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Investigative Study into Huntington's Disease: Development, Diagnosis, and Therapeutic Treatment

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Abstract

Huntington's disease is a neurological disorder characterized by rapid motor, cognitive, and physical deterioration. At the cellular level, the mutated form of huntingtin induces neuronal dysfunction and toxic protein aggregation through several mechanisms, which culminates in rapid neurodegeneration. There have been significant advances in recent years in understanding how the disease develops and progresses in the brain. There have been observable improvements concerning therapeutic strategies and clinical trials for possible treatments; however, the current strategies are subject to critical faults. Recently, researchers have begun to consider experimental strategies that are designed to target the mechanisms that contribute to mutant huntingtin overexpression, like transcription regulation, gene expression repression, and oxidative stress inhibition. While these strategies show promising results, they will need to be thoroughly studied to understand their practical applications. Furthermore, the ethics of these strategies need to be discussed to address any lingering controversies. This literature review will aim to thoroughly investigate the pathology behind Huntington's disease and current/experimental therapeutic strategies designed to treat the neurodegenerative disorder. An analysis of available articles yielded an abundance of information concerning the development, presentation, therapeutic treatment, and ethical considerations of Huntington's disease.

Keywords: neurodegeneration, neurodegenerative disorder, Huntington's disease, huntingtin, protein aggregation, neuron, gene therapy, CRISPR

Introduction

Huntington's disease (HD) is a neurodegenerative disorder that appears to operate under a genetic pattern of inheritance. Though the disorder did appear in literature in the 1840s, it was not until the 1870s that the disorder was thoroughly observed and investigated by George Huntington, from whom the disease earned its name. Huntington's disease appears to result from the extension of a CAG trinucleotide repeat in the gene encoding for the huntingtin in the human genome. Normally, there are around 35 CAG trinucleotide repeats, but those affected by Huntington's often have more than 36 repeats. It is the mutated version of this gene that induces the neurodegeneration associated with Huntington's. The disease can be characterized by rapid motor, cognitive, and physical deterioration. This deterioration culminates in an early fatality. Even though it has been nearly two centuries since the disease was first recorded in literature, there is still no treatment to reverse or slow the progression of disease. Current treatments can only endeavor to alleviate any symptoms that may arise as a result of the disease. With the continued advancement of technology and the establishment of novel therapeutic approaches, researchers should consider novel techniques that can prevent the mutant huntingtin protein from encouraging neuronal deterioration.

What is Neurodegenerative Disease?

The classification of neurological disorders as neurodegenerative diseases remains a heavily debated topic, even to this day. The etymological breakdown of the word neurodegeneration includes the prefix "neuro-" which refers to neuronal cells, and "degeneration," which refers to the process of losing structure/function (Przedborski *et al*, 2003). By basic definition, the word neurodegeneration should refer to any pathological condition that

primarily affects the function of neuronal cells. In practice, however, neurodegeneration can disturb not only the function of neurons but also the surrounding cellular pathways. Neurologists agree that, overall, neurodegenerative diseases should be classified as the progressive deterioration of neuronal cells. Because some neurodegenerative diseases have similar origins, neurologists have opted to diagnose through the clinical presentation of symptoms. Though there are hundreds of different neurodegenerative disorders identified over the centuries, the most prevalent disorders studied by neurologists are Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). This paper will focus primarily on the etiology and pathogenesis of Huntington's, and what current and experimental therapies exist for this neurodegenerative disorder.

The pathway leading to neurodegeneration depends on a variety of factors related to aging; however, the specific molecular mechanisms leading to the emergence of neurodegeneration are not fully understood by neurologists. One of the most enduring debates surrounding the etiology of neurodegenerative disorders concerns which factor majorly influences neurodegenerative development. Some neurodegenerative disorders have been observed to pass from generation to generation, suggesting that there must be an inherent genetic basis. In these cases, the disease often appears as an autosomal dominant trait, as in Huntington's. However, the disease has been observed to appear as an autosomal recessive, X-linked, or even a maternally-expressed trait. On the other hand, neurodegenerative disorders have appeared spontaneously, leading neurologists to suggest that environmental or endogenic factors may contribute to the emergence of the disease (Przedborski *et al*, 2003). There is no clear answer as to which factor influences neurodegenerative development, so neurologists have

postulated that genetic and environmental factors have the capacity to accelerate neuronal degeneration.

