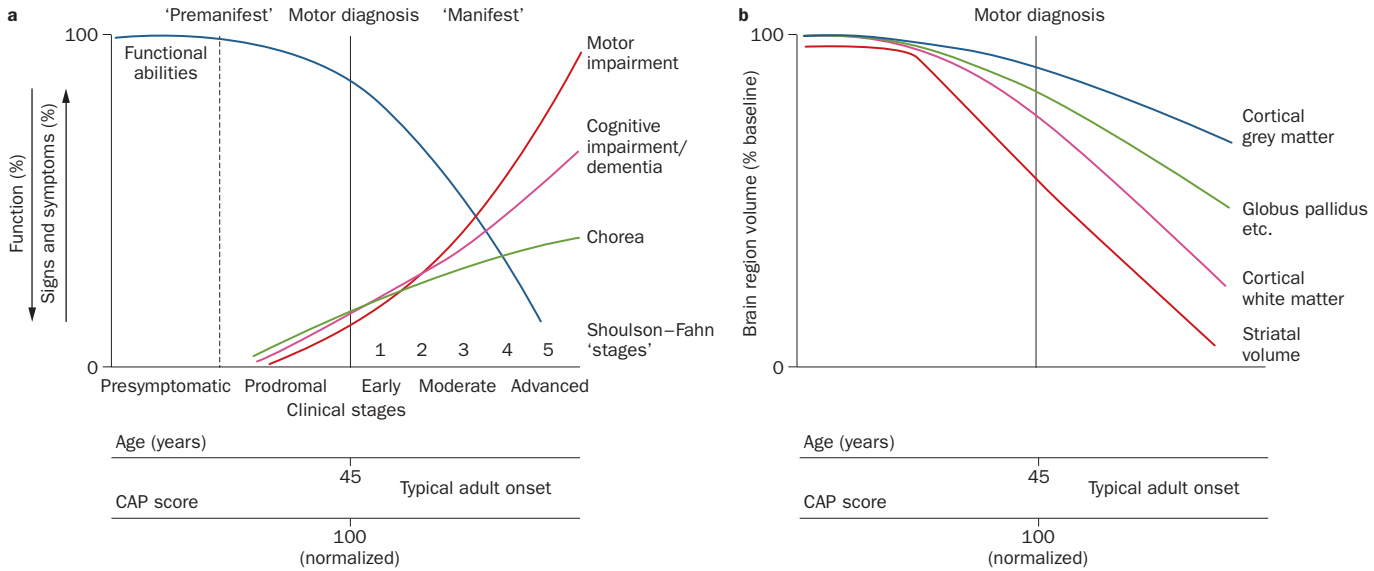

Figure 1 | Natural history of clinical HD, and hypothesized changes in imaging biomarkers. The normalized CAP score (Box 2) enables progression of many individuals with different CAG expansion lengths to be plotted on the same graph. Mean disease onset is at CAP score ~100 (typically ~45 years of age), but substantial inter-individual variability exists. Without 'normalization', the CAP score at onset exceeds 400. **a** | Natural history. The period before diagnosable signs and symptoms of HD appear is termed 'premanifest'. During the 'presymptomatic' period, no signs or symptoms are present. In 'prodromal' HD, subtle signs and symptoms are present. Manifest HD is characterized by slow progression of motor and cognitive difficulties, with chorea often prominent early but plateauing or even decreasing later. Fine motor impairments (incoordination, bradykinesia and rigidity) progress more steadily. **b** | Hypothetical trajectory of several imaging biomarkers (best estimate based on current data: the PREDICT-HD and TRACK-HD studies have not followed individuals across the entire range of HD). The globus pallidus is a representative subcortical structure. Although overall cortical grey matter atrophy occurs at a late stage, there may be more-pronounced cortical layer-specific degeneration earlier. Abbreviations: CAP, CAG age product; HD, Huntington disease.

(120 premanifest HD cases stratified by time to predicted onset, 120 early-stage patients, and 120 matched controls), with extensive annual assessments involving imaging and clinical measures.[13–16] Figure 2 shows the 36-month longitudinal data from TRACK-HD. REGISTRY is the largest multicentre study to date, with over 10,000 participants from 16 countries, though without imaging.[17] A single-site study at Johns Hopkins has followed HD families clinically for over 30 years, with some neuropsychology and imaging, and in many cases has followed individuals through the late stages of the disease to autopsy and neuropathological diagnosis.[18]

The CAP score

The age of clinical onset in HD is highly variable (with a mean of ~45 years), but is strongly influenced by the length of the CAG trinucleotide expansion within the *HTT* gene.[19] The influence of CAG repeat length on rate of disease progression is less strong but still significant.[16,20] To estimate the progression of HD pathology as a function of CAG repeat length and time of exposure to the effects of the expansion, a variable of the form AGE × (CAG – L), where AGE is the current age of the individual, CAG is the repeat length, and L is a constant, was first proposed by Penney *et al.* in 1997.[21] The authors showed that an index of this form was a good predictor of striatal pathology in the brains of HD patients at autopsy.

The terms 'disease burden' and 'genetic burden' have been used to designate the Penney *et al.* version of this statistic, but we prefer the more neutral 'CAG age product' (CAP). A form of CAP score was used in the TRACK-HD study as a premanifest HD entry criterion, and the PREDICT-HD study uses a CAP score at entry to the study to distinguish among patients predicted to be close to, or far from, predicted onset, or somewhere in between, at study entry.[22] For the purposes of this Review, we use a standardized CAP score derived from convergent evidence from several large HD data sources (see Box 2 for derivation of this score). This score provides an index of the length and severity of the individual's exposure to the effects of the mutant *HTT* gene, which is useful for conveying longitudinal data from cohorts of patients with a range of ages and CAG repeat lengths.

In Figure 3, we plot clinical measures from TRACK-HD against the CAP score, and in Figure 4 we plot a variety of clinical measures from the combined data sets of COHORT and REGISTRY against the CAP score.[17,23,24] One question raised by these data is whether there is an acceleration of changes in clinical measures around the time of onset of manifest HD. This issue will need more study in additional data sets, or in current data sets with more-sophisticated models.

