Systematic Review Article

Evaluating The Therapeutic Potential of MicroRNA and Artificial MicroRNA Overexpression in Huntington's Disease

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ABSTRACT

Huntington's disease is a neurological/genetic disorder that affects about 41,000 people in the United States alone. Those with Huntington's disease live their everyday lives with various symptoms that may greatly hinder their ability to complete specific tasks. Without a cure for Huntington's disease, people with the disease continue living their lives with these symptoms, including behavioral and movement changes. However, the overexpression of certain microRNAs and artificial microRNAs may prevent neurodegeneration induced by Huntington's disease. MicroRNAs, a naturally occurring class of non-coding RNA, can regulate the expression of specific proteins by preventing the translation of messenger RNA. Within neurological cells, the overexpression of microRNAs naturally found in the nervous system can promote neurogenesis and neuronal function. This was found to occasionally combat neurodegeneration found in various models of Huntington's disease, suggesting its therapeutic potential. Artificial microRNAs are genetically engineered microRNAs used to prevent the translation of specific genes. They are different from microRNAs in that they can be engineered to target more particular genes, such as the *Huntingtin* gene, unlike microRNAs, which usually only target genes that are involved with general cell productivity. By targeting the mutant *Huntingtin* gene among models of Huntington's disease, it was consistently found that mutant Huntingtin mRNA and protein levels were reduced, preventing neuronal death. Methods of delivery of these microRNAs and artificial microRNAs include adeno-associated virus (AAV) vectors, lentiviral vectors, and exosomes, where AAV vectors, specifically AAV5, were found to be the most effective as a delivery method.

Keywords: Huntington's disease; huntingtin; microRNA; artificial microRNA; adeno-associated virus vectors; lentiviral vectors; exosomes, injections

INTRODUCTION

There are about 41,000 symptomatic patients with Huntington's disease (HD) in the United States (1). More than 200,000 people are at risk of inheriting the disease due to having a parent with HD. HD is a well-known, autosomal dominant neurological/genetic disorder responsible for neurodegeneration and harmful behavioral symptoms, including the loss of motor skills, changes in behavior, and possible mental health conditions (2). This is because HD shrinks areas of the brain, primarily the basal ganglia and the cortex (3). HD typically manifests in patients ages 30-50, but it has been seen to manifest earlier[1]. HD is caused

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by a genetic mutation on chromosome 4, specifically 4p16.3, resulting in the production of mutated CAG trinucleotide repeats in the DNA of the *Huntingtin* (*HTT*) gene (4, 5). Normally, an individual will have about 10-35 CAG trinucleotide repeats in the *HTT* gene, with the average being about 17-20; however, those with HD have 36 or more. A successful treatment will likely target these mutant CAG trinucleotide repeats and/or proteins created by the mutation to prevent the production of mutant Huntingtin protein, which is responsible for neurodegeneration in HD. Normally, the Huntingtin (HTT) protein produced by the *HTT* gene aids in the transport of material between neurons and other cells, facilitates the production of Brain-Derived Neurotrophic Factor (BDNF) to assist in learning and memory processing, and regulates the activity of the Caspase-3 enzyme to prevent neurodegeneration (6, 7). Mutant HTT proteins have less specialized functions in these activities, resulting in motor and cognitive decline due to increased neuronal death. HD currently has no cure, meaning there are no permanent treatments for HD. Temporary treatments include therapy procedures and pill-based medications to reduce harmful motor and emotional conditions (8).

This systematic review explores the efficacy of microRNAs (miRNAs) as a potential therapy for HD. MiRNAs are naturally occurring, non-coding sections of DNA that prevent the translation of messenger RNA (mRNA), halting protein creation for the duration of the miRNA application (9). Similarly, artificial microRNA (amiRNA) is made up of molecules engineered to mimic miRNA (10). MiRNA injections are a gene-based therapy that allows miRNA to enter models to silence gene expression (11). However, since non-mutant (wild-type) HTT protein production is still required in HD patients for various cellular processes in neurological cells, allele-specific miRNA therapies will target the mutated CAG trinucleotide repeats in the mRNA of one inherited HD-causing allele, and not the unaffected allele. This will likely help heterozygous individuals with HD mitigate the production of mutant HTT proteins without compromising the production of wild-type HTT proteins. Common delivery methods for allele-specific miRNA and amiRNA for HD patients include adeno-associated virus (AAV) vectors, lentiviral vectors, and exosomes. AAV vectors utilize AAV capsids from viruses as a transport method, lentiviral vectors utilize a lipid bilayer derived from a host cell, and exosomes employ membrane-bound vesicles for transport (12-15). AAV vectors are better studied as a transport mechanism for complex, allele-specific gene-targeting miRNA *in vivo,* and lentiviral vectors are better studied for long-term gene silencing *in vitro*. Exosomes are equally studied as a transport mechanism for general neuronal-based miRNA (16).

