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Review Article

Huntington's Disease: Pathogenesis, Therapies, and Emerging Technologies

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Abstract

Huntington's Disease (HD) is a hereditary neurodegenerative disorder that is primarily manifested by motor, cognitive, and behavioral symptoms due to an expanded CAG trinucleotide repeat in the HTT gene. Currently, most of the therapeutic strategies in HD are largely centered on the pharmacological management of the symptoms. These treatments are linked to some disadvantages such as being partially effective, having adverse effects, and their inability to alter the natural course of the disease. Recent developments in HD research are exploring the use of novel pharmacological agents, nanoparticles, cell therapies, gene therapies, and RNA-based therapies, which have shown promise in preclinical and clinical studies. This literature review explores various aspects of HD, from its pathogenesis and etiology to emerging novel approaches for its treatment.

Keywords: Huntington's Disease, HTT Gene, Nanotechnology, Neurodegeneration, Emerging Therapies, Mutant Huntingtin Protein

1. Introduction

Huntington's disease (HD) is a genetic condition characterized by the progressive deterioration of the nervous system, resulting in motor, cognitive, and behavioral deficits [1]. HD is a genetic illness caused by inheriting an enlarged CAG trinucleotide repeat in the huntingtin gene on chromosome 4. It is inherited in an autosomal dominant manner. As a result, a protein product is generated with an extended polyglutamine sequence at the N-terminal, which has harmful consequences for neurons [1,2]. Typically, a typical Huntington gene contains less than 36 repeats. Individuals with 10-35 CAG repeats are considered normal, while those with fewer than 27 CAG repeats are unable to acquire the disease in their neurons [1–3]. Studies have also noted that individuals with Huntington gene repeat lengths ranging from 36 to 39 CAG repeats show lower penetrance. This means that while some people with these repetitions will acquire the disease, others will not be impacted [1,3]. Since CAG triplets in proteins encode the amino acid glutamine (Gln or Q), these disorders are classified as "Polyglutamine Diseases (Poly Q)". Polyglutamine disorders exhibit autosomal dominant inheritance and display several shared characteristics, except for SBMA (Spinal Bulbar Muscular Atrophy), which is inherited via the X chromosome. These factors include polymorphic and unstable CAG repeats, parental contradiction, new mutations, anticipation, and an inverse relationship between the number of repetitions and the age at which the disease begins. Polymorphic CAG repeat instability refers to the alteration in the number of CAG repeats passed down from one generation to the next, either by decreasing or increasing. The striatum, a brain region prone to instability, experiences deterioration that is particular to these illnesses. Parental contradiction refers to the variation in CAG repetitions and how it is influenced by the gender of the parent who passes on the allele. It has been observed that this contradiction arises when the allele is inherited from the father in this particular sickness [4].

The global prevalence of HD has risen over the past twenty years due to factors such as longer life expectancy, advancements in genetic testing, better healthcare infrastructure for HD patients leading to increased disease survival rates, and a decrease in negative

attitudes towards HD diagnosis. Another contributing component is migration or travel, in which a carrier of a mutation, or several carriers, reproduce within an isolated group. This leads to an increase in the likelihood of the allele due to the expansion of the population [5]. The frequency of HD varies among different ethnic groups, especially among those of European descent. It is estimated that HD affects roughly 18.4-18.7 individuals per 100,000 people in this population [1]. However, the occurrence of the disease is far lower in Asian and African populations, with rates as low as 0.5 per 100,000. This suggests that genetic predispositions play a critical part in the disease's development [2,6]. Research suggests that the disease tends to progress more positively in individuals within the age range of "30 to 50 years". The average age at which symptoms often begin is typically between 35 and 44 years [6]. However, juvenile forms of HD (the age of onset <20 years) and older forms of HD (the age of onset > 70 years) are also recognized. This can be attributed to the fact that as many as 90% of individuals with juvenile Huntington's disease get the mutation from their father, and the number of CAG repeats typically exceeds 60. The disease was initially identified by George Huntington and is usually known as Huntington's chorea in honor of its discoverer [3]. Adult-onset HD is characterized by 40-50 CAG repeats whereas a juvenile form is characterized by 50-120 CAG repeats [2]. HD manifests itself in both male and female individuals of all cultures and ethnic backgrounds. The chances of getting HD are 50:50 from HD parents to children [6]. It is well established that the cause of death is directly linked to the problems of staying immobile such as skin complications, infections, pulmonary complications, or cardiac events [7]. The most recent categorization by the HD Task Force of the International Parkinson's Disease and Movement Disorder Society has divided the disease into three stages based on the presence and extent of motor and cognitive symptoms: presymptomatic HD, prodromal HD, and manifest HD. Notably, it was emphasized that each diagnostic category had the potential for the development of new symptomatic and/or disease-modifying treatments [8]. An individual with an elevated quantity of CAG repeats in the HTT gene will exhibit symptoms of Huntington's disease at approximately 30 to 40 years of age. This would typically lead to end-stage disease within a period of fifteen to thirty years. At present, the onset of manifest HD is determined by the development of prominent motor symptoms. Before this time, the patient is a premanifest gene carrier. It is important to know that most patients develop some or all the cognitive, psychiatric, or subtle motor symptoms during the premanifest or prodromal state; and this may occur years, even decades before any recognizable motor symptoms begin [3].

This review presents a comprehensive overview of HD's etiology, current treatment approaches, and novel treatment strategies to inform future research directions and clinical management efforts for Huntington's Disease.

2. Pathophysiology of Huntington's Disease

Neural loss in the striatum has for many years been regarded as the most characteristic pathological feature of HD. In HD, the pathologic changes in the striatum are largely specific to a certain neuronal type, the medium spiny neurons (MSNs). The other class of neurons present in the striatum is the aspiny interneurons, which are relatively normal and least affected in HD [9]. The degeneration of a medium spiny neuron contributes to uncontrolled neurotransmission of the dopamine, glutamate, and gamma-aminobutyric acid (GABA) systems, which is why pharmacotherapy of HD is based on its alteration. While dopamine, glutamate, and GABA are believed to be the neurotransmitters most involved in HD and primarily addressed in treatment, multiple neurotransmitters and receptors are implicated at various sections of the HD brain [10]. Chorea is due to the death of the medium spiny neurons, especially those that contain enkephalin in the basal ganglia in the indirect pathway. The motor symptoms of dystonia and akinesia arise because of the additional degeneration of the substance-P-containing medium spiny neurons in the direct pathway [10]. Cerebellar degeneration in HD is best characterized by ataxia; it affects especially patients with JHD. Some studies in adult-onset HD cases showed signs of cerebellar damage and alteration in the grey matter despite being presented in the small sample population [11]. The mutated huntingtin protein affects the normal functioning of cells in several ways including proteostasis, mitochondrial dysfunction, transcriptional, synaptic, and axonal transport pathway dysfunction [1]. In essence, the disease spreads along a pattern: first, the striatal MSNs are lost, and Over time the degenerating process extends to the rest of the basal ganglia and even the cerebral cortex and substantia nigra [7].

In addition to the well-defined HD lesion in the striatum, there is growing evidence for the involvement of peripheral metabolic pathways in the pathogenesis of the disease. HD patients lose weight even when they are consuming more calories than before. Together with the phenomenon of skeletal muscle atrophy, glucose intolerance, and changes in the gastrointestinal tract, a generalized metabolic disorder has been suggested. The levels of aliphatic amino acids and fatty acids break down in the HD human blood samples are found to be abnormal in HD patients. Moreover, another recent finding is the detection of increased concentrations of urea in the human brain of HD patients after death [5].

2.1. Genetic Basis of Huntington's Disease

2.1.1.1. The Huntingtin Gene Mutation

The Huntingtin (HTT) gene is expressed throughout the human body with the highest expression levels in CNS where it is involved in neuronal activities both in the cytoplasm and nucleus [12,13]. Huntingtin is involved in many cellular operations and is important for embryonic growth as indicated by embryonic knock-out experiments [13].

The disease is inherited as an autosomal dominant trait, which implies that children of affected parents have a 50 percent chance of inheriting the abnormal allele.

Particularly, the "abnormal gene" starts at 36 CAG trinucleotide repeats, and if the CAG repeats are more than 40, the disease phenotype is fully penetrant. Moreover, a small proportion of cases may be due to mutations, if an intermediate allele of 30-38 repeats that can expand is present and, especially if it is inherited from fathers of old age [14].

The pathological basis of the HD is caused by the HTT gene mutation which is characterized by the expansion of CAG trinucleotide repeats in the HTT gene which leads to the formation of the mutant huntingtin protein (mHTT) [12,15]. This expansion is located on the fourth chromosome, precisely 4p16. 3, a locus known as 'interesting transcript 15,' or IT15. IT15 has a core body length of 210 kb and has 67 exons; the HD expansion occurs at the first exon starting from the 18th nucleotide position [14]. The mutation impacts on the caudate nucleus, putamen, the cortex, thalamus, subthalamic globus, pallidus, substantia nigra, and the cerebellum. Neuronal loss, atrophy, and gliosis are noticed in these regions [12]. The mutation affects neuronal structure, synapses, and neurotransmission circuits, causing alterations in signaling, transcription factors, and mitochondrial performance. Furthermore, the level of oxidative stress and neuroinflammation is raised in the affected regions of the brain [16].

Overall, Risk factors for Huntington's disease include three major categories: CAG repeat length, CAG instability, and Genetic modifiers. Among these factors, CAG repeat length in the mutated allele has been reported to be a vital predictor of the progression of HD, affecting the motor, cognitive, and other neurological symptoms [17].

The CAG repeats are inversely proportional to the age of onset of symptoms; hence, higher CAG repeat numbers result in early symptom manifestation [15,18]. CAG repeats are not stable and same between generations. This is the biological basis of anticipation where age at the onset of disease is noted to be progressively younger in successive generations. No other gene modifiers other than the CAG mutation that can affect the disease onset or progression have been identified, but there are genetic factors that affect CAG repeat expansions. Male children are at a greater risk of having expanded CAG repeats and some genetic haplotypes have been found to be associated with CAG repeat instability and expansion in successive generations [11].

It is noteworthy that homozygosis or the non-mutant allele does not affect the age of disease onset but can aggravate the disease course with a higher rate of neuronal loss and gliosis in various regions of the brain. The level of motor, cognitive, and behavioral manifestations depends on homozygosity, but it is not very common in patients with HD [16].