Motor disorder

The motor disorder of HD can be divided into two broad components. The first component consists of involuntary movements, especially chorea. Chorea is most prominent with adult-onset or late-onset HD, begins early in

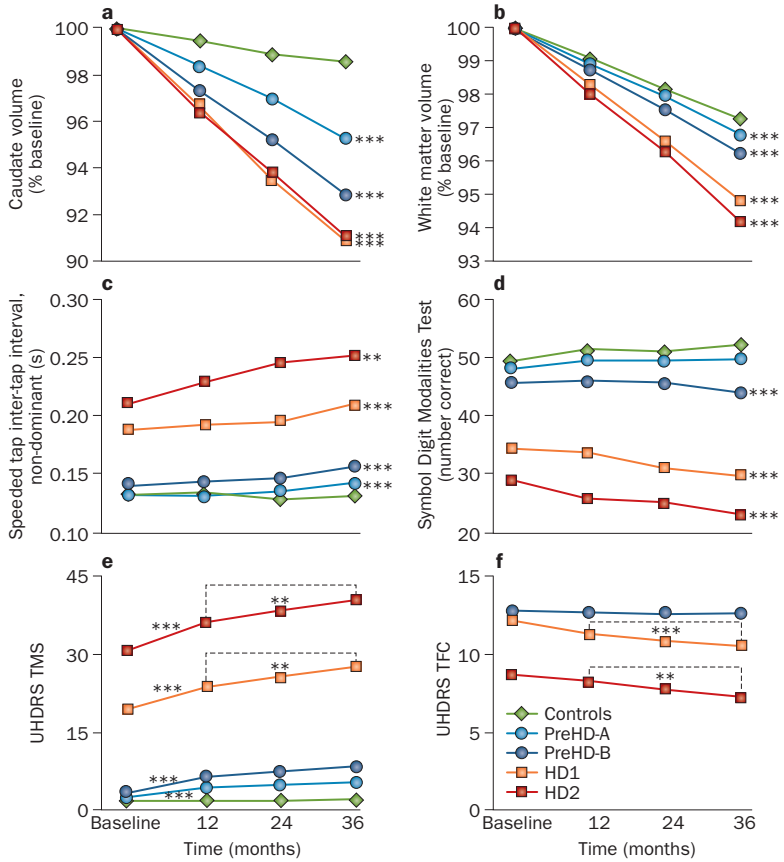

Figure 2 | Longitudinal data from TRACK-HD. Examples of the most robust changes in premanifest and early HD identified by TRACK-HD over 36 months of longitudinal study.[16] **a**,**b** | Rates of atrophy. Changes in caudate and white matter volume, seen as statistical parametric maps and presented as atrophy rates by group. **c** | Tapping test to quantify motor function. **d** | Symbol Digit Modalities Test of visual attention and psychomotor speed. **e**,**f** | UHDRS scores. Asterisks refer to levels of significance (***P*<0.01; ****P*<0.001), and dashed lines indicate specific comparisons. Abbreviations: HD, Huntington disease; HD1, early HD; HD2, later-stage HD; PreHD-A, premanifest HD far from onset; PreHD-B, premanifest HD close to onset; TFC, Total Functional Capacity; TMS, Total Motor Score; UHDRS, Unified HD Rating Scale. Reprinted from *The Lancet Neurology* **12**, Tabrizi, S. J. *et al.*, Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data, 637–649 © (2013), with permission from Elsevier.

the course of the disease, and gives HD its characteristic clinical appearance. The second component involves impairment of voluntary movements, and includes incoordination, bradykinesia and rigidity. This component tends to predominate in earlier-onset HD (including juvenile HD, which is quite rare) and in the late stages of the more common adult-onset HD, progresses more steadily than chorea,[25] and also correlates with functional disability better than does chorea.[20]

Clinical assessment of the motor deficits in HD often uses the UHDRS Total Motor Score (UHDRS-TMS).[5] The Hopkins study for many years used the Quantitative Neurological Examination (QNE),[26] a precursor to the UHDRS with different items but similar scoring. The UHDRS motor scale, like the QNE, has ratings for items including eye movements, speech, chorea, dystonia, rapid alternating movements, bradykinesia, and gait. Scores on the UHDRS motor scale range from 0–124. Subtle motor changes begin years before diagnosable HD, and by the time that motor scores reach 15–20, clinicians usually feel confident enough to assign a diagnostic confidence score of 4, indicating manifest HD.

Several measures have been developed to further quantify motor dysfunction. Simple and easily quantifiable measures of motor function can be derived from tapping on a computer keyboard;[12] speed and consistency of tapping represent a simple means to quantify motor performance. More-complex and quantified metronome-paced tapping tests can improve quantification.[27] Specialized force-transducer-based measures can also be used to quantify motor performance, as in the quantitative motor (Q-Motor) battery used in TRACK-HD.[15] Finger tapping (digitomotography) was used to assess disease progression in all subgroups of the TRACK-HD study across 2 years and 3 years.[15,28] Tongue force variability and grip force variability have also been used to quantify motor features in manifest[28–30] and premanifest[28] HD.[31] Q-Motor assessments can potentially be standardized across centres; they can be administered by technical assistants and may be applied repeatedly within a study, although they require specialized equipment, and have not been compared directly with simpler keyboard-based measures.

Cognitive disorder

Cognitive impairments emerge years before diagnosis of HD,[32] and progression of cognitive decline is gradual. In early manifest HD, significant rates of decline are detectable over 12 months in a subset of cognitive tests,[14] and more broadly after 24 months,[33] whereas in premanifest HD, significant rates of cognitive decline are detectable across 36 months, and only in those individuals estimated to be about 10 years or less from diagnosis.[16] Although cognitive decline in HD always occurs, individuals vary with respect to how the cognitive disorder manifests. Some evidence indicates that various aspects of cognition decline at different points in the disease course,[34] although this variability might be partly explained by the fact that measures of some aspects of cognition, such as psychomotor slowing, are more sensitive than are other aspects of cognition, such as executive function. Several of the frequently used 'cognitive' tests (see below) have a substantial motor component.

