Recent approaches on Huntington's disease (Review)

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Abstract. Huntington's disease (HD) is a neurodegenerative disorder characterized by severe motor, cognitive and psychiatric symptoms. Patients of all ages can present with a dysfunction of the nervous system, which leads to the progressive loss of movement control and disabilities in speech, swallowing, communications, etc. The molecular basis of the disease is well-known, as HD is related to a mutated gene, a trinucleotide expansion, which encodes to the huntingtin protein. This protein is linked to neurogenesis and the loss of its function leads to neurodegenerative disorders. Although the genetic cause of the disorder has been known for decades, no effective treatment is yet available to prevent onset or to eliminate the progression of symptoms. Thus, the present review focused on the development of novel methods for the timely and accurate diagnosis of HD in an aim to aid the development of therapies which may reduce the severity of the symptoms and control their progression. The majority of the therapies include gene-silencing mechanisms of the mutated huntingtin gene aiming to suppress its expression, and the use of various substances as drugs with highly promising results. In the present review, the latest approaches on the diagnosis of HD are discussed along with the need for genetic counseling and an up-to-date presentation of the applied treatments.

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Contents

1. Introduction

Neurodegenerative disorders have a exhibited a marked increase in incidence worldwide, thus rendering them a primary concern for the scientific society. The genetic cause of numerous disorders has already been described (1-3) and, nowadays, research focuses on the timely diagnosis and effective therapy of the most common neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease (HD). These disorders have diverse clinical manifestations; however, some of them demonstrate similarities among patients (4,5). Although in numerous cases, the onset of neurodegenerative disorders appears in middle to late adult life, there are patients who manifest symptoms of these disorders at a very early age (6,7). HD is one of the most common disorders with severe symptomatology, which affects individuals of all ages, progressively leading to severe disabilities. The genetic basis of this disorder has been established and has been known for a few decades now, and recent research has revealed promising mechanisms for eliminating HD symptoms (8). Furthermore, HD can be regarded as a model neurodegenerative disorder for the study of other cases with shared symptoms, and knowledge of other diseases may be useful for HD diagnosis and treatment.

2. Genetics and pathology of Huntington's disease

HD is a fatal, autosomal dominant, progressive neurodegenerative disorder characterized by severe symptoms, including

motor, cognitive and psychiatric symptoms, atrophy of the basal ganglia and the cerebral cortex, and an inevitably progressive course, resulting in mortality 5-20 years following the manifestation of symptoms. Typically, the motor defects include chorea and loss of coordination, and patients also demonstrate difficulty with speech and swallowing (9). Cognitive symptoms can be detected up to a decade prior to diagnosis and cognitive ability declines as the disease progresses (10). Psychiatric symptoms, such as depression, psychosis and obsessive-compulsive disorder, are also common in HD and are particularly distressing for patients (11,12). Patients with HD eventually require a wheelchair and more severe symptoms may lead to them becoming bedridden, with all the complications that may derive from that form of immobility.

From a neuropathological point of view, in patients with HD, the dysfunction and death of specific neurons within their brains are observed. There is a wide range in the age of onset of HD, as both juveniles (13,14) and adults have been diagnosed with the disorder thus far. For instance, kindred members of families that revealed a history consistent with HD autosomal dominant inheritance, took part in a 20-year study, which was published in 2004 (15). The researchers of that study found that the typical ages of disease onset were between 21 and 50 years of age (15). Although the disorder typically manifests in adulthood, juvenile HD (JHD) is also frequent among patients (16). A recent study, conducted in Argentina in 2015 (17), reported that almost 20% of the patients diagnosed with HD, revealed their first symptoms of the disorder during their childhood. It should be noted that the overall estimated prevalence of JHD of that study was higher than that in any other population recorded to date (17). The brain structure in young patients was previously assessed by Tereshchenko *et al* (18) in 2019, proposing that the morphology differs among juveniles and adult patients, as young patients revealed proportional cerebellar enlargement (18). In the same year, another study suggested that the pathogenesis of HD begins with abnormal brain development in both child and adolescent patients (19).