Spermatogenesis is more susceptible to expansion of the unstable sequence of CAG repeats during mitosis compared to oogenesis, which is why patients who inherited the disease from the father have longer CAG repeats and symptoms appear at an earlier age [13].

CAG repeat number accounts for only about 40-50% of the variability in both the age of onset and the severity of the disease. Other genetic factors that contribute to the rest of the variance are: The normal allele CAG repeat size, The Δ2642 glutamic acid polymorphism, Polymorphisms in the gene encoding for the GluR6 receptor, Polymorphisms of the gene NMDA receptor subunit 2B, Polymorphisms of the genes PPAR-γ coactivator 1α (PGC-1α), Polymorphisms of the genes ASK1 and MAP2K6, or of genes that are related to mitochondrial activity such as the CHCHD2, Genes that colocalize with the expanded CAG repeats such as the G-protein-coupled receptor (GPCR) 161 allele [13].

It appears that other environmental factors also play a role in the variation of the age of onset. In addition, dissimilar clinical presentation has been noted in monozygotic twins concluding that epigenetic factors or tissue-specific variation in CAG repeats due to somatic instability may be involved in the development of the disease [15].

Furthermore, it has been found that the length of CAG alleles rather than the length of polyQ is more significant for the development of the disease. Therefore, studies are being conducted to determine other modifying genes, especially those associated with DNA repair actions that may interact with the CAG expansion present in the HTT gene to improve the onset age and progression of HD symptoms [19].

2.2. Molecular Mechanisms of Pathogenesis

2.2.1.1. HTT Protein

HTT has 3144 amino acids and is estimated to be around 348kD in molecular mass [2]. Its amino-terminal contains a polyglutamine stretch and has consensus sequences referred to as HEAT for interaction with other proteins which include huntingtin, elongation factor 3, protein phosphatase 2A, and TOR 1. In the literal sense, HEAT motifs can be linked with the role of Huntingtin as a scaffold in the process of protein formation. Huntingtin is a protein that is mainly found in the cytoplasm and has a partial nuclear presence. In addition, Huntingtin has the nuclear export sequence at the C-terminal region of the protein. Further, it has been found that the N-terminal area of huntingtin interacts with Tpr, a nuclear protein that has a role in export from the nucleolus. Reduction of this interaction by Polyglutamine expansions results in enhanced nuclear accumulation of huntingtin [15].

HTT is a large protein and has been found to interact with many other proteins and is implicated in many cellular functions. It also participates in intracellular transport through its associations with the molecular motor protein complexes. It has been documented that HTT has been implicated in axon transport and vesicle transport via interaction with several endocytic proteins such as α-adaptin, HIP1, HIP14, HAP1, HAP40, PACSIN1, SH3GL3, clathrin, and dynamin [2].

In addition, it has been noted that HTT is implicated in cell survival and apoptosis cellular signaling pathways [12]. HTT is also associated with proteins that are related to cytoskeleton and it has functions in cytoskeleton

remodeling and cell morphology. These functions are helpful in the movement and transport of cells in living organisms [2]. Huntingtin has been shown to interact with the transcription factors, co-activators, and repressors and plays a significant role in the regulation of transcription of genes involved in the neuronal functions [12]. In embryonic development, huntingtin is required for the neurons' survival, differentiation, and neurogenesis. If it is inactivated, then the development rate may be reduced, and the embryo may even die [12].

In neurons, huntingtin undergoes a process of aggregation where it forms inclusion bodies with other soluble HTT. The mechanism of cell death is not clear yet as it is unknown whether the reason for cell death is the accumulation of the HTT aggregates or the toxic characteristic of the soluble form is responsible [10].

2.2.1.2. Impact of Mutant Huntingtin Protein

Mutant Huntington protein has an expanded polyglutamine (polyQ) tract of more than 40 glutamine residues starting from position 18 [13]. These structural changes in mHTT impair the ability of the protein to perform its normal function in the cells through protein-protein interactions and result in the toxic gain of function or loss of function in vesicular trafficking and mitochondrial functions [13,18].

The mHTT protein aggregates form nuclear and cytoplasmic inclusions in neurons and glial cells, particularly affecting striatal medium-sized spiny neurons (MSN) [16]. The reason that striatum is the first area and preferentially affected by the disease is still not obvious and requires more research. However, there is a rather strong hypothesis that lost corticostriatal connections are related to low BDNF levels and/or increased extracellular glutamate [18].

The defective autophagy process prevents these protein aggregates from being degraded; thus, leading to the atrophy and progressive loss of brain structures [16]. Mutant huntingtin protein as well as producing a toxic effect on the cell, also changes the shape and function of mitochondria. It binds to proteins like Mfn2 and DRP1 leading to the fragmentation of mitochondria which in turn causes energy metabolism dysfunction with eventual neuronal damage [13,20]. Additionally, mutant huntingtin interacts with the transport of material to mitochondria which adds to the cellular dysfunction in HD [13].

In addition, another factor that has been found to cause neurodegeneration in HD mouse models is an imbalance in calcium levels. It has been found that mHTT affects the calcium homeostasis in MSNs as well by altering the functions of the glutamate receptors, leading to excitotoxicity, apoptosis, and disruption of mitochondria functions [21].

Moreover, mHTT has been found to stimulate proapoptotic molecules like caspase 3, which in turn leads to the degradation of huntingtin protein. Then, mHTT goes into the nucleus with the degradation products and targets the genes for modulation. Furthermore, mHTT binds to signaling proteins like cAMP-response element-binding protein (CREB), specificity protein 1, and p53 to alter pathways that are crucial for cell viability and energy homeostasis [14].

2.2.1.3. Neurotrophic Factors in HD

Neurotrophic factors are proteins that can influence the differentiation, growth, and survival of neurons in different neurodegenerative diseases. Some of them are Brain-Derived Neurotrophic Factor (BDNF), Fibroblast Growth Factor-2 (FGF-2), Glial Cell Line-Derived Neurotrophic Factor (GDNF), Neurturin, and Ciliary Neurotrophic Factor (CNTF) and all of them are of significant importance in HD because of their neuroprotective properties [21].

2.3. Disease Stages and Progression

2.3.1.1. Stages of Huntington's disease

Huntington's Disease (HD) is a neurodegenerative disorder that has clinical stages where the patient exhibits different symptoms of neurological and cognitive decline. The first phase is characterized by motor dysfunction, learning disability, and psychiatric disorders including major depressive disorder. This is the early stage of the disease that is likely to develop around the age of 40, and the symptoms of the disease may become worse and change with time [16].

The onset can begin in the second decade of life in the case of the juvenile-onset variant or be as late as the seventh decade. The clinical course is usually insidious, and the early symptoms of the disease in adults might be mild and not very pronounced [14].

The disease is marked by behavioral disturbances and cognitive impairment, which are the first symptoms of the disease; among symptoms, chorea is the most noticeable one. During the later years of the disease, other motor signs such as dystonia, parkinsonism, and bradykinesia become more evident. Abnormalities in eye movements are also common and may be seen as one of the earliest signs of movement disorder in pre-manifest individuals [14].

The penetrance of HD is nearly complete by the age of 65 years, thus proving the progressive nature of the disease. The loss of neurons in the striatum area is the primary factor that enables the development of motor symptoms and the general neurological deterioration in HD patients [16].

2.3.1.2. Cognitive and Behavioral Impacts

Cognitive Impacts: cognitive decline in Huntington's disease is mild in the early stage of the disease but gradually becomes the hallmark of the disease. This comprises visuospatial and executive dysfunction as well as memory dysfunction. Before developing motor symptoms, gene-positive individuals have cognitive deficits involving memory, both short and long-term, as well as reduced IQ scores that are related to CAG length. They can abstract, calculate, and perform frontal functions but these abilities gradually reduce and result in global dementia with a decrease in verbal fluency [14].

Behavioral Impacts: Psychiatric symptoms are also observed in patients with Huntington's disease, and they include mood swings, aggression, anxiety, depression, anhedonia, poor decision-making, and impulsive behavior. Behavioral changes are observed in up to 80% of the HD subjects as a part of the disease manifestation, which may include irritability, depression, anxiety, and other behavioral changes [14].

3. Management of HD

Huntington's Disease (HD) can be described as a neurodegenerative disorder for which management mainly includes the use of strategies that are aimed at treating the symptoms to enhance the quality of life of patients who are affected by this disease, while no officially approved pharmacotherapy for modifying the course of the disease has been approved to date. Although Huntington's disease is a progressive and fatal disorder, it is not an untreatable condition [22,23].

Some of the approaches that have been put forward for the treatment of HD include small molecules, CRISPR/Cas9, RNAi, antisense oligonucleotides, monoclonal antibodies, and zinc finger repressors. These therapies include HTT gene editing, intervention at the RNA level, or blocking the aggregation of mHTT [24].

3.1. Symptomatology of Huntington's Disease

In clinical practice, HD is described as a slowly progressive neurological disorder that impacts the motor, cognitive, and psychiatric domains. The motor symptoms of HD can be divided into two categories: involuntary movements such as chorea and affected voluntary movements that result in limb incoordination and poor hand movements. Chorea is observed in the early stages of the disease and is less seen in the later stages of the disease. As the disease progresses symptoms like dystonia which is the involuntary contraction of muscles causing abnormal posture, and bradykinesia which means slowness of movements and rigidity become more prominent. If a patient has more hypokinetic symptoms (bradykinesia and dystonia) than hyperkinetic (chorea) then the patient is diagnosed with a Westphal variant of HD [3,7].

Cognitive manifestations in HD include abnormalities in executive, planning, organizing, and multitasking abilities, memory, and attention [3]. Psychiatric symptoms in HD can be as mild as depression, irritability, and anxiety to much more severe as psychotic symptoms or obsessive-compulsive disorders [1].

3.2. Pharmacological Interventions

3.2.1.1. Motor Symptom Management

Therapy is started when chorea is inconvenient or begins to affect motor coordination and work abilities. The goal of the treatment is to reduce the intensity of the involuntary movements as much as possible but not to eliminate them completely because the elimination of the movements can lead to adverse effects on balance and voluntary movements [25].