The profile of cognitive decline in HD bears similarities to other disorders associated with striatal–subcortical brain pathology (for example, vascular dementia and Parkinson disease [PD]), but it differs from Alzheimer disease (AD).[35] Cognitive deficits in HD include cognitive slowing, as well as decreased attention, mental flexibility, planning, visuospatial functions and emotion recognition.[32,33,35] Learning and retrieval of new information are impaired but, in contrast to AD, rapid forgetting is not as pronounced,[36] and language is relatively preserved. Many cognitive deficits in HD occur at the intersection between cognitive and psychiatric realms of function, including problems with initiation, lack of awareness of deficits, and disinhibition.[37] Thus,

Box 2 | CAP score and HD progression

As used in this Review, the CAP score is defined as follows: CAP = 100 × AGE × [(CAG − L) ÷ S], where CAG is the patient's CAG repeat length, AGE is the patient's current age at the time of observation, and L and S are constants. S is a normalizing constant chosen so that the CAP score is approximately 100 at the patient's expected age of onset as estimated by Langbehn *et al.*[144] L is a scaling constant that anchors CAG length approximately at the lower end of the distribution relevant to HD pathology. L has been estimated at slightly different values; for example, Zhang *et al.* use L = 33.66,[22] whereas Penney *et al.* use L = 35.5.[21]

The graphs shown in this Review use L = 30 and S = 627, which are estimates obtained by a reanalysis of the data in Langbehn *et al.*[144] presented by Warner and Hayden.[145] In this respect, it is similar to measures from Langbehn *et al.*[144] related to onset risk. The optimal value of L was also found to be about 30 for correlation with a wide variety of clinical measures as reported by Langbehn *et al.*[146] Intuitively, L might be thought of as the lower limit of the CAG lengths for which some pathological effect might be expected. Direct evidence for detectable HD pathology in the CAG range 30–35 is sparse and controversial, so the exact value of L within this range might be difficult to interpret. Nevertheless, the existence of a striking threshold for pathogenesis is reflected in the equation.

Abbreviations: CAP, CAG age product; HD, Huntington disease.

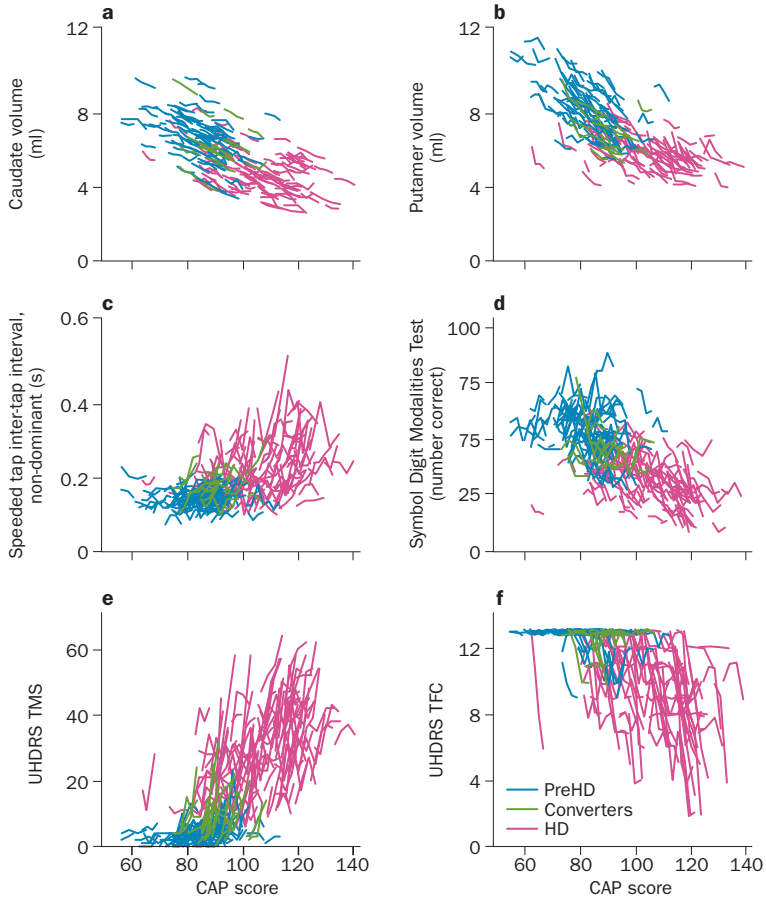

Figure 3 | Change with CAP score of clinical and imaging variables: data from TRACK-HD. **a**,**b** | Striatal volumes. **c** | Tapping test to quantify motor function. **d** | Symbol Digit Modalities Test to assess visual attention and psychomotor speed. **e**,**f** | UHDRS scores. A colour key differentiates longitudinal changes in participants with clinical HD, preHD and observed conversion from preHD to HD ('converters'). Note the very steady change in striatal volumes in all patient groups. Abbreviations: CAP, CAG age product; HD, Huntington disease; preHD, premanifest HD; TFC, Total Functional Capacity; TMS, Total Motor Score; UHDRS, Unified HD Rating Scale.

a typical picture of HD that emerges over time is one of social disengagement, low conversational participation, and slowed mentation, sometimes overlaid with lack of awareness of deficits, and impulsivity.

The number of cross-sectional HD studies far outstrips the number of longitudinal studies, making rates of progression in different aspects of cognition or at different points in disease progression difficult to ascertain. However, in the TRACK-HD study, 10 of 12 cognitive outcomes showed evidence of deterioration in early HD.[14–16] The greatest sensitivity to progression was in the Symbol Digit Modalities Test (visual attention and psychomotor speed), the Circle Tracing Test (visuomotor and spatial integration and transformation), and the Stroop Word Reading Test (psychomotor speed within the spoken context), with effect sizes (compared with controls) of up to 1.00 (95% CI 0.70–1.30). By contrast, in relatively late premanifest HD, a sample of 117 participants showed little evidence of detectable deterioration across 24 months. Many of the tests with the largest effect sizes cross-sectionally, as well as great change longitudinally, have a substantial motor or psychomotor component, emphasizing the close relationship between motor and cognitive features of HD, both of which are presumably linked to cortical–basal ganglial circuits.

