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How to Diagnose and Stage Huntington's Disease: A Brief Overview

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Abstract

Huntington's disease (HD) is a rare neurodegenerative disorder that entails progressive motor, cognitive and psychiatric dysfunctions due to a single gene mutation of CAG trinucleotide repeat expansion in the chromosome 4p16.3 in the Huntingtin (HTT) gene. An accurate diagnosis of HD is especially important due to the fact that this incurable disease inevitably leads to death. As HD is an autosomal dominant trait, a child of an affected parent has a 50% chance of inheriting the disease. Herein, a brief overview of diagnosing and staging HD is covered, including but not limited to Unified HD Rating Scale (UHDRS) for clinical testing, and modified polymerase chain reaction (PCR) and next-generation sequencing (NGS) methods for diagnostic genetic testing. Ultimately, it is the genetic testing of the CAG repeat length that confirms diagnosis. Emotional and social implications throughout the diagnostic process are discussed. In addition, the roles of various types of biomarkers including mutant huntingtin (mHTT) and neurofilament light chain (NfL) for predicting the onset of the disease, staging, monitoring the progression of HD, and management of interventions are discussed. Promising areas for future research are further mentioned.

Keywords: Huntington's disease, CAG trinucleotide repeat expansion, Huntingtin (HTT)

Introduction

Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by progressive motor disturbance, cognitive malfunctioning, and psychiatric manifestations (Flier et al., 1986). Due to the relatively late age of onset (AO), diagnosis of HD is easily overlooked and misdiagnosed as some other neurologic conditions, commonly Alzheimer's disease. While the AO is identified to be late (between 30 to 40 years of age) the symptoms of HD progressively deteriorate from abnormal involuntary movement disorders like chorea to loss of cognitive function and personality change, ultimately leading to death approximately 15 to 20 years after the first appearance of HD symptoms (Chandler et al., 1960; Naarding et al., 2001). Furthermore, although primarily a neurodegenerative disorder, HD also entails life-threatening emotional consequences; e.g. up to about 40% of patients with HD also suffer from depression (Folstein et al., 1983).

Diagnosis of HD may seem relatively simple in terms of the genetic testing method, but it needs extreme care as it involves ethical issues and entails life-threatening level implications for patients. While some argue the diagnosis of HD will worsen depression, others found that diagnosis of HD prevents suicidal ideation during certain stages of the disease (Paulsen et al., 2005). Thus, in order to best aid HD patients and their quality of life, appropriate diagnosis of the disease as well as accurate identification of their HD stage is paramount.

Previous studies indicate that HD has been more prevalent in countries with majority white populations than in those with majority Asian populations (Pringsheim et al., 2012; Rawlins et al., 2016). In the immediate local setting of Malaysia, a multicultural country that consists of a gamut of racial groups from the Malays to Chinese and Indians, establishing and tracking HD can be particularly challenging. The first case of HD was reported in 1994, and the estimated prevalence is 0.0024 per 10,000 people (Reddy, 2014). Although a rarity in Malaysia, being aware of the steps for the

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diagnosis of HD as well as its ethical and social implications is vital.

1.1 The Cause of HD

HD is caused by the elongated CAG repeat in exon 1 of the Huntingtin (HTT) gene on chromosome 4p16.3 (Roos et al., 2010). This mutation causes a polyglutamine (polyQ) expansion of the HTT protein, which affects the basal ganglia of the brain and inhibits the ability to control unwanted movements (Browne et al., 1997). HTT gene with pathogenic properties have expanded CAG repeats beyond a general threshold of 36 codons, leading to neurodegeneration (Xiang et al., 1998). Although specific lengths vary for each study, healthy individuals are thought to have a CAG-repeat number of 35 or less, whereas affected patients have more than 40 repeats (Table 1) (Kay et al., 2014).

Table 1. Trinucleotide repeat expansions of polyQ HD in the HTT gene on chromosome 4p16.3

Repeat Lengths			References
Normal repeats	**Premutation/ Intermediate repeats**	**Full mutation/ Expanded repeats**	
11-34	35-42	>42	MacDonald et al. (1993)
6-34	35-40	>40	Roos et al. (2010)
10-35	36-40	>40	Kay et al. (2014)
<27	27-40	>40	Chheda et al. (2018)

HD is inherited as an autosomal dominant trait where one copy with the expansion of CAG repeats is sufficient to cause the disorder. Thus, a child of an affected parent has a 50% chance of developing HD (Novak et al., 2011). Interestingly despite HD being an autosomal dominant trait, Ranen et al. (1995) found that unlike in paternal transmission where longer repeats demonstrated greater net expansion than shorter repeats, in maternal transmission, there is no meaningful change in the repeat length regardless of the length of repeats. Hence, it can be said that HD symptoms are more prominent in paternal inheritance compared to maternal inheritance.