In general, motor manifestations in Huntington's disease are chorea and loss of coordination; however, patients may also suffer from speech and swallowing disorders. The cognitive signs may manifest up to ten years before the diagnosis, and cognitive function worsens as the disease advances. Finally, patients with HD will ultimately need a wheelchair, and as the disease progresses, the patient may become bedridden, which is accompanied by complications related to immobility [26].

As for medicines, Dopamine Modulators, such as tetrabenazine (TBZ) and deutetrabenazine have been applied to control chorea and have been proven to have antichorea efficacy in patients with HD [24,26–28].

D2/D3-dopamine receptor antagonists such as tiapride have antichorea effects in patients with HD. The coadministration of tiapride (postsynaptic) and tetrabenazine (presynaptic) may decrease the dosage of the respective individual drugs and lessen side effects [29].

Olanzapine, an atypical neuroleptic, is used most frequently to treat chorea in HD [30]. As it is administered once a day in the evening, patient compliance is higher with this drug. Most physicians commence with olanzapine and if it does not work, then they prescribe TBZ [30].

Other drugs like sulpiride, amisulpride, and risperidone can also be used clinically in HD, but they are less often chosen because the evidence of their effectiveness is scarce [30].

For treatment of myoclonus clonazepam, levetiracetam, or piracetam and valproate acid have been suggested in practice [29]. For the treatment of dystonia in HD, the drugs that can be used are low-dose tetrabenazine and other drugs like amantadine, baclofen, tizanidine, and clonazepam [29]. Botulinum neurotoxin chemodenervation is the initial treatment of focal dystonia which is a part of HD's defining symptoms [25].

Bradykinesia and rigidity are usually manifested at a later stage of the disease, but in the case of juvenile Huntington's Disease (JHD), the symptoms are more severe. In these stages of the disease, drugs such as levodopa which enhances dopamine levels, pramipexole which is a dopamine receptor stimulant, and amantadine can be used [28,29].

Since the synaptic glutamate levels are elevated in the preclinical stage of HD, drugs like Amantadine, Riluzole, Remacemide, Memantine, and Lamotrigine could have positive effects if administered before the first sign of the disease [28]. Table 1 provides an overview of the drugs used in the treatment of movement disorders in Huntington's disease.

Table 1: Drug molecules used in the treatment of movement disorders in Huntington's Disease

No	Drug	Mechanism of Action	Pharmacological Effects	References
1	Tetrabenazine	Dopamine modulator	Antichorea Movement disorder	24,26–28
2	Deutetrabenazine	Dopamine modulator	Antichorea Movement disorder	24,26–28
3	Tiapride	D2/D3-Dopamine receptor antagonist	Antichorea	29
4	Olanzapine	Atypical neuroleptic	Antichorea	30
5	Sulpiride	D2 receptor antagonist	Antichorea	31
6	Risperidone	Dopamine antagonist	Reduce symptoms of psychosis	32
7	Clonazepam	Enhanced activity of GABA	Myoclonus Dystonia	33
8	Levetiracetam	Binding to synaptic vesicle protein 2A (SV2A)	Myoclonus	34
9	Piracetam	GABA derivative	Myoclonus	35
10	Valproic Acid	Enhanced activity of GABA	Myoclonus	36
11	Amantadine	N-Methyl-D-aspartate antagonist	Antichorea Dystonia	37
12	Baclofen	GABA agonist	Dystonia	38
13	Tizanidine	Agonist of Alpha-2 Adrenergic Receptor	Dystonia	29
14	Levodopa	Dopamine receptor stimulant	Antichorea	39
15	Pramipexole	D2/D3 receptor agonist	Antichorea	40
16	Riluzole	Sodium channel blocker	Antichorea	41
17	Remacemide	N-Methyl-D-aspartate antagonist	Antichorea Dystonia	42
18	Memantine	N-Methyl-D-aspartate antagonist	Antichorea Dystonia	43
19	Lamotrigine	Sodium channel blocker	Antichorea	44

3.2.1.2. Non-Motor Symptom Management

Psychiatric symptom management in Huntington's Disease (HD) entails the management of several neuropsychiatric symptoms that include depression, apathy, anxiety, obsessive-compulsive disorder, psychosis, aggression, sexual dysfunction, and dementia [27]. These symptoms may develop at any time of the disease depending on the stage of HD [28].

For the treatment of depression in patients with HD, antidepressants such as SSRIs that include citalopram, fluoxetine, sertraline, mirtazapine, and clozapine are often administered [27,28]. Aggressive and obsessive-compulsive behaviors can also be treated using mood stabilizers such as sodium valproate, lamotrigine, and carbamazepine [27].

Some of the typical neuroleptics that have been used in the treatment of chorea as well as psychotic symptoms include haloperidol, pimozide, fluphenazine, thioridazine, sulpiride, and tiapride. These drugs should only be used when it is necessary because of side effects. Atypical neuroleptics like Risperidone, Quetiapine, and Ziprasidone are used more than typical neuroleptics because they have lesser side effects on the extrapyramidal system compared to the typical neuroleptics [28].

The psychiatric symptom most frequently reported by HD patients is apathy. Currently, there are no medications to treat apathy in HD, although it is a common symptom. Cariprazine is an atypical neuroleptic and D3 selective D2/D3 receptor partial agonist that has been proven to be effective in a particular study. It is recommended to use individualized cognitive training and structured daily activities [29].

Medications such as haloperidol, olanzapine, risperidone, and quetiapine are useful in treating aggression and irritability in patients with HD. Furthermore, lamotrigine and lithium can enhance mood fluctuations positively [30].

Psychotic symptoms have been managed either with risperidone or amisulpride. Olanzapine is used in

treating depression, anxiety, irritability, and dealing with obsessive behaviors. Also, two tricyclic antidepressants, amitriptyline and imipramine, are rarely prescribed; Paroxetine was found to be effective in the treatment of obsessive-compulsive disorders linked to HD. Another SSRI, sertraline, may help in the treatment of obsessive ideas, dysphoria, irritability, and aggressiveness during HD [28].

Additionally, it is crucial to note that psychological treatment in the form of a meeting with a psychologist can be effective in treating cognitive and emotional manifestations in patients with HD. The use of a team of medical professionals working together is essential to provide care that is personalized to the needs of the patient [27].

Issues with weight reduction and sleep interference are prevalent in HD and can also worsen neurological issues. In patients with Huntington's Disease, weight loss is counteracted with the help of vitamin and mineral supplements and the administration of olanzapine, which is known to cause weight gain as a side effect. In the same way, information on the side effects of some drugs is used to treat insomnia in HD patients; olanzapine, amitriptyline, trazodone, or mirtazapine is often given at night. For managing insomnia in HD, some of the most used drugs that function as sedatives include zopiclone, zolpidem as well as zaleplon [30].

3.3. Non-Pharmacological Interventions

Therapies such as physiotherapy and occupational therapy can be useful for patients with HD since they can help with difficulties in coordination, balance, and mobility. Measures such as gait training, stretching exercises, as well as assistive devices can help preserve the level of motor activity and minimize dependence on the assistance of other people. In addition to the above, there are other treatments such as physical therapy, speech therapy, psychiatric therapy, and caregiver support groups for managing the difficulties that come with HD [23,27].

3.4. Neuroprotective Strategies for Huntington's Disease

HD is one of the few neurodegenerative diseases in which the diagnosis is possible years before the manifestation of the signs and symptoms of the disease, which allows interfering with the onset of the disease. Therefore, HD is an ideal model for evaluating neuroprotective interventions [25].

MSCs can renew themselves as well as they can secrete neurotrophic factors such as BDNF which has a neuroprotective effect [27]. In addition, Laquinimod is found to be neuroprotective by increasing BDNF levels; however, the exact mechanism is not known [27].

Moreover, using natural products has the possibility of providing neuroprotection and enhancing the well-being of those with Huntington's Disease. These natural products demonstrate their action as free radical inhibitors, free radical neutralizers, anti-inflammatory and anti-apoptotic agents, reducing oxidative stress, modulating autophagy, and enhancing mitochondrial function [24].

Several Natural Products exhibit their efficacy against HD with Antioxidant and Anti-inflammatory properties. For instance, the combination of cannabinoids delta-9-tetrahydrocannabinol and cannabidiol has been demonstrated to enhance levels of BNDF and phenotypes in animals. Moreover, Melatonin, due to its antioxidant and anti-inflammatory properties, exerts neuroprotective effects in HD. Also, some natural compounds with antioxidant and anti-inflammatory activity such as protopanaxatriol from Panax ginseng and celastrol from Tripterygium wilfordii, have been shown to protect striatal neurons in vitro and reduce oxidative stress in vivo. Furthermore, flavonoids such as dihydromyricetin have been found to possess neuroprotective properties through antioxidant activities. Several medicinal plant extracts because of their antioxidant activity such as Calendula officinalis, Celastrus paniculatus, and Centella asiatica have been found to possess properties that have the potential for a possible remedy for HD [24].

Likewise, the strategies aimed at preventing the formation of mutant huntingtin protein aggregates and the subsequent excitotoxicity may be useful in the treatment of Huntington's disease. Epigallocatechin-3-gallate and ellagic acid have been proven to have potential in the prevention of mHTT aggregation. Also, a combination of compounds like dextromethorphan and quinidine is currently in phase III clinical trials under evaluation for the possible treatment of excitotoxicity. Moreover, High doses of thiamine and biotin combination are being investigated as a possible treatment for improving the quality of life of patients suffering from HD by alleviating by addressing the RNA splicing issues and replenishing the cell energy [24].

Another significant strategy for the neuroprotection of HD is also the improvement of mitochondrial function. Resveratrol augments the energy metabolic process and mitochondrial function, which targets AMPK, Sirt1, and PGC-1α. Cysteamine also decreases the level of oxidative stress by up-regulating the BDNF level and has a positive impact on the mitochondria in many animal models. Some flavonoids like naringin and genistein may be beneficial in raising mitochondrial function and reducing oxidative stress. Another intervention that can be made is the regulation of apoptosis and autophagy. Neferine belongs to the group of alkaloids that enhances autophagy through the mTOR/AMPK pathway and has neuroprotective properties. Berberine has been used to improve motor function and plays a role in the mechanism of autophagy of mHTT. Moreover, trans- ε-viniferin induces autophagy and offers neuroprotection because it activates AMPK [24].