Emotional disorders

The emotional features of HD are more variable than are the motor or cognitive features. Depression is common, with depressive symptoms reported in over half of patients.[38] Major depression in HD resembles depression in individuals without HD, and is treated similarly.[39] Irritability is frequently present in HD, and might be an early symptom. Apathy is a characteristic and disabling feature of the disorder, is present in most individuals at least by later stages of the disease, and tends to worsen with time.[38] Strikingly, recent data from TRACK-HD indicate that a significant increase in apathy can be detected even in premanifest individuals over 36 months—this was the most striking single psychiatric indicator that demonstrated clear longitudinal progression.[16] In early HD, baseline apathy scores were a significant baseline predictor of functional decline, and neuropsychiatric symptoms associated with frontal lobe function, such as affect, irritability and apathy, were significantly associated with functional decline in early HD.[16]

Biomarkers for HD

Relevant biology of HD

Many pathogenic mechanisms have been hypothesized for HD, but some are likely to be more relevant than others for biomarker development (Figure 5). HD is potentially a good model for development of biomarkers of direct relevance to pathogenesis, since it is caused by a single gene mutation and has an increasingly well-understood pathogenic pathway. A great need exists for target engagement biomarkers; however, they tend to be treatment-specific, and will not be the focus of this Review. Most attention in the past has focused on the CNS, but it is becoming clear that some peripheral tissues are also

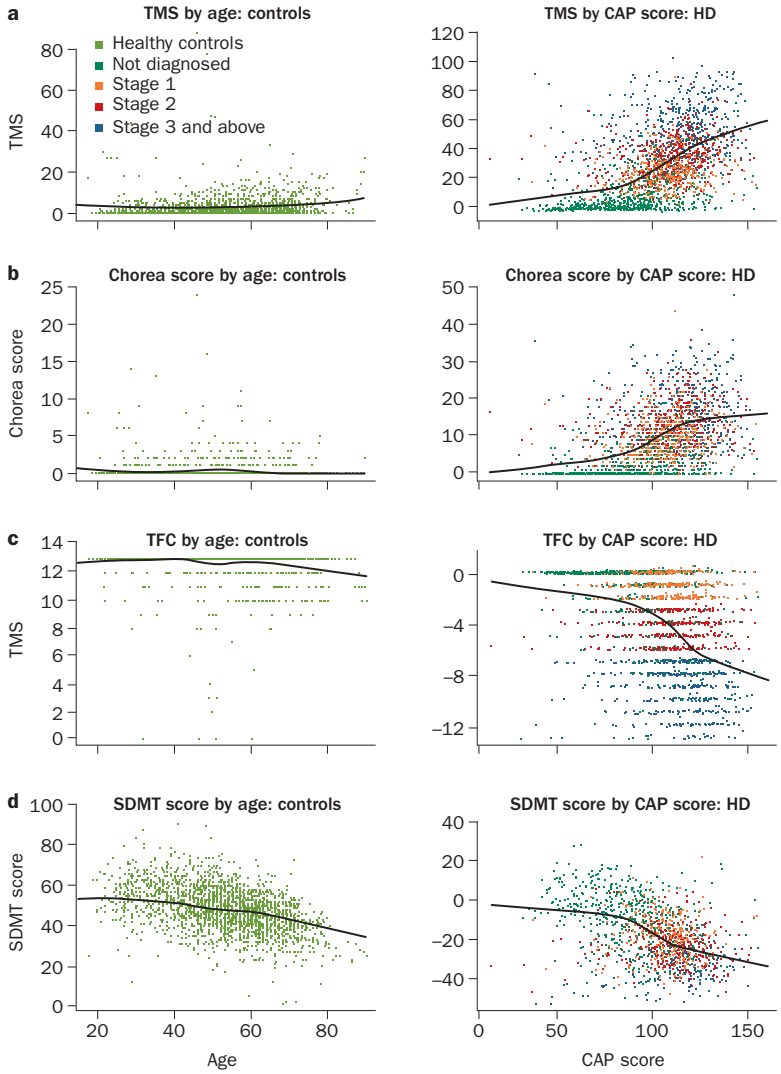

Figure 4 | Change in clinical features: data from COHORT and REGISTRY databases. **a** | TMS. **b** | Chorea score. **c** | TFC. **d** | SDMT score. Clinical features are plotted against CAP scores for gene-positive individuals (HD) and against age for healthy controls. Data for patients with HD are age-adjusted. Trend lines are based on a mixed effects nonlinear model under development by the Model-HD project. Colour coding indicates diagnostic status and Shoulson–Fahn stages. Only baseline values for patients with HD are shown. Modelling data set excludes healthy controls with fewer than two visits, and HD patients with fewer than three visits or CAP scores >160. Patients and controls both show considerable variability in clinical features, and the slopes of the trend lines increase around the time of expected HD diagnosis in patients with HD (CAP score ~100). Flattening of trend lines for CAP scores >120 might be attributable to under-representation of the sickest patients. Abbreviations: CAP, CAG age product; HD, Huntington disease; SDMT, Symbol Digit Modalities Test; TFC, Total Functional Capacity; TMS, Total Motor Score.

affected in HD. Consequently, peripheral biomarkers, such as inflammation, hold some promise.[40–43]

Some of the earliest steps in the pathogenic cascade of HD include misfolding of huntingtin to a β-sheet structure,[44] and post-translational alterations, such as cleavage or altered phosphorylation. Specific antibodies could be developed to monitor these events. The mutant huntingtin protein has many effects in cells, including abnormalities in cellular proteostasis mechanisms, for which reporters might be available.[45] The mutant protein can enter the nucleus and alter gene transcription,[46] the consequences of which could be measured. Mutant huntingtin can also affect cellular metabolism; in particular, mitochondrial function, which may lead to the production of abnormal metabolites and markers of oxidative stress.[47]

Age of onset and rate of progression of HD are both likely to be influenced by environmental and genetic modifiers.[11,48] CAG repeat length explains about 50–70% of the variance of age of motor onset, and the residual variance has a heritability of over 0.50.[49]

Thus far, the attempts to find genetic factors other than the CAG repeat length that modify age of onset by examining the HD locus or specific candidate genes,[50,51] or through genome-wide linkage analyses,[52] have yielded negative results, or intriguing but inconsistent leads. Novel and robust genetic modifiers will, hopefully, emerge from new approaches, such as the use of genome-wide association studies for large series of cases, or application of whole-genome sequencing to small pedigrees with at least two affected individuals in different generations.[53] Additional modifiers may emerge from careful examination of rare cases of HD that appear to develop with repeat lengths below the canonical threshold of 36 CAG triplets.[1] Identification of genetic modifiers might, in turn, provide leads to biomarkers.