1.1.1 *Genetic anticipation*

Genetic anticipation in HD is the concept that, over generations, the size of polyQ HD stretch gets lengthier (more CAG repeats), and thus AO of HD decreases. A study by Andrew et al. (1993) demonstrated a significant correlation between the number of CAG repeats and the AO of HD, which implies a faster progression of HD as it gets passed down to following generations. Especially when it comes to juvenile onset HD, there is a larger amount of CAG repeats and it is possible that at times, the children of HD-gene carriers may develop HD before their parents (Cheuk-Wa et al., 2018).

1.2 Pathogenetic mechanism of CAG repeat expansion leading to HD

The expanded polyQ stretch in the HTT protein encoded by the expanded CAG repeats can affect several cellular processes such as transcriptional deregulation, impaired vesicle transport, and mitochondrial dysfunction (Ross and Tabrizi, 2011). The longer polyQ domains induce conformational changes of the HTT protein, causing it to form intracellular aggregates that manifest as nuclear inclusion in the nucleus, cell body, and neurons, and associate with organelles such as the Golgi apparatus and mitochondria (Harjes and Wanker, 2003). This alteration of the HTT structure ultimately leads to its cytotoxicity. As a result, the striatal medium-sized neurons undergo selective degeneration in HD, accompanied by progressive chorea and dementia (G. Vonsattel and DiFiglia, 1998).

Mutant huntingtin (mHTT) RNAs with expanded CAG repeats also contribute to neurotoxicity in HD at several levels (Nalavade et al., 2013). Mutant HTT RNAs give rise to small, neurotoxic CAG-

repeat RNAs (sCAGs) that interfere with cell functions by silencing the expression of genes that are complementary to each other (Bañez-Coronel et al., 2012). The findings of Khan et al (2019) suggest that this small RNA–dependent mechanism contributes to HD neuronal cell loss through the sequestration of proteins by expanded CAG repeat mRNAs and the repeat-associated non-ATG translation (RAN translation). Although the mechanism is not fully known, expanded CAG repeat of untranslated RNAs can alter the transcription pathways of several components of the Akt/Gsk3-β signaling (Bañez-Coronel et al., 2012). Whereas RAN translation induces the expression of proteins with expanded polyQ, polySerine (S) or polyAlanine (A) tracts (Nalavade et al., 2013). Thus mHTT RNA adds another level of toxicity contributing to the disease pathology.

1.3 HD development phases and progression stages

The onset of "manifest" HD is defined as when patients develop definitive motor signs indicative of HD (Ghosh et al., 2018). For many years prior to manifesting HD, patients may go through the "prodromal" phase of HD wherein they exhibit subtle motor, psychiatric or cognitive symptoms (Tabrizi et al., 2012). Representative neurobiological signs during this period include striatal degeneration and the loss of corticostriatal connectivity (Unschuld et al., 2012).

Before the development of manifest HD, patients with a positive family history of HD and with HD gene mutation are deemed to be "premanifest." Decade(s) before the onset of HD, premanifest individuals exhibit no distinguishable characteristics that deviate from those of the controls; however, as the onset approaches, patients suffer from prodromal HD symptoms (Ghosh et al., 2018).

Following the disease onset, HD progression consists of five stages classified by five parameters from the Shoulson-Fahn Staging system; engagement in occupation, capacity to handle financial affairs, capacity to manage domestic responsibilities, capacity to perform activities of daily living, and where care can be provided at (Ghosh et al., 2018). However, recently, more general terms of 'early', 'moderate' and 'late' are used (Ross et al., 2014).

2. Methods of diagnosing HD

HD is ultimately diagnosed by genetic testing that measures the number of CAG repeats in the HTT gene. Nonetheless, holistically, diagnosis of HD adheres to the sequential process of clinical testing followed by diagnostic or predictive genetic testing.

2.1 Clinical Testing

For genetically defined diseases like HD, genetic testing should be performed for definitive diagnosis. However, clinical screening holds importance as advances in diagnosis will be dependent upon clinical consensus and guidelines (Paulsen et al., 2014). Currently, the Unified HD Rating Scale (UHDRS) is the most common clinical tool for the assessment of HD that includes motor, cognitive, behavioural, functional and emotional components (Kieburtz et al., 2001). While the disease consists of considerably heterogeneous clinical phenotypes, motor onset emerges at the forefront as the most consistently agreed HD feature.