Finally, there is another field that has started developing and it is the use of natural products to stimulate neurohormetic response. Sulforaphane, naringin, and resveratrol are compounds that activate the Keap1/Nrf2/ARE pathway that increases the cellular stress response and provides neuroprotection. These compounds are useful in regulating the levels of oxidative

stress, the efficiency of the mitochondria, and the general health of neurons [24].

4. Recent Advances in Treatment

4.1. Development of Small Molecules

Novel approaches in the management of Huntington's disease are the use of novel investigational small molecules, repurposing drugs that have not been previously used for HD but have been developed for other diseases, and gene therapies. Currently, various investigational molecules are undergoing clinical trials for Huntington's disease including Neflamapimod (targeting enzyme mitogen-activated protein kinase 14 MAPK/p38α), PBT2 (reduce Aβ plaque formation), SRX246 (targets vasopressin 1A (V1AR) receptor), OMS643762 (PDE-10A inhibitor), Mardepodect (PDE10 inhibitor), BN82451 (blocking the Na+ channel and inhibiting cyclooxygenases), Mavoglurant (inhibiting metabotropic glutamate receptor 5), Bevantolol hydrochloride or SOM3355 (targets the vesicular monoamine transporter 2), SAGE-718 (targets NMDAR), PBF-999 (targets PDE-10A and A2AR), and GSK356278 (PDE4 inhibitor) [45].

Some of the dopaminergic stabilizers like pridopidine that target the sigma 1 receptor (SIG1R) to reduce toxicity to MSNs and increase neuroprotection have been observed to be effective in some of the studies [46]. Furthermore, other researchers have suggested substances like Congo red, Trehalose, Compound C2-8, and rapamycin which have proven efficient in rodent models addressing HTT aggregation and clearance as an innovative approach for treating HD [47]. Moreover, some evidence suggests that selisistat, a SirT1 inhibitor (silent information regulator T1) could increase the clearance rate of mHTT from the body [48]. Interestingly, Meclizine which is generally used to treat allergic reactions, targets mitochondrial dysfunction through the suppression of oxidative metabolism and apoptosis and has been found to show neuroprotective effects in a drosophila model and could be used as a candidate for future research [47]. Because Mithramycin and Chromomycin target Transcriptional Dysregulation by inducing epigenetic histone modifications, they can be other potential drugs for future trials [47]. Another compound that is thought to increase the level of BDNF and thereby potentially delay the progression of HD is transglutaminase inhibitor cysteamine [49].

The repurposing of currently used drugs for the management of rare diseases is important because it takes time for new drugs to be discovered and developed. Currently, several drugs such as Fenofibrate, Ursodiol, Cysteamine, Riluzole, Sodium phenylbutyrate, Atomoxetine, Minocycline, Ramelteon, Nilotinib, Ethyl-EPA, Valbenazine, and Triheptanoin are some drugs which are under investigation for repurposing them for the treatment of HD [24,45].

4.2. Therapeutic Potential of Natural Compounds

Recently, many naturally occurring small molecules that have been identified to have neuroprotective effects because of increased mitochondrial function, promotion of autophagy, and suppression of apoptosis have been investigated for the management of HD. For instance, resveratrol, and melatonin are among the compounds that are currently being evaluated in clinical trials. Further, blends of delta-9-tetrahydrocannabinol with cannabidiol, quinidine with dextromethorphan, and thiamine with biotin have been found to have positive results in clinical trials [24]. Likewise, a few plant extracts have been shown to have activity against HD due to their antioxidant activity including *Calendula officinalis*, *Celastrus paniculatus*, *Centella asiatica*, *Convolvulus pluricaulis*, *Luehea divaricate,* and *Withania somnifera* [24].

Apart from small molecules and repurposing drugs, autophagic degradation of mHTT in HD could be helpful. Gossypol is a phenolic compound, which induces autophagy to target the damaged mHTT protein for degradation [45].

4.3. Stem Cell-Based Therapies

The use of stem cells in cellular replacement therapy is being explored as a possible treatment for Huntington's disease to improve symptoms in affected patients [23,26]. Stem cell therapies can be applied in the replacement of damaged neurons, in the promotion of neurogenesis, and in the protection of neurons from further degradation. Some of the stem cells that have been identified to possess the possibility of treating HD are Mesenchymal stem cells, Embryonic stem cells (ESCs), neural stem cells (NSCs), and induced pluripotent stem cells (iPSC cells) [50].

4.4. Surgical Interventions

Another approach to managing HD is through Deep Brain Stimulation (DBS), which has been noted to positively impact chorea's symptoms without worsening the bradykinesia. It has also been pointed out that DBS enhances motor function, and voluntary and involuntary eye movements in HD patients [27,28].

4.5. Additional Emerging Treatments

Exercise has also been found to play a role in improving motor function, gait speed, balance, and the quality of life of patients with HD [26].

Also, some interventions have been made using probiotics to treat gut dysbiosis. Although probiotics did not enhance gut dysbiosis or lack the effects on cognition, mood, and gastrointestinal symptoms, the therapeutic potential of the gut in HD cannot be entirely dismissed [51].

Other strategies are the involvement of histone deacetylase (HDAC) inhibitors and flavonoids; these have shown curative effects on motor symptoms of HD according to recent studies [52].

5. Limitations of Current Therapies

Current interventions in Huntington's disease have certain challenges. Non-human primate large animal models present certain ethical, practical, and cost concerns that can influence the translation of preclinical cell therapy plans into clinical practice. In addition, few targeted medications can be administered to Huntington's disease patients because of the lack of comprehensive knowledge about the mechanisms of the

disease. The significant problem with cell therapy delivery is that the intact blood-brain barrier is impermeable and spatial constraints result in the inefficiency of systemic delivery. Also, there is a need to design appropriate clinical trials that meet regulatory requirements and consider the complexities of cell therapy [53].

The currently available therapies are mostly palliative and do not affect the onset age or the disease progression rate. Tetrabenazine and deutetrabenazine are drugs that are used to treat chorea, but they also come with side effects that include depression, anxiety, and even suicide. The major limitation of existing therapies is their lack of specificity, modifying only some targets and letting the pathological processes develop where they are not blocked. Questions regarding the safety of non-selective mHTT reduction, the frequency of off-target effects in large populations, and the safety of CRISPR-based therapies remain unanswered [54].

Presently available symptomatic treatments are directed at the moderate phase of the disease or, in the case of cognitive and neuropsychiatric signs, early to moderate phases only. Further, there is a need to develop HD-specific treatments for all types of symptoms that manifest in the later stages of the disease. As of now, there are no treatments for cognitive symptoms that are specific to HD but there are treatments that are still under development [49].

At present, the medications are mainly directed towards chorea and there are no treatments that effectively ameliorate the motor, behavioral, and cognitive symptoms of the disease. There are no disease-modifying treatments available for Huntington's disease to date, despite the extensive research and advances made in this field [55].

Moreover, there is no sufficient research on medicines utilized in HD cases. Many of the treatments for HD have not been assessed in RCTs, which means that the treatments are based on the opinion of experts and practice [56,57].

Moreover, since HD is an orphan disease with a prevalence of less than 10 per 10,000 population, it presents financial risks for pharmaceutical companies as well as makes it challenging to enroll enough patients for any study [57].

Furthermore, existing huntingtin-lowering approaches are unlikely to be useful in all cases, or they will halt the progressive loss of neurons in all instances. Another question that needs to be resolved in cell therapies is how to obtain appropriate donor cells from (nonfetal) cell sources [58].

6. New Approaches and Technologies

6.1. RNA-based Therapeutic Approaches

Treatments aimed at RNA or DNA hold the potential to address the root of the problem by inhibiting the production of the protein or correcting the mutated gene [59,60].

The mHTT in HD can be targeted at a post-transcriptional level by either selectively degrading the mHTT mRNA or reducing the efficiency of its translation. These strategies avoid the formation of mHTT, lower its concentration and the likelihood of its aggregation, and decrease the mHTT's toxic effect on cells. mRNA molecule is easier to target since it is located both in the nucleus and the cytoplasm and it does not have repair mechanisms [61].

RNA-based strategies in HD involve the use of antisense oligonucleotides (ASO), RNA interference (RNAi), and small molecule splicing modifiers [59,60]. The ASOs cause the degradation of pre-mRNA in the nucleus, RNAi targets the mature mRNA in the cytoplasm, and small molecules target the pre-mRNAs' splicing pattern to produce a protein that is not functional [62]. These approaches lower the production of the mutant huntingtin protein by suppressing the gene expression [26,27,52].

6.1.1.1. Antisense Oligonucleotides Therapies

ASO therapies are becoming increasingly popular for their application in the treatment of HD [27]. ASOs are synthetic single-stranded oligonucleotide analogs. They range in length from 16 to 22 nucleotides and interact mainly with pre-mRNA through Watson-Crick binding [61]. Their effects are easily reversible which is beneficial in the case of side effects, but they need to be administered frequently [62].

ASOs target pre-mRNA or mRNA and exert their effects via different mechanisms including RNA degradation, translation repression, and the alteration of splicing events to modulate protein production [27]. Based on the location where they bind to the mRNA, ASOs can trigger various consequences. ASO binding can lead to the degradation of mHTT pre-mRNA by the action of RNase H1 endonuclease or cause translational arrest through which the bound oligonucleotide causes a halt in the translation on the mRNA, or they can modulate pre-mRNA splicing where ASOs can occlude splice sites and result in the production of a non-toxic but different protein [61].

ASOs are either HTT allele-specific, affecting only the mHTT gene, or HTT allele non-specific, affecting both the mHTT and wtHTT genes [27]. Allele-specificity can be obtained either by targeting the CAG repeat itself or other genetic changes co-inherited with it [62].

ASOs do not depend on viral vectors to penetrate the cells, but because of their size ASO molecules cannot cross the blood-brain barrier [62]. Presently, efforts are focused on enhancing the delivery across the blood-brain barrier by designing new ASO architectures, such as the tricyclo-DNA ASO molecules, or by encapsulating the ASOs into nanocarriers [61].

Presently, there are three ASOs in clinical trials for HD namely Tominersen, WVE-120101, and WVE-120102 [27]. Tominersen, WVE-120101, and WVE-120102 bind to their target pre-mRNA through complementary base pairing and thus initiate degradation through RNase H1. This helps in lowering the mRNA of mHTT and hence the formation of the mHTT protein. These ASOs are given intrathecally through lumbar puncture to permit the

drug to circulate in the CSF and target the CNS [27]. Tominersen is the most developed HD antisense oligonucleotide that targets misfolded and clustering huntingtin and its mutant form. Currently, it is being investigated as it holds the potential to slow down the progression of HD by reducing the levels of mutant huntingtin protein [26,27,52]. Unfortunately, clinical trials were stopped due to its poor performances [63].