Neuronal death is the hallmark of HD, but neuronal dysfunction manifesting in clinical features probably occurs before actual cell death. Chorea has been suggested to reflect neuronal dysfunction,[54] while motor impairment (bradykinesia/fine motor dysfunction) seems to be best correlated with neuronal cell death. This idea would be consistent with the observation that chorea tends to predominate early in the disease course while motor impairment supervenes later in the course. Supporting this hypothesis, motor impairment—but not chorea—has been found to correlate with both the Vonsattel score (a measure of neuropathological severity) and loss of neurons as determined by stereology in postmortem striatum.[55]

Evidence for neuronal dysfunction, including synaptic dysfunction, is plentiful in animal models of HD,[56] and evidence that such dysfunction can be reversible comes from both conditional knockout models[57] and nucleotide-based gene silencing in mice.[58,59] Reversal of dysfunction seems possible even to the extent that reversal of both histopathological and neurological abnormalities is seen when production of mutant huntingtin is reduced. Thus, biomarkers relating to both neuronal dysfunction and neuronal cell death are likely to be important.

Another important issue relates to cell-autonomous versus cell-interaction mechanisms in HD pathogenesis. Mutant huntingtin is likely to have cell-autonomous toxic effects, but there may also be elements of cell interaction, which could be mediated in several different ways, including excitotoxicity, spread of abnormal mutant huntingtin from cell to cell in a prion-like fashion,[60] and loss of trophic support from brain-derived neurotrophic factor or other trophic molecules. Whatever the biological mechanism, the implication is that localized changes might be propagated in a topographic manner (Figure 6),

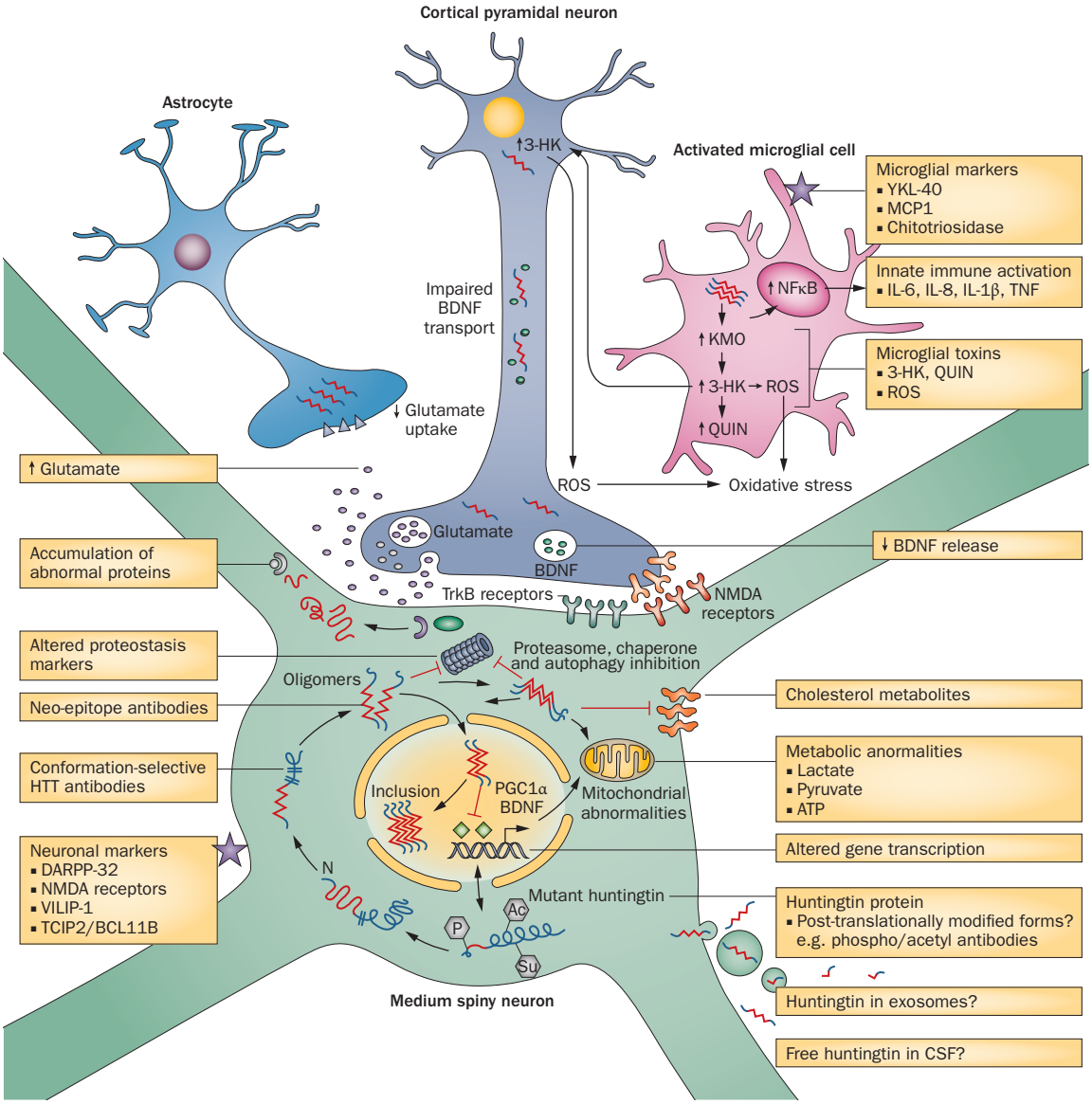

Figure 5 | Schematic diagram of Huntington disease cellular pathogenesis. Yellow boxes highlight pathways with potential for biomarker development. In some cases, the molecule might be involved directly in pathogenesis, as with huntingtin itself, and might, therefore, also be a therapeutic target and serve as a pharmacodynamic marker, as well as a marker of disease status. Abbreviations: 3-HK, 3-hydroxykynurenine; Ac, acetyl group; BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; KMO, kyurenine mono-oxidase; NMDA, *N*-methyl-D-aspartate; P, phosphate group; QUIN, quinolinic acid; ROS, reactive oxygen species; Su, SUMO post-translational modifications; TNF, tumour necrosis factor.

which could have profound implications for the design of therapeutic interventions, especially those involving localized huntingtin lowering.