Motor disorder of HD consists of two components: involuntary movements (including chorea) and the impairment of voluntary movements (including incoordination and rigidity) (Ross et al., 2014). UHDRS Total Motor Score (TMS) is used to quantify motor deficits in HD. The motor scale includes ratings for domains including but not limited to eye movements, speech and dystonia and ranges from 1-124. Usually when the motor scores reach 15-20, patients are given a diagnostic confidence score of 4 which indicates manifest HD. Whereas for cognitive assessment, the UHDRS has three tests of executive functions (letter fluency test, symbol digit modalities test and stroop test) (Huntington Study Group, 1996; Sheridan et al., 2006).

Recent studies support the importance of motor signs and cognitive signs in diagnosing HD; e.g. Paulsen et al. (2014) demonstrated that there were subtle motor abnormalities long before the acceleration of progression when genetic testing was performed. Although clinical findings along with a positive family history of HD are only suggestive of the diagnosis, Paulsen et al. (2014) suggest that several clinical domain measures can improve the prediction of HD diagnosis (e.g. TMS and Stroop word test) and that these clinical predictors might also open up new avenues for early disease intervention.

2.2 Genetic Testing

For a genetically defined disease like HD, genetic testing is by definition the gold standard for diagnosis (Harbo et al., 2009). Although HD is an autosomal dominant trait, accurate clinical information about past generations is often difficult to obtain, and new HD mutation events can happen. Thus, instead of pedigree gene tracking, a molecular analysis of CAG repeat expansions in the associated HTT gene is warranted. Genetic testing for HD is done through measuring the CAG trinucleotide repeats in the HTT gene (*IT15*) which encodes for the Huntingtin protein. The region that harbors the HD mutation is amplified from the DNA. The DNA is collected either from blood (leukocytes) or tissue samples (e.g. buccal mouth swabs). However, as a positive diagnosis has consequential implications not only for the patients themselves but also for their family members (who share some of the same DNA sequences), it is crucial to take an informative and cautionary approach in offering genetic testing (Novak et al., 2010).

In the currently established guide of genetic testing, "pretest counselling requirements differ for diagnostic and predictive genetic testing" (Harbo et al., 2009; Craufurd et al., 2015). Genetic testing is used either to (a) confirm a clinical diagnosis of HD in manifest (symptomatic) patients or (b) predict the progress/ onset of HD in people at risk (Craufurd et al., 2015). However, it should be taken into account that the measurement of CAG repeat length holds meaning as a trait marker rather than a state marker (Craufurd et al., 2015). Detection of an abnormal CAG repeat length does not provide any indication of one's immediate clinical state.

2.2.1 *Diagnostic genetic testing*

A diagnostic genetic test is carried out on already symptomatic HD patients. It is a prerequisite that their symptoms must include motor signs in order to justify a diagnostic genetic test (Craufurd et al., 2015). Even for patients with a positive family history of HD who exhibit psychiatric or cognitive symptoms of HD, if they do not exhibit any motor signs, they should be referred to a clinical geneticist for predictive testing rather than diagnostic testing (Ghosh et al., 2018).

Despite its diagnostic ability, a significant drawback of such genetic testing is that the majority of patients with early HD are not aware of their behavioral symptoms and consequently do not receive genetic testing until HD has already significantly advanced (Caine et al., 1983; Deckel et al., 1996; Ho et al., 2006; Hoth et al., 2007). Craufurd et al. (2015) suggested the following as patients who should consider diagnostic genetic testing: patients with a positive family history who either show definitive motor symptoms or prodromal symptoms suggestive of the onset of HD, and patients who show HD-likely symptoms although with no positive family history of HD. Especially as Almqvist et al. (2001) estimated that 24% of the diagnoses of HD were individuals who did not have any family history of HD, confirmatory genetic testing is becoming increasingly appropriate for those without a positive family history.

2.2.2 *Predictive genetic testing*

On the other hand, a predictive genetic test is for those who are at risk of developing HD, especially due to positive family history. A positive predictive test result indicates a certain development of HD later in one's lifetime. In accordance with the internationally established guidelines, pretest counselling (which often includes neurological evaluation) with a clinical geneticist followed by a period of reflection, then a second counselling session is required in advance of the testing (IHA, 1994). Predictive testing on children is illegal to be performed on children, as informed consent from a parent would remove the child's right to autonomy in choosing whether or not to be aware of the information early in his or her lifetime (Novak et al., 2010).