WVE-120101 and WVE-120102, which are allele-specific and stereopure ASOs have been developed to selectively target HD-related SNPs. WVE-120101 binds to rs362307 (SNP1), and WVE-120102 binds to rs362331 (SNP2). Since These ASOs are allele-specific they do not reduce the levels of wtHTT which could cause adverse effects in the long run. Given that WVE-120101 and WVE-120102 focus on SNPs, they cannot be used to treat all HD patients; however, if used in combination therapies, they could potentially address 80% of European HD patients [27]. Unfortunately, clinical trials were stopped due to their poor performances [63].

6.1.1.2. RNAi Technologies

The RNAi-based technologies can be employed as therapeutic strategies to decrease mHTT levels since they harness the physiological RNAi pathway [61]. Currently, there is evidence that this technique can lead to a decrease in HTT mRNA by ~80% in different HD models in preclinical studies [63].

RNAi therapies employ transgenes that generate RNA molecules, including micro-RNA (miRNA), short hairpin RNA (shRNA), and short interfering RNA (siRNA). Afterward, the cellular machinery engages with these molecules and identifies their target mRNA for destruction. The process is carried out by enzymes present in the RNA induced silencing complex (RISC). They impede the process of mRNA translation, hence halting the production of proteins. RNAi therapeutics are transported via a viral vector and are supplied via intracranial injection. Like every form of medicine, RNAi treatments have advantages and disadvantages. ShRNA treatments, while having the disadvantage of challenging regulation of shRNA production, are less prone to causing off-target effects compared to other RNAi therapies. Additionally, they have a longer duration of action [27]. Novel approaches are being devised to further reduce the already quite low occurrence of off-targeting events (OTEs) caused by shRNA. Tough decoy RNAs (TuDs) are competitive RNA molecules that closely resemble the sense strand of shRNA. They serve as an effective method to reduce unintended gene suppression. The purpose of these TuDs is to specifically attach to the sense strands of shRNA before they may attach to undesired genes [64].

6.1.1.3. siRNA

In the RNAi pathway, RNAi is mainly mediated by small interfering RNA (siRNA) that is derived from a double-stranded RNA (dsRNA) which undergoes a process of endoribonuclease cleavage. The dsRNA is then cleaved to the siRNA guide strand and liberates the non-functional passenger strand. The siRNA then combines with the RNA induced silencing complex (RISC) and binds to the target mRNA and causes its degradation. This endogenous RNAi pathway is manipulable by the introduction of an artificial siRNA to degrade the mHTT mRNA [61].

To target mHTT mRNA, a synthetically produced siRNA can be introduced into the cell. The siRNA may not need any further processing because as soon as the siRNA is within the cell, it may cause the degradation of the mHTT mRNA at once. Due to their small size and negative charge, siRNA molecules need to be chemically modified or coupled to enhance the delivery into neurons, increase their efficiency, and reduce their toxicity. The delivery of siRNA involves injecting it directly into the CSF through the intracerebroventricular route since it cannot cross the blood-brain barrier. Studies on animals reveal that siRNA prevents the synthesis of mHTT and therefore leads to the formation of smaller IB, increased life expectancy of the striatal neurons, and reduced HD motor dysfunction [61].

In order to retain the efficacy of siRNA, it has to be stabilized. For sustained gene silencing in vivo, the molecule must be trapped in endosomes and stabilized. Stabilization is achieved by the combination of sugar, backbone, and terminal phosphate modifications. The longer effect can be attributed to the uptake into endosomes which acts as a reservoir and maintains a continuous release of siRNA. A new chemically modified siRNA was recently developed in one of the research studies that can provide extensive distribution and long-term knockdown in the brains of rodents and non-human primates. Di-siRNA, chemically modified divalent, had efficient neuronal uptake and slow CSF clearance. Also, a single ICV delivery of Di- siRNA that targets HTT mRNA reduced the expression for up to six months [65].

6.1.1.4. shRNA

shRNAs are synthetic RNA molecules that possess a short hairpin secondary structure. The use of shRNA is better than direct siRNA delivery because it can provide long-term knockdown of mHTT [61]. shRNA may be expressed for months or years as it is incorporated into a DNA plasmid [64]. The level of shRNA is controlled by the expression promoter which leads to high levels of shRNA thus enhancing mHTT mRNA silencing. The siRNA production from the shRNA scaffold involves the shRNA being transcribed and processed into siRNA before it can show its effect on mHTT mRNA suppression [61]. After transcription, the result resembles pri-microRNA and is cleaved to generate pre-shRNA. Then this pre-shRNA is exported from the nucleus by Exportin 5. Later, the pre-shRNA is processed and then it binds to the RISC complex. When the passenger strand is cleaved, the anti-sense guide strand directs RISC to degrade the target mRNA [64]. Based on several studies, when compared head-to-head, siRNA has been reported to show higher off-target effects than shRNA [61]. Studies in mice show that shRNA expression decreases mHTT mRNA levels resulting in an amelioration of the HD phenotype [61]. Studies have shown that shRNA is more potent and accurate than siRNAs. One explanation is that shRNA when compared to siRNA, its process is remarkably similar to the pri-miRNA hairpins that exist in the cells naturally. Since siRNA does not go through this process, it

must be delivered at higher frequencies and in higher concentrations to achieve the same level of gene silencing. In the cytoplasm, the unprotected siRNA can be modified and degraded, therefore it can reduce its target binding capacity [64].

To date, the major challenge that exists for shRNA is intracellular overdosing, or the inhibition of the miRNA processing machinery. This problem can be addressed by altering the level of the shRNA construct expression. A key factor that determines the silencing efficiency and cytotoxicity of the transfected cells is promoter strength. Class III promoters are commonly used promoters that are considered to be strong. However, overexpression of shRNA from class III promoters is toxic in vivo and can lead to cell death and activation of the innate immune response within one month of continuous expression. To overcome the problem in one study, researchers have changed the viral vector shRNA dosage, and also weakened the promoter sequence, and applied this strategy with great success for the hepatitis B mice model in vivo [64].

6.1.1.5. miRNA

Artificial mi RNAs, also known as amiRNA, miRNA mimic, shRNA-miR, or pri-miRNA-like shRNA consist of a primary miRNA (pri-miRNA) scaffold and a small interfering RNA (siRNA) insert. AmiRNAs resemble endogenous pri-miRNAs and have a bipolar structure containing a double-stranded stem and two single-stranded basal segments at the 5p and 3p ends and an apical loop [66]. They are introduced into the miRNA biogenesis pathway at the early stage and are cleaved by DROSHA and DICER in two steps. Since the levels of siRNAs are tightly controlled by endogenous machinery, amiRNAs are considered safe and efficient RNAi triggers. Compared with synthetic siRNA or antisense oligonucleotides (ASOs), amiRNA can exert a longer duration of knockdown when they are introduced into cells using viral vectors [66].

Because the pri-miRNA sequence and its structure affect the processing of the miRNA, it is challenging to determine the consequences of replacing the miRNA sequence with an external siRNA sequence. Also, miRNA biogenesis is regulated posttranscriptional at several stages, and several factors that act in cis and trans can influence this process. Poor amiRNA design could lead to poor cleavage, emergence of siRNA variants with altered seed sequences, or arm switching which causes the release of the passenger strand of siRNA [66].

Viral vectors such as Adenoviruses (Ads), Lentiviruses (LVs), and Adeno-Associated Viruses (AAVs) are the most frequently used methods for amiRNA delivery. Different types of vectors and promoters can be used in the construction of amiRNAs, and this will enable the delivery of the siRNA to a tissue of choice and the expression can be either stable or regulated [66].

The lower interest and utilization of amiRNAs can be attributed to the fact that amiRNAs are more complex in design and their processing is less reliable compared to shRNA which may lead to low efficacy in the silencing process. However, the properly designed amiRNAs are as efficient as shRNAs. They are stable and safer than other RNAi triggers [66].

shRNA and artificial miRNA approaches for siRNA expression are quite similar. Both produce dsRNA molecules and require intrastriatal injection and an rAAV vector delivery system. Compared to shRNA, artificial miRNA seems to have higher tolerance in the mouse cerebellum. Due to the elimination of the undesired off-target effects that may occur because of the elimination of the passenger strand, other modifications to the miRNA scaffold structure can also enhance its safety even further. In several species of animals with different-sized brains, such as mice, rats, mini pigs, and sheep, the application of miRNA has been indicated to lead to a reduction in the level of mHTT [61].

6.1.1.6. Current RNAi therapies

AMT-130 is RNA interference-based gene therapy that is still in the experimental stage. AMT-130 consists of a gene that expresses miRNA and is delivered through adeno-associated virus vector serotype 5. AMT-130 sets off RISC and leads to the translation inhibition of mHTT (and wtHTT). The administration of AMT-130 is through intrastriatal injection. [27]. It is currently being assessed in a sham-controlled randomized trial for early HD in humans [63].

VY-HTT01 is an RNAi therapy that utilizes allele non-specific RNAi to target genes. It acts by expressing a miRNA which mediates the degradation of HTT mRNA. It is delivered through the adeno-associated virus vector serotype 1 (AAV1) and is given intracranially through injection [27]. Voyager Therapeutics has suspended its clinical program as it is working on the development of a proprietary adeno-associated virus capsid-containing gene therapy product that can be given intravenously to get to the affected tissue [63].

RNAi-based therapies for Huntington's disease have not advanced to the stage of clinical trials even if they have positive results in animal tests. Since RNA-based medications are new to the human brain, the patient's response and side effects may vary, and the impact of the drug on the cellular machinery is still unknown. The primary concern is that they need to be delivered by intraparenchymal injection of viral vectors into specific regions of the brain which may result in an immune response and may cause irreversible damage [62]. Another disadvantage of RNA medications is that they cannot pass through the blood-brain barrier. The two main strategies for avoiding this problem are to employ adenoviruses or to directly introduce the cure into the brain or CSF [61]. Another viable way of achieving the delivery of agents across the blood-brain barrier (BBB) might involve the opening of the tight junctions by modulating the claudin-5 expression in tight junctions with siRNA treatment, which leads to increased BBB permeability. Also, RNAi. shRNA and siRNA can be transported to CNS through nanoparticles. Liposomes, exosomes, and other similar nanoparticles have certain benefits over viral vectors. These nanoparticles are generally less immunogenic, and they can be readily engineered and tailored. But their

transduction efficiency remains lower than that of viral vectors [64].