MRI methods

Macrostructural brain imaging

To date, structural imaging has been the source of the most robust biomarkers for HD.[61] Structural MRI methodologies have demonstrated strong cross-sectional and longitudinal changes in volumes of the striatum, in both premanifest and manifest HD.[13,62–70] Large longitudinal studies (PREDICT-HD and TRACK-HD) have shown significantly faster rates of decline in striatal volume in premanifest and manifest HD individuals compared with age-matched controls, even in those individuals who are more than 15 years from estimated onset of diagnosable signs.[71,72] Studies using raw volumes to calculate longitudinal change suggest that once atrophy begins, the rate remains fairly constant, and is significantly faster in those with higher CAG repeat lengths.[62,71,72]

Other regions, such as the globus pallidus, thalamus and hippocampus, also undergo atrophy, though less attention has been paid to these structures. Cross-sectional and longitudinal studies indicate that the magnitude of volume reduction is smaller in these regions than in the striatum.[71,73,74]

Cortical grey matter atrophy occurs later than striatal atrophy in premanifest HD,[71] and is less dramatic than

striatal atrophy in both manifest and premanifest stages of disease.[13,75] Results from cross-sectional studies differ regarding specific areas of cortical involvement.[71,76,77] Longitudinal data from PREDICT-HD suggest that rates of change in cortical volume do not distinguish individuals with premanifest HD from controls.[71] Longitudinal studies in manifest HD indicate significant change over 1–2-year periods,[72] and faster rates of change as compared with controls.[78]

White matter volume is significantly reduced long before motor onset in HD,[13,71,73,79–81] and atrophy continues into the manifest period.[13,75,82] Longitudinal studies show significant atrophy over 1–2-year periods in premanifest HD[61,62] and early manifest HD.[14,78] Using Statistical Parametric Mapping methodology, the TRACK-HD group[14] found that the most prominent changes in white matter occurred around the striatum and within the corpus callosum and posterior white matter tracts. Longitudinal atrophy of the corpus callosum in both premanifest and early HD cohorts has been confirmed by a recent volumetric study.[83] Aylward *et al.*, using lobular regions of white matter, found the greatest volumetric change to reside in the frontal lobe.[71,83]

Changes in subcortical structures can also be detected using shape analysis.[74,84] This approach might be more sensitive than volumetric analysis, and gives additional information about which structure subregions could be affected.

Microstructural brain imaging

Diffusion tensor imaging (DTI) has revealed abnormalities in neuronal fibre orientation and integrity in white matter and subcortical grey matter structures in both premanifest[85–88] and manifest[68,70,86,89–94] HD. In white matter, the greatest differences are generally found in the corpus callosum. As yet, it has not been established which processes—for example, a reduction in neuronal density and/or demyelination—contribute to volumetric loss in this structure, but recent advances in diffusion imaging are likely to further elucidate the relevant mechanisms. Abnormalities in the cortico-cortical fibres in the corpus callosum could result in cortical 'disconnection' effects.[91]

Several studies have shown increased fractional anisotropy (or reduced diffusivity) in the basal ganglia—in particular, the putamen—in manifest and premanifest HD,[68,70,86,87,89,92,94] with less-consistent findings in the globus pallidus and caudate before diagnosis. The interpretation of increased fractional anisotropy in grey matter is uncertain. It has been proposed to reflect the microstructure and organization of fibre tracts, but another possibility is that as neurons die, white matter tracts passing through are proportionately overrepresented, resulting in increased fractional anisotropy. Other measures from DTI, including mean diffusivity, and radial and axial diffusivity, have also been found to be abnormal in HD.[70,91]

Functional and chemical MRI

Imaging methods that probe functional and metabolic disturbances might be especially useful early in the HD course, perhaps even before structural changes begin, and could be more responsive to therapeutic interventions than are structural imaging measures.

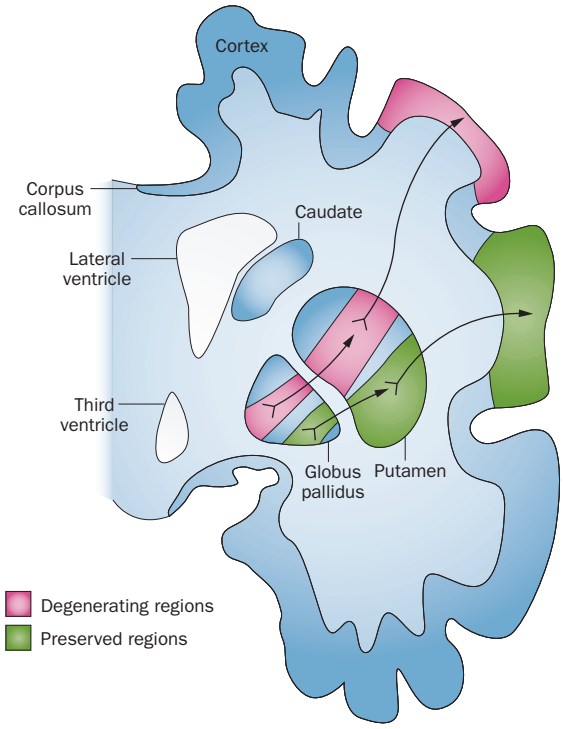

Figure 6 | Conceptual diagram of possible circuitry-related degeneration in Huntington disease. The hypothesis is that pathogenesis spreads via some form of intercellular communication, which could involve transmission of mutant huntingtin from cell to cell in a prion-like fashion. Excitotoxicity and/or loss of trophic support could also be involved in pathogenesis involving cell–cell interactions. If imaging measures can be used to track the initiation and spread of such a process, it may be possible to target huntingtin-lowering interventions to the initiating regions of the brain at the optimal time in order to minimize spread.

Functional MRI (fMRI) incorporating blood oxygen level-dependent (BOLD) contrast can provide a reflection of neuronal activity, and might be capable of identifying changes in premanifest HD even before structural brain damage.[95–99] Functional changes may include regional overactivation and underactivation, which could be interpreted as signs of dysfunction, compensatory overactivity, or both. Consequently, interpretation of fMRI data can be complex, and it is not clear whether increases or decreases would be expected in response to therapeutic intervention. Functional connectivity can also be determined using fMRI, by measuring synchrony of the BOLD signal in spatially remote brain regions. In premanifest HD, functional connectivity has been reported to be abnormal in the motor system[100–102] and in systems related to cognitive processing.[97,98,103–107]

An advantage of using functional connectivity is that it can be measured while the patient is at rest, so interpretation does not require consideration of differences in task performance. Nevertheless, further work validating the

test–retest reliability of fMRI data, the consistency across multiple sites, and the presence of progressive longitudinal changes, is required before this technique is adopted for clinical trials.