The first attempt to discover the genetic cause for HD by Gusella *et al* (20) revealed that the HD gene is linked to a polymorphic DNA marker that maps to human chromosome 4, in particular 4p16.3. A decade later, a Huntington's Disease Collaborative Research Group discovered that mutations in the Huntington (HTT) gene encoding the huntingtin protein, a large protein of 3,144 amino acids, led to the neurodegenerative disorder (21). In particular, they suggested that the disorder is caused due to a cytosine-adenine-guanine (CAG) trinucleotide expansion in exon 1 that codes for polyglutamine (polyQ) in the N-terminal of the *HTT* gene (21). The CAG sequence is normally repeated 9 to 35 times, with an average median of between 17 and 20 repeats. However, patients with HD usually reveal a CAG expansion exceeding 35 repeats (22). Above a threshold of ~35 CAG repeats, the age of onset of HD is inversely associated with the length of the expansion. A recent study conducted by Schultz *et al* (23) demonstrated that the development of verbal skills appeared to plateau earlier as CAG repeat length increased. The repeats are usually between 36 and 39, depending on the age. Juveniles with HD exhibit high repeat lengths (24). In certain rare cases, patients exhibit less repeats in their genome, 27-35, demonstrating an endophenotype (25).

Of note, the huntingtin protein is expressed in all cell types of the body, both at the tissue and subcellular level, in all developmental stages. Recently, research has focused on the investigation of the HTT structure via cryo-electron microscopy contributing to a better comprehension of its morphology and form abnormalities (26). It has been described as a 350-kDa HEAT-repeat protein which interacts with hundreds of other proteins (27) and participates in numerous cellular processes. Although the cellular functions of HTT protein are not yet completely understood, it appears to play a crucial role during early embryonic development and neurogenesis. In particular, Saudou and Humbert (22) described the human huntingtin protein sequence and its post-translational modifications in detail. They suggested that it coordinates cell division, as it participates in the proper mitotic spindle positioning and it regulates ciliogenesis. They also noted that huntingtin mediates endocytosis, vesicle recycling and endosomal trafficking, as it interacts with other proteins which are related to these mechanisms. Other functions of the protein include autophagy and transcription (22). It should be underlined that the *HTT* gene physiological expression is essential for organism homeostasis as it plays a neuroprotective role as well, even against mutant HTT (mHtt) toxicity (28,29). Moreover, HTT protein plays a crucial role in mitochondrial structure and function in the embryogenesis and oxidative metabolism, and HTT mutations have been linked to mitochondrial abnormalities (30-32).

The role of both wild-type Htt and mHtt in gene silencing studies has been investigated for the development of an effective therapy. As regards mHtt molecules, they form toxic aggregates into the central nervous system, depending on the length of polyQ expansion. For instance, mHtt co-aggregates with other proteins which play a crucial role in the cell, leading to misfunctioned phenotypes. Numerous studies have focused on the effects of *HTT* gene knockouts and knockdowns in cellular function. For example, a study published in 2017 suggested that mutations in HTT protein are related to nucleocytoplasmic transport disruption, leading to the improper function of cells (33). During initial experiments performed on mice with *HTT* knockdown mutants, the mice succumbed after 8 days of gestation (34). Other studies have demonstrated that *HTT* deletion in the mouse central nervous system leads to a phenotype similar to that of HD (35,36). It is worth mentioning that a recent study suggested that *HTT* variants are also linked to another disorder with similar symptomatology with HD, the so-called Lopes-Maciel-Rodan syndrome (37). In addition, other studies have revealed the ability of various molecular chaperones, such as the heat shock family proteins, HSP40, HSP70, HSP90 and HSP105, to combine with misfolded mHtt and inhibit aggregate formation, leading to cell survival (38,39).

It is clear that the loss of HTT function contributes to HD pathology and for this reason, it is essential for survival. The reduction of mHtt levels should be accompanied by regular *HTT* expression.

3. Diagnosis and genetic counseling

It has already been mentioned that the age of onset of HD is inversely associated with the length of the expansion in the *HTT* gene. For instance, rare carriers of 36 to 39 CAG repeats

have lower penetrance and a later onset of the disease than those with 40 or more CAG repeats. Additionally, Keum *et al* (40) found that, along with clinical onset, the age of patients with HD at the time of death was well determined by an expanded CAG-repeat length. However, they claimed that the overall duration of the disease was independent of the length of the mutation's (40). These data may be useful, not only for the molecular diagnosis of the disorder, but also for the prediction of the outset of HD symptomatology. For the molecular diagnosis of the disorder, various PCR methods have been demonstrated in order to detect CAG expansions (41). A recent study presented a novel triplet-primed PCR-based assay aiming to improve the test reliability and accuracy by detecting CAG expansions in samples with sequence variations in the *HTT* gene (42).