6.1.1.7. RNA Targeting Splice Modulators

RNA Targeting small molecules can either promote the inclusion or exclusion of specific target exons in the pre-mRNA, thereby changing the protein, which is known as splice modulation. The advantages of these drugs are that they are taken orally while other treatments might require invasive administration. However, this also poses a threat of side effects resulting from their systemic circulation and non-selective targeting. PTC518 and Branaplam are two of the RNA splice-modulating agents that are currently being explored. PTC518 is an RNA splice modulator that includes a faux exon with a premature stop codon. This results in an accelerated breakdown of mHTT mRNA, resulting in a reduced production of mHTT protein. Branaplam is an orally administered small chemical that modifies RNA splicing. It is taken weekly. Studies have demonstrated the ability to decrease mHTT protein levels in animal models of Huntington's disease. In addition, due to the essential role of transcription elongation cofactors like Spt4 in the transcription process of the enlarged CAG repeats, there have been endeavors to create an RNA splice modulator that can effectively hinder elongation transcription factors such as Spt4. Spt4 inhibition would lead to a decrease in mHTT protein levels [27]. Moreover, the use of radiolabeled small interfering RNA (siRNA) and Epigenetics Therapies are still under investigation for the treatment of Huntington's Disease (HD) [23].

Delivery is a critical problem for all HTT-lowering therapies and can greatly affect the potential of each method. New delivery methods such as intraventricular and subcutaneous catheters may potentially enhance the safety, tolerability, and efficacy [62].

6.2. Gene Editing and DNA Therapies

These treatments either modify the HTT gene or modify its expression in the body. These compounds are usually comprised of two components; a DNA-binding moiety that recognizes the HTT gene, and an effector that either modifies the DNA sequence or regulates gene expression [62]. DNA targeting therapies function by modifying the mHTT gene through the introduction of protein-coding sequences into the brain parenchyma, where they specifically recognize and attach to a DNA region. A viral vector is required for delivery, which is administered through the intracranial route [27]. There are different techniques of gene targeting such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 systems [46]. Some recent works have noticed that DNA repair processes significantly influence the course of this disease. The FAN1 gene which codes for a nuclease that helps in the cutting of DNA during the repair of crosslink between two DNA strands was the most notable gene that influenced the disease as a modifier gene. FAN1 has been also suggested to be a protective factor in HD, which may work through enhanced repair of the loop-outs that occur at CAG repeats [23].

6.2.1.1. Zinc Finger Nucleases

Zinc Finger Nucleases (ZFN) was developed by Kim and his co-workers in 1996 as a chimeric nuclease having two components. The first component is a DNA binding domain that consists of three to four zinc finger motifs. The second part is the nuclease domain of endonuclease FokI [67]. Zinc finger domains are commonly found in DNA-binding motifs in eukaryotic transcription factors. In zinc finger proteins the zinc ion has a structural role as a stabilizer [62].

It consists of DNA binding parts that can interact with three to five nucleotides of the target DNA. These molecules target and interact with the expanded CAG repeats to decrease the expression of the mHTT gene which reduces the levels of mHTT without changing the DNA of the gene. Also, zinc finger nucleases (ZFNs) are capable of modifying the DNA thus making it possible to correct or knock out the mHTT gene [27]. It has been used for gene editing in pluripotent stem cells animals and plant cells [67].

ZFPs for therapeutic applications are generally designed with a zinc finger array that corresponds to a specific DNA sequence of interest. Each zinc finger in the array represents three nucleotide base pairs, and it is connected to a domain that enables the specific biological output. Some of the examples include the DNA cleaving zinc finger nucleases (ZFNs) and the c gene expression regulating zinc finger transcription factors (ZFTRs) [23].

Preclinical studies with ZFPs revealed a reduction in mHTT levels, HTT protein aggregates, and the amelioration of the HD-like phenotype in mice models. They were also effective in fibroblasts derived from patients, and human neurons derived from induced pluripotent stem cells [63]. Two primary ZFPs are under development for HD, namely TAK-686 and ZF-KOX1 [27].

Although being novel, a possible disadvantage of this approach is producing non-human proteins which may elicit an immune response. Also, the DNA binding of ZFPs may not be precise enough, thus leading to possible off-target binding [62]. Moreover, DNA-binding domain construction is a complex and costly process [67]. To achieve stable expression, ZFPs have to be packaged into an AAV or lentiviral vector and injected intraparenchymal [62].

6.2.1.2. TALENS

TALEN consists of two parts: a synthetic DNA-binding domain and a DNA-cleavage domain of endonuclease FokI [67]. The DNA-binding domain is based on transcription activator-like effectors (TALEs), which are proteins naturally found in plant pathogenic bacteria. TALEs are known for their unique ability to bind to specific DNA sequences [68]. Certain domains in TALENS with repetitive peptides help them to bind to DNA nucleotides. These domains are composed of monomers with tandem repeats of 34 amino acids and the two at the 12th and 13th positions are highly variable and are involved in nucleotide recognition [67]. TALENS Bind to specific regions of the mHTT allele to inhibit the transcription of the gene [27,59]. When compared to ZFNs

TALE has higher specificity due to its ability to recognize a single nucleotide while ZFNs recognize a three-nucleotide sequence. Also, TALENs have better attributes in terms of reduced cytotoxicity and enhanced design versatility as opposed to ZFNs and thus offer a higher targeting range [68].

TALENs and TALE-SNPs have been used to reduce the production and aggregation of mutant huntingtin (mHTT) protein in fibroblasts generated from HD patients. The TALE-SNPs specifically suppressed the mHTT gene by targeting SNPs, whereas the expression of the normal allele, the wtHTT gene, remained unaffected. TALENs were used to specifically target the gene, inducing double-strand breaks. Subsequently, the repair process deleted the CAG repeats. Research has discovered that many TALE-SNPs can reduce mHTT expression by as much as 20% without affecting the levels of endogenous HTT [27].

Currently, TALENs being designed for use in the treatment of Huntington's Disease (HD) are still in the preclinical or discovery stage. The effects of TALENS on phenotype, their toxicity, and an effective delivery system have not been determined yet. Research showed that in the yeast cells, TALENs have been used to knock out the expanded CAG repeats through the process of double-strand breaks without altering the mutation rate and without any genomic reorganization [27].

6.2.1.3. CRISPR-Cas9

Earlier, DNA strategies for genetic diseases were focused on using zinc finger nucleases and transcription activator like effector nucleases, but these have been replaced by CRISPR-Cas9-based strategies [59,60].

Cas9 is an enzyme that is natively found in certain bacteria and has been harnessed for genetic manipulation [27]. There are three main types of CRISPR/Cas systems (I-III) and at least ten subtypes [67]. Class 1 CRISPR systems including Type I and Type III are more sophisticated and they require several proteins to help them in the identification of target DNA. Class 2 systems which include Type II and Type V use single, multi-domain Cas proteins [68]. In genome engineering II-A type system SpCas9 is the most widely used system [67]. The reason for the interest in Cas9 in gene editing is because it is highly effective, flexible, and can target the genome with high specificity thus making it suitable for therapeutic use [68]. Although having two components CRISPR/Cas9 functions as a monomer [67]. The CRISPR-Cas9 system has two components: a guide RNA that recognizes the target DNA sequence and a Cas9 nuclease that cuts the DNA at the target site, creating double-strand breaks [62]. These double-strand breaks are usually repaired by non-homologous end joining (NHEJ) which leads to frameshifts that alter gene expression [27]. In addition, a special PAM sequence in the 3'end of the target is also required for cleaving DNA [67]. All the CRISPR strategies are viral vector-mediated and injected intracranially [27]. The CRISPR-Cas9 can be packaged and delivered to cells in three ways co-delivery of Cas9 and sgRNA, delivery of DNA plasmid containing Cas9, or as a ribonucleoprotein complex (RNP).

Delivery of Cas9 by using DNA plasmids is more common as they are relatively cheap to produce and purify for mass production. However, plasmid DNA can be easily degraded by nucleases, which makes it less stable and less efficient. It also raises the risk of off-target effects, particularly when the Cas9 protein is still being produced even after the intended gene editing has been accomplished [68].

In in vitro and in vivo studies CRISPR/Cas9 has been formerly applied for plants, animals, and human cells and has helped to establish human iPSC-based genetic disease models [67].

In the CRISPR-Cas9 genome editing system, RNA-DNA interactions are involved in the identification of the DNA sites in the genome [26,27,52]. For Huntington's Disease (HD) CRISPR/Cas9 can be employed to perform HTT transcription inhibition, CAG repeat excision, or SNP targeting [62].

The effectiveness of the CRISPR/Cas9 system in eliminating the mutant allele and preventing the production of the mHTT protein has been seen in several studies in cell cultures derived from HD patients [63]. In various experiments on cell lines, it has been found that the use of paired Cas9 Nickase removed the expanded CAG repeats in exon 1 of the HTT gene in three different HD patient-derived fibroblast lines and effectively knocked out HTT. Irrespective of the CAG repeat lengths, cell lines that were treated with Cas9 Nickases had approximately a 70% reduction in the HTT protein levels. Also, CRISPR-Cas9 with piggyBac transposon was effective in HD human-induced pluripotent stem cells by correcting the mHTT allele and reducing the impact of HD phenotypes. Also, the mHTT-linked SNP CRISPR/Cas9-based strategy provokes a deletion mutation that causes the inactivation of the mHTT allele while the other normal HTT allele is preserved. By using this strategy, in HD patient-derived cell lines, the removal of CAG repeats linked to mHTT alleles has been achieved [27].

Unfortunately, CRISPR-Cas9 has several problems that require more attention. One disadvantage is that repair mechanisms are not completely accurate and can therefore allow for mutation to occur [62]. Also, Off-target effects may lead to genomic instability and impair the normal function of genes that were not intended to be edited and this limits the application of CRISPR/Cas9 is [67]. Another concern is that since it is derived from bacteria there might be a risk of an immune response in humans [62].