Another promising magnetic resonance-based approach to identify early brain changes in HD is magnetic resonance spectroscopy (MRS), which has been used to identify alterations affecting *N*-acetylaspartate (NAA), glutamate and glutamine.[108–110] Levels of myoinositol, a marker of astrocytosis, have also recently been found to be elevated in the putamen of patients with early HD, correlating with motor dysfunction.[110] Recent MRS studies using high-field-strength MRI have confirmed the results of earlier studies, especially the alterations in NAA and glutamate levels.[111] MRS at high field strength, with its increased signal-to-noise ratio and spectral resolution, can be used for investigation of additional metabolites, such as lactate, gluthatione and γ-aminobutyric acid, and might increase power for identification of physiological measures associated with early brain change in HD.[111] Potential MRS markers of interest could subsequently be assessed at the more-routine field strengths available with clinical MRI scanners, perhaps using spectral editing methods.

MRI can also be used to assay brain iron and other transition metals. Brain iron levels have been reported to be altered in HD.[112]

Clinical–imaging correlates

Striatal volumes correlate with CAP scores and estimated time to disease onset in premanifest HD,[12,67,73,113,114] as do white matter measures.[73,80,115] Measures of motor dysfunction also strongly correlate with the volume of the striatum[66,84,116] and white matter.[86,116,117] Using digitomotography, the TRACK-HD group[27] found significant correlations between motor scores and volumes of the caudate, putamen and grey matter in the right superior temporal and left precentral gyrus, as well as cortical thickness in the occipital and parietal lobes and primary motor cortex.

Measures of cognitive function show a strong correlation with imaging variables.[65,69,73,82,116,118] The association of corpus callosal atrophy and impairment on a visuomotor integration task in early HD suggests that a reduction in interhemispheric communication may have a direct impact on HD symptomatology.[83] By contrast, little or no correlation is observed between structural imaging measures and psychiatric symptoms.[73,90,116,118] Measures of functional capacity correlate with total grey and white matter volumes[75,80,119] and striatal volumes in manifest HD.[84] Patterns of cortical thinning have been linked to other specific phenotypes that represent heterogeneity in clinical presentation and rates of progression.[76,120,121]

White matter DTI measures correlate with estimated years to HD onset,[88] cognitive measures,[86,90,93,94] motor measures,[90,94] and apathy.[94] MRS and fMRI measures have been studied less extensively, but correlations with clinical variables have been reported in very small cross-sectional studies.[106,111] One fMRI study reported reduced activation in the dorsolateral prefrontal cortex associated with increasing working memory load in premanifest HD,[107,122] and in another study, premanifest individuals who performed at a similar level to controls on a motor task employed a compensatory network in the supplementary motor area.[87] However, few longitudinal fMRI studies are available, and a recent study failed to show change in activation over a 2-year period.[102]

PET methods

Initial ^{18}F-fluorodeoxyglucose (FDG)-PET studies in patients with HD showed glucose hypometabolism in the striatum, with a suggestion of possible hypermetabolism preceding the decrease.[123] A recent longitudinal study reported a decline in glucose metabolism in patients with rapidly progressing early HD.[124] An alternative approach is to delineate a network of regions with altered metabolism.[125,126] These findings show that FDG-PET, in combination with network analysis tools, may identify specific patterns of abnormal brain function in prodromal stages of HD. Patterns of metabolic alterations in preclinical HD might be used as measures for quantifying the rate of disease progression during the earliest disease phases. FDG-PET analyses might also provide suggestions of possible spread of HD-related pathology. A recent study suggested that alterations in metabolic network measures could provide useful markers for clinical trials,[127] although interpretation of network pattern changes and their impact on clinical performance may be complex.

Prediction of key clinical changes

For imaging measures to be candidate surrogate measures, they should ideally not only correlate with clinical measures, but also be able to predict these measures. Studies have shown that striatal volumes can predict motor onset and add predictive power beyond age and CAG repeat length alone.[16,128] Another study has shown that FDG-PET hypometabolism is also a predictor, although whether it adds additional predictive power beyond striatal volumes has not been determined.[129]

Other biomarkers

Biochemical measures of pathogenically relevant processes in accessible biofluids would be highly desirable as biomarkers for HD. Despite the ubiquitous expression of mutant huntingtin, the development of biochemical biofluid biomarkers for HD has proved challenging.[7] Hypothesis-driven and 'omics' discovery approaches have yielded a multitude of candidate biomarkers,[130,131] but none can be said to have been 'validated'.[7]

An example of the difficulties is 8-hydroxydeoxyguanosine (8OHdG), a product of oxidative DNA damage, which was reported to be elevated in plasma from patients with HD, and to be responsive to treatment with the antioxidant creatine.[132] However, in a larger patient cohort in whom 8OHdG was quantified by the original laboratory, only a subtle alteration was found in patients with HD.[133] In a separate study (PREQUEL), no relationship was observed between CAP scores or projected years to onset and 8OHdG levels, and no change in levels of this compound were seen after treatment with coenzyme Q_{10} (CoQ_{10}), another antioxidant. Furthermore, a rigorous,

two-laboratory, blinded analysis recently reported no disease-related alterations in 8OHdG levels at any stage of HD, or any significant change with longitudinal progression.[134] The authors concluded that 8OHdG is not a useful biomarker for HD onset or progression. This work emphasizes the importance of independent replication of results, blinded sample analysis, use of multiple analytical methods, and rigorous biosample quality control for future HD biomarker studies.

Future work in biofluid biomarkers is likely to focus on pathogenically relevant molecules in the cerebrospinal fluid (CSF). Unbiased omics discovery approaches in CSF have not yet identified good candidate biomarkers in HD.[135] Hypothesis-driven studies will focus on functional correlates and neurobiological underpinnings of detectable changes already reported, such as immune activation,[40–42] transcriptional dysregulation[136] and cholesterol biosynthesis.[137] Another possibility would be to attempt to track striatal degeneration using CSF markers such as DARPP32 or TCIP2, which would be predicted to be released into the CSF by dying medium spiny neurons.

Direct quantification of the mutant huntingtin protein itself shows promise as a pathogenically relevant marker.[138,139] Mutant huntingtin levels are seen to rise with disease progression, owing to the accumulation of N-terminal fragments, and the concentration of mutant huntingtin correlates with both CAP score and brain atrophy rate, indicating potential functional relevance.[138–140] If work that is currently underway to further improve these assays is successful, accurate quantification of mutant huntingtin in CSF might be useful, analogous to the current use of amyloid-β peptides and tau isoforms in AD.[141] A more valuable approach, however, might be to identify specific post-translational modifications or abnormal conformations of huntingtin that correlate with disease pathogenesis.