It is known that miRNAs are involved in the biological processes of development, proliferation, inflammation and apoptosis, and their expression has been linked to HD diagnosis and symptomatology. For instance, Langfelder *et al* (43) found that the abnormal expression of miRNAs played a critical role in HD pathogenesis. For this reason, apart from the direct quantification of mHTT itself, which is the main disease-related biomarker, other miRNAs may be useful tools as biomarkers for HD prognosis (44).

Furthermore, numerous diagnostic tests have been proposed thus far, based on criteria related to inheritance and the symptomatology of the individual; however, these methods need to be improved. Patients who experience certain cognitive and behavioral symptoms may have HD (45,46). A recent study proposed the Enroll-HD dataset for estimating disease onset and its diagnostic confidence level (47). The results of that study were not promising, suggesting that it is important to develop more reliable diagnostic criteria (47). Another diagnostic approach suggested that the concentration of trace elements in the blood of patients with HD differs from that of healthy individuals. Researchers found increased levels of the essential elements iron, chromium, selenium and zinc and of the non-essential element, arsenic, in the blood of patients with HD, suggesting that the blood metal profile may be used as an easy tool for the disorder's medical detection (48).

HD follows the Gregor Mendel's principles of inheritance, as it is inherited in an autosomal-dominant manner. The offspring of an individual with a pathogenic variant, heterozygote, have a 50% chance of inheriting the disease-causing allele. Genetic counseling includes predictive testing in asymptomatic adults and prenatal testing in order to reveal the mutated allele (49). The prevalence of HD is ~1 in 10,000 individuals in the USA, as well as in Europe (50,51). In the year 2000, Sobel and Cowan (52) conducted predictive testing on asymptomatic individuals at risk of developing HD in the context of genetic counseling. Family members were requested to describe their communication and interactions with the social environment, and provide concerns about their future care. Members in 50% of the families experienced changes in patterns of communication and 56% of the participants reported changes in current relationships. The researchers suggested that families may benefit in pretest sessions by examining their patterns of dealing with illness issues, both past and present (52).

Migliore *et al* (53) suggested different approaches of counseling, depending on the genetic condition of the individual. For instance, in the case of intermediate alleles (27-35 CAG repeats) the experts should explain the potential risk of mutations and other members of the family should also be tested. In the case of low penetrance alleles (36-39 CAG repeats), individuals should be informed about the risk of HD symptoms manifesting at any age. Counseling for all family members is also required when juveniles are diagnosed with JHD. When the HD mutation is detected in a prenatal genetic test, the parents should be informed for the risk of the newborn manifesting the disease and should be given the option of terminating the pregnancy (53). This approach utilizes the current knowledge of the molecular basis of HD with the inclusive genetic counseling of all relatives. Recently, MacLeod *et al* (54) proposed a family systems approach to genetic counseling, which uses the narrative model. With the narrative resources, the genetic counselor can contribute to generate new meanings that the person may give to their experience of the genetic condition and help the patient adapt to living with the disorder or its risks (54). Another interesting comparative study, that was performed over the past two decades, on how parents inform their children who are at risk about their genetic risk demonstrated that, although testing is performed more often, the overall attitude towards information and testing has not changed significantly (55). This ascertainment proposes that new methods for more comprehensible information and accessible genetic counseling need be developed.

4. Treatment

Research focusing on understanding the underlying molecular mechanisms leading to the *HTT* gene mutations is highly promising, aiming to find a cure for HD. However, current treatments for HD are still limited. The therapies applied focus on the treatment of symptoms, as neuroprotective therapies to prevent disease onset and to attenuate the progression of the disease are not yet available. For instance, it has been proven that HD is caused by toxic properties of mHTT, rather than merely the decrease of wild-type HTT; for this reason, approaches focusing on mHTT expression, such as lowering HTT mRNA and mutant huntingtin protein, appear to be promising (56). The main strategies which have been demonstrated thus far as treatments for HD are presented in Fig. 1.