During the normal aging process, neurons experience increased amounts of oxidative stress, protein aggregation, and incidence of apoptosis. As previously mentioned, these neuronal changes during normal aging are worsened by genetic, environmental, and endogenic factors. In the later stages of life, progressive oxidative stress can induce cellular and neuronal damage, which cannot be reversible to the rapid decline of cell production. As cell production decreases with age, neurons tend to accumulate masses of damaged proteins which form aggregates within cellular pathways. These aggregates can spread easily in the brains of elderly individuals, contributing to accelerated neuronal dysfunction and apoptosis (Mattson and Magnus, 2006). These aggregates, in conjunction with external influences, induce observable symptoms in afflicted individuals, like cognitive decline, memory loss, and varying behavioral changes.

The observation that normal aging correlates with increased incidence of cellular stress and apoptosis, in conjunction with the finding that the most common neurodegenerative diseases have been observed predominantly in elderly individuals, indicates that the risk of neurodegenerative development increases with age. Interestingly, Huntington's is one of the few neurodegenerative disorders that does not present predominantly in elderly individuals. It has been observed that symptoms begin to manifest in middle-aged individuals with the age on onset decreasing in subsequent generations, thus contrasting the theory that the elderly are most at risk for neurodegenerative development (Jimenez-Sanchez *et al*, 2017).

History of Huntington's Disease

Suspected cases of Huntington's disease can be traced back to as early as the Middle Ages; however, it was not until the nineteenth century that the disease began to appear in medicinal literature. Literature concerning this neurological disorder referred to it as "choera," for the jerky, involuntary movements associated with the disorder (Bhattacharyya, 2016). Though the disorder would appear more frequently in the 1840s, it was not until the 1870s that the disorder was thoroughly observed and investigated by George Huntington. Huntington was born into a family of physicians and grew up with daily exposure to a plentiful amount of disease. When he eventually completed his medical training and began practicing, he noticed that there were instances of neurological dysfunction present in several generations of families that seemed to follow the pattern of autosomal dominant inheritance. By 1872, Huntington had released the first investigative report into "choera" and detailed the symptoms and methods of transmission associated with the disorder (Bhattacharyya, 2016). It was his logical presentation of his findings that brought the disease international attention, with researchers calling it "Huntington's disease". By the end of the nineteenth century, there was a spike in international interest regarding the etiology, pathology, and iamatology of the disease.

Several biologists over the next few decades would further cement the autosomal dominance theory of inheritance for Huntington's through investigations of the pedigrees of afflicted individuals. By the early twentieth century, the first study concerning the etiology of Huntington's was commissioned in the United States by Charles Davenport. He went on to publish his findings that expanded upon the age range when symptoms would manifest and the kinds of symptoms afflicted individuals could experience (Davenport and Muncey, 1916). He publicized the idea that many cases of Huntington's in the United States could be traced back to

several founding families. By 1968, this disorder was further popularized when the Hereditary Disease Foundation was founded to conduct research on the causes and treatments for genetic neurological conditions (*Hereditary Disease Foundation*, 2021). By 1983, there was a breakthrough by the US-Venezuela Huntington's Disease Collaborative Project. Over ten years, they had analyzed populations in South America with high rates of disease expression. Through genetic linkage analysis and DNA-marker visualization, they were able to determine that the gene responsible for the disease was located at human chromosome 4 (MacDonald, 1993). Later that decade, experiments with transgenic mice would reveal that the disease manifested with the presence of misfolded fragments associated with the gene, which would be called the huntingtin protein (MacDonald, 1993). Advances in the past few decades have provided opportunities for more extensive research concerning potential drug treatments, genetic therapies, and the protein itself.