Conclusions and future prospects

Current clinical trials in manifest HD have required large numbers of participants (for example, 600 individuals over 5 years for the 2CARE study of coenzyme Q_{10}). Clinical trials in premanifest HD with clinical outcomes such as motor onset could require even larger numbers of participants if selection is not based on age and CAG repeat length. Use of structural imaging biomarkers as outcome measures in clinical trials could potentially decrease the number of participants needed for efficacy trials of neuroprotective agents in HD, as the effect sizes for these structural imaging measures are large relative to clinical measures.[15,16]

Until biomarkers can be established as surrogate markers, phase III clinical trials must have relevant clinical end points. Nevertheless, biomarkers could be extremely useful for phase II clinical trials in which the goal is to assure safety and gather initial evidence that an agent has neuroprotective properties and, thus, merits being taken to larger phase III trials with definitive clinical end points.

A recent phase II biomarker treatment trial suggests the power of this approach. A study of creatine in individuals at risk of HD showed striking slowing of progression of structural brain atrophy in the drug-treated group compared with controls.[142] By contrast, there were no change in clinical outcomes, suggesting that imaging may be more sensitive to change. However, the numbers of participants were very small, and a significant number could not tolerate the treatment, so the study will need to be repeated with much larger groups. Nevertheless, this study shows the potential for structural imaging as a biomarker in phase II studies.

HD can provide a model for other neurodegenerative disorders, since it is caused by a single mutated gene and has a characteristic and well-known neuropathology, and also allows the study of the premanifest phase of neurodegeneration in humans, when therapeutics are most likely to be efficacious at slowing or reversing the disease. The relationship between CAG repeat length and age of onset provides a unique opportunity to predict the age of onset in premanifest cases, in a fashion not possible even for the rare single-gene causes of AD or PD. Furthermore, since HD is a protein misfolding disorder, like PD and AD, insights from HD studies might help to identify potential biomarkers for use in these disorders. In addition, the close relationship between neuronal cell death and functional disability makes correlation of neuroimaging markers with neuropathology and clinical features feasible. Striatal atrophy seems to be a remarkably stable and useful biomarker over essentially the entire course of the disease, with atrophy beginning 15 years before diagnosable onset, and progressive atrophy continuing throughout the manifest period. As in AD, and possibly also in PD, the changes of HD begin very early in the disease course.[143] Therefore, treatment can have the goal of delaying or preventing clinical onset, as well as slowing progression of established disease. Biomarkers are likely to be most relevant for clinical trials in these early presymptomatic and prodromal periods.

Different biomarkers might be more useful at different points in the course of HD. Steady progression of atrophy is observed in the striatum and other brain regions, and has the potential for utility over long periods. Cortical grey matter and hippocampal volumes might be more useful markers later in the disease course, especially when correlated with cognitive variables.

A number of questions remain to be answered. For example, which functional and chemical measures will be most useful and most responsive to therapeutic interventions? Do neurobiological features accelerate, resulting in biomarker changes, just before onset of HD? Which biomarkers correlate best with which clinical features of the disease at each stage in the longitudinal course? A general biological question is whether biomarkers can be expected to correlate with CAG repeat length in the HD range only, or whether the CAG repeat length even within the normal range[2] could be relevant in some cases.

A major—and potentially therapeutically important—question is whether imaging biomarkers can be used to trace out circuits and determine the role of cell–cell interactions (Figure 6). The combination of several MRI methods might be especially powerful. For instance, it may be possible to use tract-tracing DTI and fMRI

functional connectivity (or PET correlation analysis) to trace changes in pathways between subregions of brain structures defined as atrophic by shape analysis. This analysis may guide therapeutics. In one scenario, if HD neuronal degeneration begins in the striatum and then progresses to other brain regions, it is conceivable that injection of RNA interference reagents into the striatum very early in the course might be sufficient to interrupt pathogenesis. Conversely, if HD pathogenesis begins in the cortex and progresses via anterograde mechanisms to the striatum, then superfusion of antisense oligonucleotides over the cortex might be sufficient to interrupt pathogenesis. If the pathology is largely cell-autonomous and occurs simultaneously in cortex and striatum, however, then several interventions together would be indicated. Of course, highly brain-penetrant small molecules are likely to be effective no matter which of these mechanisms is most relevant.

In summary, the validation of biomarkers for future trials of disease-modifying therapeutics to delay the onset and slow the progression of HD seems increasingly feasible. These biomarkers could be useful as outcome measures in phase II studies, and in the future might even be developed as surrogate markers for phase III studies. In turn, the methods developed for HD may be useful for development of personalized preventive therapeutics for other neurodegenerative diseases.

Review criteria

Articles were selected based on searches of PubMed using a number of different search terms, such as "Huntington's disease" plus "biomarker", "MRI", "CSF", "motor exam" etc. Selected papers were full-text papers. We only searched the English language literature. We also searched the reference lists of identified papers for further leads.

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Acknowledgements

We acknowledge the contributions of the REGISTRY and COHORT study investigators for their work in collecting the data displayed in Figure 4. A complete list of the COHORT study investigators appears in Huntington Study Group COHORT Investigators & Dorsey (2012),[23] and a complete list of the REGISTRY study investigators appears in Orth, M. & The European Huntington's Disease Network (2010).[17] In addition, we thank the European Huntington's Disease Network, the Huntington Study Group, the CHDI Foundation, and HP Therapeutics for providing financial support for the data collection efforts that led to Figure 4. We thank all the study participants and their families, including the Venezuela study, the JHU study, and the TRACK-HD, REGISTRY, PREDICT-HD, and COHORT studies, who made this work possible. We thank Simon Noble, Cristina Sampaio and Robi Blumenstein for comments and suggestions. We thank Sharon Blackburn for figure drawing.

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C.A.R., E.H.A, E.J.W., D.R.L., J.D.L., J.H.W., R.I.S., B.R.L., J.C.S., R.R., P.G.U., R.L.M. and S.J.T. researched the data for the article. C.A.R., E.H.A., E.J.W., D.R.L., J.D.L., J.H.W., R.I.S. and S.J.T. provided substantial contributions to discussions of the content. C.A.R., E.H.A., E.J.W., D.R.L., J.D.L., J.H.W., R.I.S., B.R.L., J.C.S., R.R., P.G.U., A.W., R.L.M. and S.J.T. wrote the article. All authors reviewed and/or edited the manuscript before submission.