Employment of Cas9 orthologs is one way to enhance the selectivity of the CRISPR system as they are associated with other PAM sequences and have higher cleavage specificity. Another way is to modify the Cas9 sequence to improve hydrogen bonding between Cas9 and the target DNA strand [67].

6.2.1.4. SGN and NgAgo

In 2016 two new genome editing tools were developed that hold great promise. One of them is NgAgo which is an Argonaute endonuclease from Natronobacterium gregoryi. This tool employs 5'-phosphorylated short

single-stranded 24nt DNA (ssDNA) as a guide and does not necessitate a specific PAM sequence to target and cleave DNA. NgAgo is more advantageous for targeting GC-rich sequences than other tools including the CRISPR-system [67]. Another promising new tool for genome editing is SGN composed of two parts: the first part is FEN-1 which binds to DNA-DNA 3'-flap structures and the second compartment is nuclease domain FokI. FEN-1 is involved in replication and DNA repair which catalyzes the removal of 3' unpaired flap through phosphodiester [67]. Off-target effects and cytotoxicity are among the concerns of using these tools therefore future studies and improvements shall be done for their safe usage [67].

6.3. Monoclonal Antibodies

Injection of a monoclonal antibody that specifically binds to the HTT protein to reduce the protein's count within the cell has also been studied. The results support the concept that monoclonal antibodies can prevent the pathological processes of the in vivo propagation of mutant huntingtin. Currently, there is an ongoing effort to use monoclonal antibodies to lower the levels of huntingtin protein in cells and slow down the development of the disease by inhibiting the formation and spread of the mutant huntingtin protein in the body [23,26]. VX15/2503 or Pepinemab, antibody-based immunotherapy targeting semaphorin 4D is under consideration for Huntington's Disease [48]. Unfortunately, a recent clinical trial (NCT02481674) with VX15, did not prove useful for HD patients [63]. Another promising antibody is ANX-005 which was developed by Annexon Biosciences to maintain synaptic connections in HD. Also, an intrabody named INT41 was developed by Vybion to target mutant huntingtin protein in HD [63].

6.4. Phage Therapy

Phage therapy may offer a new modality of treating HD and hence may be of great benefit in managing the disease. Phages can be used as vectors to transport ASO across the Blood-brain barrier. They can also be engineered with genetic manipulations. In addition, phages engineering stem cells with the help of filamentous phages can increase neurogenesis [50].

7. Nanotechnology in Huntington's Disease Treatment

Nanotechnology offers special approaches to tackle the challenge of ND and to develop efficient ND-altering treatments [69]. Nanomedicine is the use of nanotechnology in medicine and health. [70]. Huntington's Disease preventive strategy has benefited from the nanotechnology-based approach in a significant way. It involves the design and preparation of nanoparticles capable of crossing the BBB and having the ability to regulate the nucleation of fibrillar proteins, degradation of mature fibrillar proteins, and targeting protein aggregation in HD [70].

Nanoparticles (NPs) are described as solid particles with a size ranging from 1-100 nm and characteristics such as small size, large surface area, hydrophobicity, strong adsorption capacity, surface modifiability, and high reactivity [71]. Nanomaterials can be given through the respiratory system, skin, GIT, and intravenous injections, which are then transported to the organs where they function metabolically [72].

Medications and therapeutic compounds can be improved in terms of efficacy and use by encapsulating them in NPs. This in turn increases their half-life, decreases the rate of drug resistance, shields them from enzymatic degradation, and allows them to be released at the site of interest [71].

Nanoparticles have been analyzed and are currently being used as delivery systems in numerous in-vitro and in-vivo studies related to CNS disorders. Several nanoparticles have been reported to exhibit that they enhance CNS pharmacokinetics and distribution in brain regions compared to free drugs. Most of the nanoparticle-mediated treatment approaches in CNS diseases center around targeted delivery of therapeutic agents at the affected part of the brain after crossing BBB [73]. Nanocarriers with 20–100 nm diameter are deemed appropriate for neurodegenerative treatments because they can cross the BBB and are not rapidly eliminated from the body [71]. In addition, these methods reduce peripheral toxicity because the rate of drug release is reduced in the brain [69].

The surface of NPs can be modified with certain ligands, which makes it possible for NPs to target certain cells or organelles, enhance the transport of drugs through the BBB, minimize the interaction of NPs with other cellular components, and avoid accumulation in other organs and tissues. This provides better drug delivery, minimizes the side effects, and lowers the overall body load of the drug [71,74].

In addition to the ability to target specific cell types, they are also able to impact specific compartments in the cell, they can also impact specific compartments in cells. Consequently, the employment of the nanocarriers enhances the chances of the medication penetrating the cells [74].

7.1. Nano-sized Formulations for the Treatment of Huntington's Disease

In the past years, diverse types of nano-sized formulations have been used for drug delivery in the treatment of HD. These nanosized formulations include Polymeric NPs, lipid-based NPs, micro/nanoemulsions, self-emulsifying drug delivery systems (SEDDSs), liposomes, dendrimers, nano gels, solid lipid NPs, micelles, metal and metal oxide NPs, quantum dots, magnetic NPs, nanocrystals, and fullerenes [71].

Magnetic nanoparticles are one of the nanoparticles which can be controlled by an external magnetic field. MNPs consist of metals such as iron, nickel, and cobalt and their oxide forms. MNPs have been employed in magnetic resonance imaging, targeted drug and gene delivery, cell labeling, tissue engineering, and as a diagnostic tool due to their ability to function under an external magnetic field. This characteristic makes it easy to control the drug delivery at a given site, time, and amount. In one study, the authors investigated the

potential therapeutic role of MNP-labeled mesenchymal stem cells in a rat model of Huntington's disease which was characterized by selective loss of striatal GABAergic neurons [75].

Lipid nanoparticles are categorized as liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) [76]. Liposomes are divided into three categories: small unilamellar vesicles (SUV) with a size less than 100 nm, bilayer large unilamellar vesicles (LUV) with sizes greater than 100 nm, and bilayer and multilamellar vesicles (MLV) [72]. The main components of LPNs include phospholipids, fatty acids, monoglycerides, triglycerides, fatty alcohols, and waxes. The three types of LNPs differ mainly in the lipid layer organization, shape, encapsulation capacity, size, and surface charge. Some of the disadvantages of LNPs include instability, difficult production, polymorphism changes, and drug release in storage [76].

SLNs are colloidal particles with sizes ranging from 10-1000 nm and consist of a unique lipid matrix. SLNs are colloidal dispersions in an aqueous medium of biodegradable solid matrix lipid, which is of high melting point and coated with aqueous surfactant [75]. The drug can be dissolved or dispersed within SLN which has a solid hydrophobic lipid core. The potential routes used by SLN to enter the brain include endocytosis, passive diffusion, active transport, and opening of tight junctions in the microcirculation of the brain [72]. As the lipid matrix of SLN consists of physiological lipids, the chances of acute or chronic toxicity are low. In addition, it was discovered that solid lipid nanoparticles can regulate the rate of drug delivery and enhance the stability of lipophilic compounds that are prone to decomposition [75]. Studies have shown that rats with HD received intranasally administered SLNs containing rosmarinic acid, to have enhanced motor dysfunction and reversed oxidative stress. It also brought back the endogenous antioxidant capability of glutathione and catalase. Also, it has been found that repeated injections of g7-NPs-Chol cholesterol-loaded nanoparticles in HD mice had a positive impact on the amelioration of synaptic dysfunction and cognitive deficit. These cholesterol nanoparticles target various areas of the brain and diverse types of brain cells and release cholesterol slowly. In another work, thymoquinone-incorporated SLNs (TQ-SLNs) caused improvements in behavioral abnormalities and had a neuroprotective impact by preventing oxidative stress. Thymoquinone-incorporated SLNs downregulated the inflammatory cytokines and reduced NMDA receptor sensitivity [70].

Polymeric nanoparticles (PNP) play a vital role in drug delivery for the control of neurodegenerative diseases. The biodegradable and biocompatible polymers that are used in the synthesis of PNP include polylactic acid, polylactide- co-polyglycolic acid, polyglycolic acid, polycaprolactone, polymethyl methacrylate, as well as natural polymers like chitosan, alginate, gelatin, and albumin [72,75]. Nanospheres and Nanocapsules are the two primary structures of polymeric nanoparticles that are mostly observed. Endocytosis or transcytosis using the endothelial cells are the main possibilities for the mechanisms underlying the uptake and release of drugs from polymeric nanoparticles [72]. In one study a novel hybrid polymeric nanoparticle based on PLGA and cholesterol with a heptapeptide (hybrid-g7-NPs-chol) was developed. It was found that these nanoparticles can cross BBB and increase endogenous cholesterol synthesis and thus counteract cognitive impairment. Also, studies indicate that polyD nanoparticle, L-lactide-co-glycolide, containing a polyglutamine aggregation inhibitor peptide PGQ9 decreases the amount of toxic protein aggregates [70]. Moreover, in an experiment, researchers employed a specific combination of polymer (g7-PLGA and PLGA [Poly D, L-lactide-co-glycolide]) and cholesterol to form hybrid nanoparticles called MIX NPs. These MIX-NPs were readily taken up by neurons and could release cholesterol into cells which leads to a change in the expression of synaptic receptors that could be therapeutic in HD [70].

Since exosomes can functionally transport small RNAs, they can be an alternative to enhance neuronal targeting and uptake while avoiding the activation of neuronal innate immunity or cell death. In one study it was found that exosomes containing hydrophobically modified siRNAs (hSIRNAs) can be used to knock down the huntingtin gene and decrease mHTT levels in primary neurons [70].

According to studies selenium levels are deficient in most cases of patients suffering from neurodegenerative diseases such as HD. Selenium deficiency in the brain was associated with the mutant huntingtin aggregation, increased oxidized glutathione level, and brain dysfunction [73]. Se NPs have been proven to exhibit antioxidant properties with reduced toxicity risk even in concentrations below 0. 5×10^{-3} m[69]. Nano-Se shows enormous potential in the treatment of HD by decreasing neuronal loss, ameliorating behavioral dysfunction via protecting neurons from oxidative damage, preventing huntingtin protein aggregation, and decreasing the mRNA levels of histone deacetylase family member [77].