Huntingtin to Huntington's Disease

Neuronal deterioration is associated with the accumulation of misfolded proteins, either in the form of aggregated mutations, prions, -amyloid, or tau. In Huntington's disease, mutations in the HTT gene cause -amyloid growths that induce neurodegeneration. Encoded from HTT, the huntingtin protein measures approximately 350kDa with a polyglutamine sequence and consensus sequences important for regulating protein-protein interactions. Huntingtin is widely expressed throughout but can be found most predominantly in cells belonging to the central nervous system. Normal levels of huntingtin can influence embryonic development, transcription regulation, and synaptic connectivity. The polyglutamine tract located in the HTT gene plays an important part in regulating many of these cellular pathways.

Normally, the tract contains around 35 CAG trinucleotide repeats, whereas those affected by Huntington's often have more than 36 repeats (Jimenez-Sanchez *et al*, 2017). It is the mutated version of this gene that induces the aggregates and cellular interference responsible for Huntington's.

Mutations of huntingtin can cause protein aggregation via nucleated growth polymerization, leading the polyglutamine tract to convert into -sheets held together by hydrogen bonds in an amyloid structure. Neurologists have determined that these aggregates are not necessarily always deleterious in terms of function, but cannot agree on the intrinsic nature of the aggregates. Some studies have shown that aggregates of the polyglutamine tract have been found in increased quantities in individuals afflicted by accelerated neurodegeneration, suggesting that there is a direct link between aggregation and cell death (Hackam *et al*, 1998). On the other hand, some studies have shown an inverse correlation between aggregation and cell death, suggesting that the increased presence of aggregates may serve a supportive role by protecting the neuronal cells from toxic exposure (Arrasate *et al*, 2004). The consensus, however, by those studying neurodegeneration in Huntington's is that these -amyloid growths disrupt the daily functioning of neurons, thus accelerating the rate of neurodegeneration.

Mutation of the huntingtin protein does not only induce simple protein aggregation, but toxic fragmentation, transcription interference, and gene expression alterations. When huntingtin is mutated, the protein often experiences impromptu fragmentation. These mutant fragments tend to adopt toxic characteristics, due to their tendency to form nuclear aggregates (Hackam *et al*, 1998). Mutant huntingtin has been known to interfere with cellular transcription, as well. Because activation domains in several transcription factors tend to contain glutamine-rich

regions, transcriptional mechanisms should expect to see effects from the expanded quantities of polyglutamine via mutant huntingtin. Researchers have seen the effects of mutant huntingtin on transcriptional regulators, like CREB-binding proteins and cAMP response element-binding proteins (Jimenez-Sanchez *et al*, 2017). Gene expression dysregulation has been observed as a result of mutant huntingtin. In one study, researchers discovered the presence of mutant huntingtin in several RNA structures that influence genetic expression posttranscriptionally, like P bodies, stress granules, and dendritic RNA granules (Savas *et al*, 2010). It suggested that each of these toxic effects by mutant huntingtin possibly play a role in accelerating the rate of neurodegeneration, thus culminating in the prognosis of Huntington's disease.

Current Therapeutic Strategies

Researchers cannot agree, like with many neurodegenerative diseases, exactly how the aforementioned mutation accelerates neuronal atrophy; however, evidence suggests that targeting the overexpression of huntingtin could halt the disease progression. Current trials to repair the mutation utilize antisense oligonucleotides [ASOs] and RNAi have yielded encouraging results in clinical trials, but these experimental techniques are subject to faults.