3. Current genetic testing methods

3.1 PCR Methods

As CAG repeats are very CG rich, the standard polymerase chain reaction (PCR) method is difficult to accurately measure the number of CAG repeats. A variety of the polymorphic CCG repeat also attaches to the 3' end of the gene, complicating the process (Mahjoubi et al., 2011). Hence, different PCR methods of visualizing are designed and used depending on specific situations. The most common is a combination of CAG flanking PCR and triplet-primed PCR (TP-PCR) to differentiate normal and mutated alleles in the HTT gene and quantify CAG repeats (Chheda et al. 2018). As the detection of pathologic alleles expanded over thousands of trinucleotides is a challenge for a one-step standard PCR testing, standard PCR amplification with target repeat flanking primers is used to first detect alleles up to 100 repeats; then, TP-PCR is used to detect larger expansions (Falk et al., 2006). This two-step PCR is

not only more sensitive and reproducible but also more time-efficient as it does not require further Southern analysis (Falk et al., 2006).

3.2 Next generation sequencing (NGS)

More recently, NGS approaches have been used in research settings to diagnose HD (Schwarze et al., 2018). Sequencing through NGS is significantly faster and cheaper, making the possibility of whole genome sequencing (WGS) and whole exome sequencing (WES) more feasible (Goldman et al., 2020). WGS is a potential test that can alone identify "nearly all forms of genomic variation without bias" (Gilissen et al., 2014), whereas WES interpret the protein-coding exons of thousands of genes (Han et al., 2020). Comparing the sequence of a patient's exome with a normal reference sequence, the causes of his or her medical disorders can be discovered.

Before the rise of NGS technology, genetic analysis was considered the final stage of the diagnosis of a rare disease. However, NGS revolutionized the existing diagnostic workflow by providing a more rapid, effective, and cheaper alternative for genetic analysis in the early stages of the process (Mastrokolias et al., 2015). Containing genes with a broader phenotypic spectrum, NGS not only drastically reduced the time consumed in the diagnostic process but also increased the accuracy of diagnosis. Specifically for HD, validation has been particularly challenging due to the disease presenting itself through a variety of symptoms and progression rates, so conducting WGS or WES using NGS technology would aid a patient's genetic diagnosis (Mastrokolias et al., 2015).

3.3 Negative testing cases: genes predicted to cause HD-like diseases

Approximately 1% of patients test negative for HD gene mutation (Ghosh et al., 2018). In such cases, individuals harbor different genetic mutations that are collectively referred to as "HD phenocopies" (Wild et al., 2008). Table 2 lists known genetic mutations that cause HD-like diseases.

Table 2. HD-like diseases and their genetic causes (Adapted from Ghosh et al. (2018))

Disease	Mutation
HD- like syndrome (HDL) 1	PRNP: octapeptide insertion in gene encoding prion protein
HDL2	JPH3: triplet repeat expansion in gene encoding protein junctophilin-3
HDL3	mutation unknown
Spinocerebellar ataxia (SCA) 17 (HDL4)	TBP: triplet repeat expansion in gene encoding TATA-box binding protein
SCA1/2/3	ATXN 1/2/3 0 triplet repeat expansion in gene encoding Ataxin-1/2/3 respectively
Chorea-acanthocytosis	VPS13A: mutation in gene encoding chorein

4. Prediction of the HD onset and staging

An improved prediction of onset would be advantageous for prognostic counselling and early diagnosis of HD (Paulsen et al., 2014). However, currently CAG repeat lengths are not independently associated with specific clinical features of HD (Andrew et al. 1993). In the future, genetic testing should stretch beyond the diagnosis of the disease and provide further insight in relation to clinical symptoms of HD, e.g. whether psychiatric symptoms of HD are related to CAG repeat lengths.