A recent study proposed that targeting of CAG repeat-dependent mechanisms, through gene-silencing approaches, may affect the rate of functional, motor and cognitive impairment, but not weight loss, in manifest HD mutation carriers (57). The standard approaches to DNA targeting use some form of specific DNA-binding element combined with nucleases, epigenetic modulators, or transcription factors. Zinc-finger transcriptional repressor approaches may lower mHTT levels by targeting DNA without altering it, whereas zinc-finger nucleases can add to the repressive effect of Zinc-finger proteins that reduce the levels of gene expression by simply binding to DNA and preventing gene transcription by actually disrupting or correcting the mutant gene (58). Along with similar techniques to other direct genome editing strategies, such as CRISPR/Cas9, strategies that are targeted in lowering huntingtin and HTT genome editing have immense

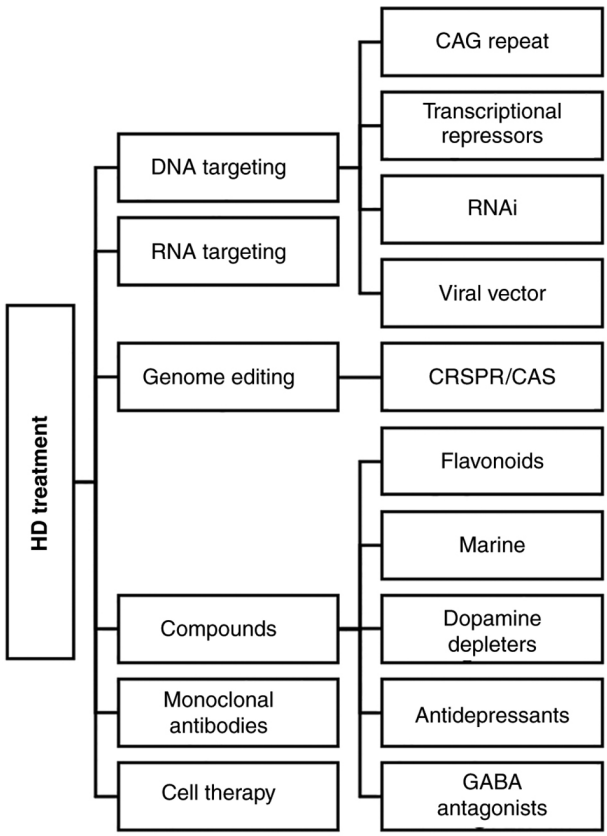

Figure 1. Box diagram of the main strategies which have been demonstrated thus far as treatments for HD. HD, Huntington's disease; CAG, cytosine-adenine-guanine.

potential for the treatment of HD. The main advantage is the permanent correction of the disease-causing CAG expansion. An antisense mechanism targeting HTT RNA, using synthetic antisense oligonucleotides (ASOs) that bind to the specific sequence of ribonucleic acid, may reduce the mRNA translation to the HTT disease-causing protein (59). The ASOs are widely distributed throughout the central nervous system and they do not require a viral or lipid carrier, resulting to an effective and simple to develop treatment. Tabrizi *et al* (60) used the antisense oligonucleotide IONIS-HTTRx designed to inhibit HTT mRNA by triggering the RNase H1-mediated degradation of the target mRNA, in order to minimize the concentration of mutant huntingtin in cerebrospinal fluid. They conducted an extended research with 34 patients who were treated with increasing dose levels of 10 to 120 mg and the observations were compared with individuals who received the placebo. The results revealed that the reduction in concentrations of mutant huntingtin was dose-dependent (60). The strategy for post-transcriptional gene suppression using non-coding double-stranded RNA sequences is known as the RNA interference (RNAi) mechanism. The RNAi pathway has been used thus far to suppress specific genes of interest and the results are highly promising for numerous diseases. There are various molecules which can be used for this purpose, such as siRNAs, shRNAs and artificial miRNAs that have been used to eliminate the HD symptoms. The first trials were performed two decades ago in rodents. In 2005, Harper *et al* (61) used a shRNA molecule to target the *HTT* mutant gene and the results were satisfactory, as the reduction in mHTT synthesis, prevented inclusions, gait deficits and rotarod dysfunction. In another case, a siRNA molecule was injected into the mouse striatum, and the reduction in mHTT synthesis prolonged striatal neuron survival, reduced aggregates and prevented motor dysfunction (62). The application of siRNA approaches has been successful in multiple animal systems (63). In a recent study for example, a single-stranded siRNA (ss-siRNA) was used for RNAi, resulting in a selective decrease of CAG-expanded HTT protein in various regions throughout the mouse brain (64). Other ribonucleic acids, such as miRNAs have been used for the suppression of mHTT in genetically modified mice and the results have been promising; in one case, this strategy led to the prevention of regional cortical and striatal atrophy, and reduced weight loss (65). It is worth mentioning that the majority of the previous technics, apart from gene editing, effectively reduce, but do not completely eliminate the production of mHTT.