Antisense oligonucleotides [ASOs] are small, synthetically engineered nucleic acids that utilize Watson-Crick base pairing to bind to RNA sequences within the genome. This binding can result in gene silencing or RNA modification. Gene silencing by ASOs can occur either through degradative mechanisms in which an artificially manufactured endonuclease cleaves the target RNA or non-degradative mechanisms in which where binding of the ASO sterically obstructs subsequent translation and replication. Chemically, ASOs function through the degradative mechanism, where an RNaseH molecule is bound with an oligonucleotide–RNA

heteroduplex. Once bound, RNA that is bound to DNA in a heteroduplex will be cleaved by the RNaseH, thus freeing the DNA. Any remaining ASO will essentially catalyze the degradation of the subsequent RNA products, effectively silencing the genetic expression. When adapted for individuals with Huntington's disease, researchers hoped that this technology could be adapted to target HTT, the gene responsible for the disease development. In one study, researchers found promising results that suggested that silencing the overexpression of HTT through the use of ASOs could successfully slow disease progression in transgenic mice. However, they did note that ASOs would only temporarily silence the expression of HTT, and recipients would require continued intermittent treatment to counteract the return of the neurodegenerative symptoms and possible toxic effects (Southwell *et al*. 2012).

Another promising genetic therapy option utilized against Huntington's is RNA interference (RNAi), which Thermo Fisher (2019) defines as a phenomenon where small RNAs bind to messenger RNAs (mRNA) that encode for specific proteins in order to inhibit subsequent protein translation. RNAi begins when double-stranded RNA (dsRNA) is introduced into the cell, whether that be by natural or artificial means. When the cells recognize the dsRNA, they signal for an immune response to destroy this potentially harmful foreign body, which results in dsRNA cut into smaller pieces (siRNA). These smaller pieces can bind with other cellular components in the cell, thus creating new single-stranded RNA (ssRNA). These new ssRNAs can then bind to target mRNA and inhibit the production of proteins encoded by those mRNAs. When adapted for individuals with Huntington's, researchers hoped that they could engineer siRNA to target the HTT mRNA encoding for huntingtin, which would decrease the expression of that protein. In one study, researchers found that RNAi could successfully be manipulated to

target the HTT gene encoding for huntingtin. However, they noted that this therapy frequently did not succeed; in several cases, they observed several off-target effects and severe organ toxicity resulting in fatality in experimental models (Aguiar *et al.* 2017).

Because the proposed genetic therapeutic strategies cannot be fully controlled and frequently induce off-target mutations, researchers have begun to investigate a *priori* strategies in the literature that appear promising in neurodegeneration.

Experimental Therapeutic Strategies

One alternative for neurodegenerative treatment presents itself in the form of the genomic editing technique called CRISPR. CRISPR, an acronym for clustered regularly interspaced short palindromic repeats, can be defined as a family of DNA sequences found in regions of the prokaryotic genome. It can be altered to search, remove, and replace specific DNA sequences to repair any unwanted mutations; it could then either remove the mutation or signal the body to repair that specific segment. The CRISPR-Cas9 system consists of two components: the Cas9 endonuclease that will ligate the target sequence and a single guide RNA (sgRNA) that guides the Cas9 to the target site. Upon binding to the targeted site, Cas9 induces a double-strand break in the DNA that employs the non-homologous end joining (NHEJ) repair method, which will facilitate the introduction of random nucleotides into the target site that encourages frameshift mutations, thus disrupting any subsequent gene expression. In the case of Huntington's, researchers suggest that this system could be delivered into the brain *in vivo* and disrupt the continued production of the HTT gene. When Huntington's was simulated in mouse models expressing the HTT gene and its CAG trinucleotide repeats, researchers hypothesized that the therapeutic strategy would target HTT production and halt disease progression. They found that

there was a reduced formation of neurotoxic fragments, increased expected lifespan, and improved subsequent motor dysfunction in the mouse models. They admit that this therapy will not restore lost neurons, implying that this technology would be most beneficial on those early in their diagnosis; however, the neuronal cells could be recovered via cell replacement therapy (Ekman *et al*. 2019). This preliminary study indicates that this therapy could yield more benefits than traditional methods of treatment when used in conjunction with cell replacement therapy. Some researchers nonetheless criticize this method of treatment because of the varying limitations associated with genomic editing.