In one study a non-toxic, water-soluble, nanozyme named citrate functionalized manganese-based biocompatible nanoparticle (C-Mn3O4 NP) was synthesized. It could scavenge neuronal reactive oxygen species and replace the glutathione peroxidase enzyme in the physiological environment. In several animal models that modeled HD symptomatology motor functions were improved in the 3-nitro propionic acid intoxicated mice and behavioral alterations were prevented [70].

In another study, researchers studied the protective role of Epigallocatechin-3-gallate (EGCG). It is derived and EGCG is chemically unstable. Therefore, encapsulation in nano-sized capsules increases its stability. They found that EGCG in nano-sized capsules is much more effective in alleviating motor deficits and reducing neuronal damage in the HD mouse model [70].

In one study layered double hydroxide (LDH) nanoparticles were loaded with siRNAs and their effectiveness in gene suppression was evaluated. LDH nanoparticles have low cytotoxicity and high

biocompatibility. The complex was rapidly and dose-dependently uptaken by neurons and showed good delivery of siRNA into the neuronal cytoplasm. It was found that LDH-mediated siRNA delivery efficiently suppresses neuronal gene expression [70].

Some published works also emphasized the fact that several treatments based on NP were effective. In one research it was found that MnFe2O4 nanoparticles enhance clearance of mutant huntingtin as well as showing neuroprotective effect through the ubiquitin-proteasome pathway in Neuro 2A/GFP-Htt (Q74) cell lines. ubiqinlin-1 functioned as the ubiquitin receptor that allowed MnFe2O4 Nanoparticles to more effectively degrade Htt (Q74) [70]. Furthermore, cyclodextrin nanoparticles containing siRNA were evaluated in vivo, and in vitro silenced HTT mRNA[73]. Moreover, in one study curcumin conjugated SLNs were applied to target mitochondrial dysfunctions that affect the activity of the striatum complex II, and the activity of the striatum complex II was found to have decreased in the treatment of rats with HD [72,73].

Nitrendipine is a calcium channel blocker indicated for dementia in HD. It has poor absorption and cannot easily penetrate the BBB. SLNs of nitrendipine were prepared and characterized, and the uptake of bulk medication and nano-formulations was compared. The findings indicate that the drug was absorbed at a higher rate when encapsulated in SLNs [73].

Europium hydroxide nanobonds [EuIII (OH) 3], with wortmannin and chloroquine, were able to promote autophagy by degradation of the specific substrate of the autophagy/p62/SQSTM1 load receptor and increase production of the characteristic autophagic marker protein LC3-II and enhance the elimination of mutant huntingtin protein aggregates without cytotoxicity [70].

Specifically, TiO2 NPs can catalyze the oxidation of methionine at the N-terminal domain of the mutant huntingtin protein, which can then form a sulfoxide, and reduce the tendency of the protein to aggregate [78].

Poly(trehalose) NPs have also been found to be highly effective in arresting the progression of HD through the reduction in the aggregation of mutant huntingtin protein [78].

Dendrimers are the smallest nanoformulations, which can be employed in the treatment of neurodegenerative diseases [76]. Dendrimers are polymeric structures with branching structures. Polyamidonamine, polypropyl-enimine, and polyaryl ethers are three classes of dendrimer molecules that are commonly used. Actin or tight junction occludin proteins are reversibly regulated during cellular absorption [72]. Dendrimers can be designed to have varied sizes, core-shell composition, and surface functional groups to create various nanocarriers for drug and gene delivery to the brain [76]. Evidence shows that PAMAM dendrimers exhibited a remarkable ability to travel into the CNS tissue and to enter living neurons after injections into the parenchyma [72].

CeONPs, also known as nanoceria, are biocompatible nanomaterials with antioxidant and radical-scavenging activities that can be used in the treatment of neurodegenerative diseases. It has been reported that CeONPs with a size of less than 5 nm can easily cross the BBB [76].

Quantum dots are classified as nanomaterials with zero dimensions. They possess excellent optical and electrical characteristics. The functionalized QDs hydrodynamic size (10-20 nm) appears to mimic the labeling of neurons and glial cells and tracking. In various studies, QDs have been used to investigate their possible employment as therapeutic or imaging agents for diseases of the CNS [72].

Nanogels are prepared through the crosslinking of polyethylene glycol with polyethyleneimine (PEG-PEI). They have sizes of less than 200 nm and are crosslinked through physical or chemical covalent bonds. The types of nanogel network structures include hollow, hairy, multilayered, core-shell, and core-shell core cross-linking. They have several advantages such as high colloidal stability, large loading capacity, core-shell structure, permeability, and sensitivity to external stimuli. In both in vitro and in vivo models, the penetration capabilities of these nanoscale drug carriers in the brain parenchyma are better than the conventional nanomaterials. These particles can transport antisense oligonucleotides [76].

7.2. Challenges of Nanoparticle-Based Treatments

Many studies showed that nanoparticles through oxidative stress induce inflammation in the liver, lungs, and brain. This inflammation was initiated via nanoparticle uptake by the reticuloendothelial system [74]. There is a possibility of change in the shape and size of nanocarriers which leads to various physicochemical interactions and may lead to poor delivery [79]. The ability of nanocarriers to cross the blood-brain barrier is an advantage in the treatment of neurodegenerative diseases. However, if the site of action is another organ rather than the brain, it may lead to neurotoxicity. Moreover, nanoparticles may cause immunomodulatory effects [74].

Stimuli-responsive nanoparticles are another type of nanoparticles that are sensitive to different external stimuli. To enhance the selectivity, precision, and reproducibility of such dosage forms more research and analysis should be conducted. Additionally, currently, for nano-drug delivery and stimuli-responsive nanoparticles and functional biomaterials, there are no guidelines. Therefore, the development of Regulatory guidelines for nano-drug delivery systems is required [79].

Evidence indicates that silver nanoparticles can induce ECM breakdown by upregulation of matrix metalloproteinases (MMPs) through activation of an inflammatory signaling pathway. Furthermore, in vitro studies show that depending on the type of endothelial cells, the toxicity status of multiwalled carbon nanopipes might change after they encounter the cells [80]. Inadequate data on the nanomaterial's toxicity, immunological compatibility, and insufficient in vivo

trials of nanoparticles are some of the obstacles before the introduction of nanoparticles into clinical practice [80].

Although many nanoparticles have been designed in recent years and the approach holds great promise only a limited number of currently available nanoparticles have been assessed and discussed in HD. Nanomedicine toxicity is a big issue, and it should be investigated before usage in clinical practice. Nanotechnology as a typical treatment for neurodegenerative diseases is also expensive and therefore novel approaches for the development of affordable solutions must be sought [76].

8. Future Perspectives

The current research trend of Huntington's disease (HD) demonstrates a promising future for HD patients with the continuous advancements in therapeutic strategies targeting primarily the core cause of the disease [27]. RNA therapies, especially antisense oligonucleotides, present satisfactory results and are now among the most attractive perspectives for the future of HD therapeutics. On the other hand, DNA therapies also offer hope for curing HD but when using these therapies, especially CRISPR therapies, considerations regarding the safety of these therapies for human use shall be taken [27]. Current ongoing interest in therapies aimed at mHTT, DNA, and RNA, together with approaches that target glutamatergic neurotransmission, BDNF signaling, or mitochondrial function, will most likely lead to improvements in both treatment of the disease as well as symptoms associated with it [54].

Additionally, research on cell therapy may have a significant role in the treatment of Huntington's disease. In this regard, new frameworks and cooperation initiatives like SC4HD improve the translational efficiency and success of those new therapies [53]. Moreover, intrabodies have been an area of ongoing research as an option for the treatment of HD [81]. Furthermore, advancements in nanotechnology hold promise to achieve great enhancements in therapeutic agent delivery. Nanoparticles can be engineered to cross BBB and deliver the drug right to the cells of therapeutic interest in the brain [69,71,74].

Identification of new therapeutic targets is one of the promising ways to provide potential targets for therapeutic intervention. Recent studies discovered that some new routes and proteins participate in HD pathogenesis including G protein-coupled receptor 52 (GPR52). Preclinical models have demonstrated small molecule antagonists of GPR52 are effective in decreasing mHTT levels as well as improvement of the HD-related symptoms [82].

Lastly, enhancements in designing clinical trials and improvements in regulatory frameworks might help in the development and approval of new therapies. Ongoing clinical trials, collaborative frameworks, and novel therapeutic approaches have brought the current therapeutic landscape of HD on the verge of dramatic changes in multiple aspects of disease treatment to provide better outcomes for individuals with HD.

9. Conclusion

Huntington's Disease (HD) is one of the challenging neurodegenerative disorders that is genetically inherited and exhibits motor, cognitive, and psychiatric symptoms. The disorder is of a monogenic autosomal dominant nature and results from a mutation in the HTT gene which eventually leads to expanded CAG trinucleotide repeats that translate and give rise to mutant huntingtin protein. This protein is central in the pathogenesis of the disease since it contributes to many cellular dysfunctions in the brain, particularly in the striatum. Our review elucidated complex processes of HD including disruption of proteostasis, mitochondrial anomalies, transcriptional dysregulation, and synaptic dysfunction, which result in neurodegeneration in patients.

Since most of the available treatments are considered palliative, the demand for disease-modifying therapies is on the rise. At the moment, new targeted genetic therapies such as RNA interference, antisense oligonucleotides, and CRISPR/Cas9 systems have the potential to either halt or to some extent reverse disease progression.

In addition, the nanoparticle-based drug delivery system improves the therapeutic effectiveness of the drugs by increasing the bioavailability of the drugs and facilitates the drugs to cross incredibly challenging barriers such as the blood-brain barrier. Also, in various preclinical studies, the plant-derived molecules have shown neuroprotective potential due to antioxidant, anti-inflammatory, and autophagy-inducing effects.

Despite all these advancements, there are still some challenges that require more attention. For instance, for the development of disease-modifying therapies, a better understanding of HD pathogenesis is required. Also, identifying biomarkers that help early diagnosis and monitoring is essential.

Finally, future treatment strategies for HD should be multidimensional and should encompass various genetic, pharmaceutical, and nanotechnological methods. Further in-depth research of various aspects of the disease and more expanded collaboration between various researchers and clinicians is essential in addressing the current limitations and resolving the issues concerning the treatment of Huntington's Disease.

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Conflict of Interest

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