4.1 Biomarkers

As genetic testing does not hold sufficient predictive power for accurate staging of HD, biomarkers of HD have been identified for staging (both onset and progression) of HD. Potential biomarkers include but are not limited to the followings: volume of fractional anisotropy in basal ganglia (Sanchez-Castaneda et al., 2012) monitored through diffusion tensor imaging (DTI); metabolic markers such as cholesterol and isoleucine (Markianos et al., 2008; Mochel et al., 2007); endocrine markers such as cortisol and

leptin (Hubers et al., 2015; Mochel et al., 2007); and oxidative stress markers such as the level of 8-hydroxydeoxyguanosine (8OHdG), a product of oxidative DNA damage, in blood plasma (Hersch et al., 2006). Henley et al. (2012) even suggested the use of emotion recognition as a biomarker for the onset and progression of HD. Two non-invasive staging biomarkers used in HD are mHTT, which showed considerable association with the clinical phenotype (Wild et al., 2015), and neurofilament light chain (NfL), which well reflected neuronal and glial degeneration (Silajdžić et al., 2018).

4.1.1 mHTT

mHTT itself in affected tissues works can be used as a biomarker of HD. However, specifically quantifying mHTTs has been challenging due to their extremely low concentrations in biofluids and difficulty distinguishing between central nervous system (CNS) generated mHTT from those derived peripherally. mHTT in cerebrospinal fluid (CSF), the fluid surrounding CNS, was found to be more readily quantified than that in the blood (Huhmer et al., 2006). However, in 2013, Moscovitch-Lopatin and colleagues were able to measure levels of mHTT in the peripheral blood mononuclear cells of prospective HD subjects using a homogeneous time-resolved Förster resonance energy transfer assay, and predict AO within 2 years.

In 2015, Wild et al. developed an ultrasensitive single-molecule counting (SMC) mHTT immunoassay based on the 2B7-MW1 antibody pair which made it possible to specifically quantify soluble mHTT in CSF in two cohorts (from the UK and Canada). In this study, mHTT was found to have the ability to independently predict phenotypic features of HD. A higher level of mHTT load was associated with proximity to disease onset, diminished cognitive function and more severe motor dysfunction (Wild et al., 2015). There were close associations of mHTT with HD stages: in premanifest subjects, mHTT level in CSF correlated with their probability of HD onset; in manifest subjects, mHTT level correlated with clinical parameters of HD progression (severity), such as UHDRS TMS; and mHTT measures showed associations with neurofilament light chain (NfL) and total tau (Wild et al., 2015).

Later on, Fodale et al. (2017) further validated the SMC immunoassay for the measurement of mHTT in human HD CSF and for its application as both a HD progression biomarker and a pharmacodynamic readout for HTT-lowering therapeutic approaches. In addition to the original assay, Fodale et al. (2017) recommended the quantification of hemoglobin levels in the CSF in order to exclude contaminated samples prior to the measurement of mHTT. Furthermore, in accordance to Huhmer et al. (2006), Fodale et al. recommended ancillary biomarkers (i.e. total protein amount in the CSF) to be identified as additional characterizations of the testing matrix aimed to interpret the mHTT concentration levels. However, more replications in longitudinal studies are needed to further buttress the clinical predictive power of mHTT (Rodrigues et al., 2018).

4.1.2 NfL

Integral components of neurons, neurofilaments are types of intermediate filaments mainly located in the myelinated axons of the CNS and peripheral nervous system (PNS) (Marti-Martinez et al., 2022). Neurofilaments not only sustain neuronal structure but also ensure axonal integrity and aid the spread of neural transmission. Amongst its types, neurofilament light chain (NfL) is the most soluble and most promising biomarker for HD onset and progression.

In general, NfL is utilized as a marker of neurodegeneration in a gamut of diseases (including Parkinson's and Alzheimer's). Specifically in HD, NfL levels show a correlation with both motor and cognitive decline (Przybyl et al., 2021). Compared to mHTT levels, NfL concentrations hold an advantage that premanifest and manifest HD stages are more clearly distinguished and provide good correlations with direct brain and clinical features of HD (Zeun et al., 2019).

In 2017, Byrne and colleagues successfully quantified neurofilament light chain (NfL) in blood plasma as a prognostic biomarker of disease onset and progression in HD. Byrne et al. (2017) revealed that even in the premanifest HD group, NfL levels in the blood plasma significantly increased at every disease stage compared with controls. The findings of Byrne et al. (2017) showed a significant association between the baseline NfL concentration in plasma and disease onset in premanifest HD; baseline plasma NfL held a predictive value of HD onset within 3 years in premanifest mutation carriers.