Researchers have considered the possibility of treating the disease by repressing huntingtin transcription by binding zinc-finger domains of the expanded CAG repeats. Zinc finger proteins (ZFP) can be engineered to include a second domain that can activate [or repress] a target gene's promoter to regulate its transcription. This strategy would be able to target any desired sequence, including CAG repeats, thus overcoming one of the restrictions inherent to CRISPR-Cas9 that prevents it from recognizing CAG repeats. In one study, when these ZFPs were modeled in neural stem cells (NSCs) differentiated from embryonic stem cells expressing the CAG repeats. In the NSCs, the engineered zinc proteins successfully repressed the transcription of the diseased HTT gene corresponding with the CAG repeats without affecting the transcription of normal HTT (Zeitler *et al*. 2019). In a similar study modeled in mice, different configurations of these zinc proteins were shown to repress transcription at different levels of efficiency. Longer zinc constructs were more successful in repressing mutant mRNA expression and protein levels. Analysis of the brains further indicated that the introduction of the zinc proteins slowed the rates of cranial decay in mice in the later stages of the disease, implying

that this therapeutic strategy successfully slowed neurodegenerative progression as well (Garriga-Canut *et al*. 2012). However, one shortcoming of this technique is that researchers have had difficulty reproducing the results of similar experiments, meaning that this technique is not stable enough to progress into clinical trials.

One last alternative for neurodegenerative treatment concerns the role of oxidative stress on neuronal degeneration. Free radicals, like reactive oxidative species (ROS) for example, regulate several pathways within the brain. However, an abundance of free radicals produced as a result of these pathways can overwhelm the antioxidant response and induce cranial oxidative stress. Oxidative stress occurs in the body when there is an imbalance between free radicals and antioxidants that detoxify these products. This imbalance can be further influenced by certain environmental, genetic, and age-related factors. For example, glial cells, found primarily in the brain and spinal cord, generate large amounts of ROS during the glial inflammatory response. Increased production of these free radicals can inhibit the antioxidant system response, which is regulated by glial cells as well. As a result, glial cell dysfunction by oxidative stress will result in reduced antioxidant production, leading to increased neurotoxicity in the brain. Researchers have suggested that the inhibition of free radical production could prevent the accumulation of oxidative stress, thus preventing the progression of neurodegeneration. However, none of the past suggestions that focus on oxidative stress prevention have developed into viable therapeutic strategies. Current research has demonstrated several barriers are hindering oxidative stress reduction therapy, such as substandard clinical models and lack of access to certain brain regions. Some neurologists have suggested that this strategy could work if treatment was personalized to each individual; for example, if an individual suffers from reduced glutathione function, a major

producer of antioxidants, then this therapeutic strategy could target these enzymes to enhance their production. However, there has been no practical application of this strategy to date. Researchers nonetheless are certain that personalized approaches when targeting oxidative stress could lead to improved clinical outcomes (Neal and Richardson, 2018).

Ethics Concerning Neurodegenerative Detection and Treatment

Current efforts to detect and treat neurodegenerative disease, like the aforementioned therapeutic strategies, are designed to identify and repair the responsible pathologies by genetic alteration. Genetic therapy has been shown to correct genetic mutations at their source, often without any serious side effects to the recipient. However, several groups have opposed the use of genetic testing and editing for ethical reasons. Discussions concerning the use of these technologies generally focus on the ethical, legal, and social consequences in humans. The scientific and bioethical communities have observed debates concerning all facets of the future of neurodegenerative identification and treatment for decades. Yet, they are no closer to a consensus than they were when the technology was first introduced.