In addition, the therapeutic approach of overexpressing wild-type HTT has been investigated. Early trials of inserting the wild-type HTT into mammalian cells which expressed mHTT have led to reduced cell death (26).

Numerous research studies have used viral vectors, such as adeno-associated virus, which encapsulate the RNA molecules, and their genome is combined with enhancers and promoters, in order to deliver these agents by injection into the body (66).

Different compounds may be candidates for the treatment of neurodegenerative disorders, including HD. A recent study proposed the utilization of flavonoids, which may reduce cellular stress and play an anti-inflammatory and anti-apoptotic role in the cell (67). In another case, researchers proposed that marine compounds may be used for the treatment of various neurodegenerative diseases, as they also demonstrate antioxidant, anti-inflammatory and anti-apoptotic properties. Tetrabenazine (TBZ), which is an inhibitor that blocks dopamine uptake into vesicles, has been shown to exert antichorea effects in patients with HD and was the first approved drug for medication (68). Since then, studies on the optimization of drug delivery and bioavailability of TBZ in patients have been conducted based on latest nanotechnology technics (69). Several molecules have been suggested for the treatment of Parkinson's disease, such as fucoidan and xyloketal B, and fucoxanthin and cerebrosides for Alzheimer's disease, and have also been investigated for other disorders, such as HD for effective treatment (70). In 2020, Jabłońska *et al* (71) suggested that pridopidine, a dopamine stabilizer, may be a promising drug for HD symptoms. It is well-known that there is an association between the amount of dopamine in the central nervous system and the stage of the disease, as the causes of HD are dopaminergic conduction disorders, and experiments on animal models have demonstrated the protective effect of pridopidine on nerve cells (72,73). The main advantage of drug treatment for HD is that the effectiveness and tolerance of each active compound is well-studied for other neurodegenerative diseases with similar symptomatology. Thus, it is easier to design a suitable medication personalized to patient diagnostics. Another study proposed that the application of monoclonal antibody, which targets the HTT protein may deplete its concentration in the cell, proving that monoclonal

antibodies can interfere with the pathological processes of mHTT spreading *in vivo* (74).

Cell replacement therapy for HD using stem cells may be another opportunity to alleviate symptoms in patients (75). Furthermore, some case studies have indicated that exercise and physical activity may be beneficial for patients in terms of motor function, gait speed and balance, and social benefits have been also identified (76). Thus, exercise may play a complementary role in the treatment of the disorder.

5. Conclusions and future perspectives

HD is the first trinucleotide disease that was described and the first autosomal-dominant disease with a possible diagnosis prior to the manifestation of symptoms. Since 1983 and the localization of the gene, knowledge of the disorder has markedly increased, which is necessary in order to improve the quality of life of patients and improve therapeutic strategies by discovering novel molecular targets. The pathophysiology of HD is significant for designing and developing proper treatments (77). Science offers possibilities for attenuating the symptoms of the disease, and even the onset; however, it is also critical to identify effective biomarkers that may help prevent HD manifestation by early detection and blocking its course. Modern therapeutic trial design also vastly relies on identifying and examining biomarkers relevant to each disease.

The next step may be to evaluate and use data from genome-wide association studies and account for their clinical utility. Studies (as aforementioned) towards this direction have contributed to the existing knowledge concerning the association of genetic variations to the onset of symptoms and the progression of HD. The combination of early testing in order to predict the possible HD onset and new targeted and personalized medicine represents the future in preventing and hopefully, eliminating neurodegenerative diseases. The path for science ahead to help patients with HD is a long one. Until then, finding the optimal care for patients and caregivers is significant.

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