For those with manifest HD, NfL concentrations correlated with clinical progression, indicating neurodegeneration (Constantinescu et al., 2009; Vinther-Jensen et al., 2016). NfL concentration was very closely associated with CAG repeat length, being the first biomarker showing a relationship with the genetic expansion factor in HD

(Constantinescu et al., 2009; Byrne et al., 2017). HD patients with higher CAG repeats contained earlier and steeper increases in plasma NfL. Furthermore, in an independent cohort, NfL levels in plasma and CSF were strongly correlated, and NfL concentrations were indicative of the likely rate of "worsening of cognition, functional ability, and brain atrophy beyond age and CAG repeat count" (Byrne et al., 2017). In 2018, Johnson et al. further revealed the longitudinal association between baseline NfL in blood plasma and cortical and subcortical gray-matter and widespread white matter, corroborating NfL as a dynamic marker of brain atrophy. As a side note to NfL's use as a biomarker of HD, it should be taken into account that a study by Byrne et al. (2017) showed a closer association between NfL concentrations in plasma and the rate of whole-brain atrophy than that of the striatal atrophy, suggesting that NfL in the blood is more reflective of the global rate of neuronal damage. Overall, NfL has been substantiated as a reliable yet not HD-specific biomarker of neurodegeneration.

4.2 The Role of Neuroimaging in the Diagnosis and Staging of HD

Neuroimaging is often done for patients with movement disorders like HD to monitor disease progression. Magnetic Resonance Imaging (MRI**)** has been widely used to predict clinical onset before diagnosis in HD (i.e. to measure brain volume). Through the voxel-based morphometry (VBM) approach, MRI measures the volume of gray matter, white matter, and cerebrospinal fluid (CSF) (Ashburner and Friston, 2000). According to Zande et al (2022), the volume loss seems to begin in the striatum a few years before the development of motor signs. A newer MRI technique, diffusion tensor imaging (DTI) studies have identified diffusion abnormalities in certain areas in the same stage and shed light on the correlation with certain genetic and clinical variables, helping researchers classify and understand HD phenotypes according to the differences observed in diffusion maps (Zande et al., 2022).

Neuroimaging of biomarkers has also been found to be advantageous as they are convenient and relatively less invasive. For example, several radioligands that target mHTT are used in HD imaging studies in the brain of suspected patients to be compared to healthy controls (CHDI Foundation, Inc. and Universitaire Ziekenhuizen KU Leuven, 2022). Positron emission tomography (PET)-scans have been used to detect early pathophysiological changes in HD before structural changes occur (e.g. neuroinflammation).

5. Implications

Despite the relatively straightforward diagnosis of HD through measuring CAG repeat length, it is of particular concern that the diagnosis is made late in the disease course after patients show significant motor impairment, and at a time when, on average, over half of their striatal volume is lost (Aylward et al., 2012). Clearly, an earlier diagnosis of HD and subsequently earlier therapeutic intervention seems to be beneficial for future HD patients (Rothstein et al., 2012).

Ross et al. (2014) highlighted that different biomarkers may be more useful at different points in HD progression. For instance, later in the HD when cognitive symptoms become salient, cortical gray matter and hippocampal volumes are potentially more useful markers. Although objective biomarkers are discovered to accurately track HD progression, a number of questions remain unanswered: which specific biomarkers would be most responsive to therapeutic treatments and to what extent would the biomarkers show correlation with the CAG repeat length?

Going further, in order to address the important question of whether or not biomarkers can be used to trace out circuits, Ross et al. (2014) suggested the potential of combining several MRI methods. In accordance with the clinical complexity of HD, a combination of biomarkers (such as biochemical markers and neuroimaging markers) should be utilized to aiding each other (Tippett et al., 2017). For example, Mason et al. (2018) created a polymarker that combined imaging metrics (such as brain function and structure) to predict the onset of HD and the diagnosis at an appropriate timing.

6. Conclusion

In contrast to the seemingly simplistic way of diagnosis, HD requires a cautionary approach leading from clinical screening to genetic testing, from diagnosis to its inheritance. Especially as HD is incurable, diagnosis of HD entails serious implications for the patient and his or her family members. Potential HD patients need to be closely monitored prior to the onset of clear diagnostic symptoms; at the same time, however, the ethics and future consequences of the diagnosis should also be taken into consideration. As an attempt to

go further beyond simple diagnosis and for the staging of HD, numerous biomarkers have been explored. Yet, they are not yet "validated" and there are still many questions that need to be addressed especially in regard to their predictive and prognostic value as well as the interplay between themselves. Both cross-sectional and longitudinal studies should be conducted and replicated in order to validate the biomarkers that may further provide insight into designing effective therapeutic interventions in order to predict and delay the onset of the disease and slow the progression of HD.

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