In the case of genetic testing, there have been discussions concerning the ethics of predictive testing for Huntington's disease. Predictive testing produces three different types of ethical dilemmas: test accuracy and reliability, misuse of test information, and effect of test information on affected individuals. The information from the predictive testing will be used by those tested to make important decisions about their futures, and it is, therefore, essential to know if the tests can be completely trusted. What are the possibilities that the results could lead to false-positive results? Even with the advances in technology in this decade, there cannot be a guarantee that these predictions will not be prone to occasional error. This ethical dilemma could

further influence social dynamics since the results will be of interest to those close to the individual tested. Prospective employers, marriage partners, and family members may demand to be privy to the results, as they will often want to know whether this person will be around for the long term. What if the afflicted individual does not wish to disclose this information? Does their child not have the right to know that they may be a carrier for this disease as well? The most important ethical consideration for predictive testing concerns the effect of the results on the individual. Informing the individual of their disease could lead to increased rates of risky behaviors like depression, recklessness, and suicide. The conflict between honesty and the pledge to do no harm has always presented a clinical dilemma to healthcare providers when no treatment is available. Providers tend to favor honesty when considering requests from patients for information; the sooner they come to terms with their disease, then the sooner they can start treating any symptoms (Coustasse *et al* 2009). However, consider a hypothetical case where a provider tested an individual for several disorders as part of a theoretical standard annual exam. He discovered and then informed the individual that he was a carrier for Huntington's. Unbeknownst to the individual, he would never exhibit symptoms and live a long life. Yet, after finding out about his condition he decided to take his own life instead of risk the possibility of developing the disease. Is the provider ethically bound to disclose to his patient the findings of the exam, if they did not specifically request to know? There is currently no technique that would allow researchers to predict how individuals will react psychologically to their predictive testing results. Thus, it is of the utmost importance to researchers to establish and refine a code of ethics concerning predictive testing.

In the case of genetic editing, there have been discussions concerning the application of these strategies in humans. Specifically concerning the CRISPR-Cas9 system, all therapeutic utilization of this technology was studied in somatic cells before 2015. Genetic editing in somatic cells generally does not require much ethical assessment because of the low risk/high benefit balance. It is when this technology is applied to germline cells that we begin to consider the multitude of risks associated with this application. The utilization of CRISPR-Cas9 in the germline risks the manifestation of off-target effects in both current and future generations (Ayanoğlu *et al*. 2020). Some argue that these studies should continue only in somatic cells, where the risk/benefit balance can be established, as there is a high risk of off-target effects developing even with controlled application. Conversely, some believe that new developments by CRISPR-Cas9 could prevent off-target effects and that it should be further discussed (Yumlu *et al*. 2019). Further issues have arisen concerning the application of this technology, like equal access in terms of cost and social status; what kind of genetic flaws will this technology be used for; and informed consent for those undergoing genetic therapy by this technology (Ayanoğlu *et al*. 2020).

Despite being thoroughly debated for decades, the scientific community cannot agree on the ethics of predictive testing on humans for neurodegenerative disease. As with ethics and philosophy, there is never a clear answer on the correct course of action. Researchers, healthcare providers, and at-risk individuals should consider all options regarding testing in order to avoid any ethical or social complications.

Implications for Future Work

A thorough investigation into the aforementioned experimental therapeutic strategies will be of high relevance for the biomedical community. Currently, all these experimental methods exist only theoretically because of the ethical and functional limitations placed upon these techniques. For these techniques to progress to clinical trials, they would need to show safe, stable, reproducible results in a lab setting. An analysis of the effects that these strategies incur *in vivo* regarding mutant huntingtin expression, disease development and progression, and other important pathological ramifications hold immense value, especially for the future of neurodegenerative treatment. Even experiments with undesired results will be beneficial nonetheless since little is known about the practical application of such strategies. Furthermore, the current and experimental strategies will be subject to extensive ethical debate to determine their applicability in future clinical trials.

Conclusion

Huntington's disease is a progressive disease that devastates at-risk individuals and those closest to them. Over the last decade, there has been a rapid advancement in our understanding of the pathogenesis of Huntington's development and presentation. Currently, there are few treatments available that seek to prevent the progression of the disease; furthermore, several existing therapeutic strategies suggested by researchers have failed the clinical stage. There is a desperate need for the development of novel therapeutic strategies capable of targeting the mutant HTT gene responsible for inducing neurodegeneration. With researchers now considering new genetic therapy strategies that show *a priori* promise, there is a real possibility that one day an HD diagnosis will not be a death sentence.

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