As HD has no viable treatment yet, and the separation of mutant and wild-type protein levels has not been fully studied in HD models, miRNA-based therapies serve as a potential method for gaining insight into both of these topics and for treating HD.

METHODS AND MATERIALS

This systematic review involved conducting a literature search on the function of miRNA in HD models and how procedures, such as AAV vector, lentiviral vector, or exosome-bound miRNA, can be used to regulate mutant HTT mRNA and protein levels. A three-step search strategy was employed, consisting of identifying advanced search agents in the first step, identifying keywords in the second step, and searching for relevant studies in the final step.

PubMed and Google Advanced Search agents were utilized to find sources including specific keywords; input keywords included ("Huntington" OR "microRNA" OR "artificial microRNA" OR "AAV" OR "Lentiviral Vector" OR "Exosome"). PubMed was used to look for credible, recent studies on HD, miRNAs, amiRNAs, and delivery methods for miRNA-based therapies. This allowed for the collection of specific information relevant to the evaluation of miRNAs and amiRNAs as potential treatments for HD. Google Advanced Search was utilized to search for a broader range of sources relevant to HD. Google Advanced Search was used to search for background information on HD, miRNAs, amiRNAs, and delivery methods for miRNA-based therapies to create audience understanding of this systematic review paper. PubMed and Google Advanced Search were used to search for relevant and credible books, documents, original research papers, review papers, and systematic review papers between 2005 and 2025. This time frame was set to ensure that studies used in this systematic review paper would provide accurate information related to modern applications of RNA-based therapies and HD progression.

After studies that met these requirements were identified, they were further analyzed for their relevance to HD. Studies including ("microRNA" OR "artificial microRNA" OR "AAV" OR "Lentiviral Vector" OR

"Exosome") within their text were specifically analyzed to see if laboratory results related to HD were observed, or if they included background information about the mechanisms behind miRNAs, amiRNAs, AAV vectors, lentiviral vectors, or exosomes. Any studies that did not meet these requirements were excluded from being used in the creation of this paper.

RESULTS

MicroRNA Efficacy

MicroRNA Canonical Biogenesis Pathway

MiRNAs are a form of non-coding, single-stranded RNA that are transcribed from DNA, typically found in intronic regions of DNA (9, 17). The canonical biogenesis pathway for the production of miRNAs is illustrated in Figure 1. Pri-miRNAs serve as the initial precursor for miRNA creation. Once pri-miRNAs are transcribed from the DNA, Drosha and DiGeorge syndrome critical region 8 (DGCR8) proteins synthesize the strand into precursor-miRNA (pre-miRNA). Pre-miRNAs are exported into the cytoplasm of cells via Ran-Guanosine Triphosphate (Ran-GTP)-bound Exportin5 transport factors, where Dicer proteins further process them to remove the "short-hairpin" structure from pre-miRNAs, creating the miRNA duplex18. Argonaute 2 (AGO2) proteins bind to the miRNA duplex and remove passenger strand (miRNA*) sequences (9). To prevent protein translation, mRNA must undergo the miRNA-induced silencing complex (miRISC), as illustrated in Figure 2. AGO-bound miRNAs bind to specific mRNA sequences depending on miRNA location and function, resulting in the miRISC. AGO2-bound miRNAs either work to degrade mRNAs or reduce gene expression by blocking ribosomes from translating specific mRNA sequences to prevent protein production (9, 17).

Once transported into the nervous system, neuronal-based miRNAs such as miR-124 and miR-22 are processed (9, 17, 18). Since transported miRNAs do not undergo the miRNA canonical biogenesis pathway in comparison to transported pri-miRNAs, direct miRNA transport is becoming more favorable for HD treatment. When directly transported, miRNAs begin undergoing the miRISC, and therefore, there is less risk of error compared to the transport of pri-miRNAs as a treatment for HD. However, as naturally-occurring miRNAs do not target the *HTT* gene, they cannot guarantee reductions of mutant HTT protein and mRNA levels, and with the potential for degrading the incorrect mRNA, may cause systemic complications among *in vivo* systems (17, 18). This can include toxic side effects depending on the mRNA that is silenced, as well as

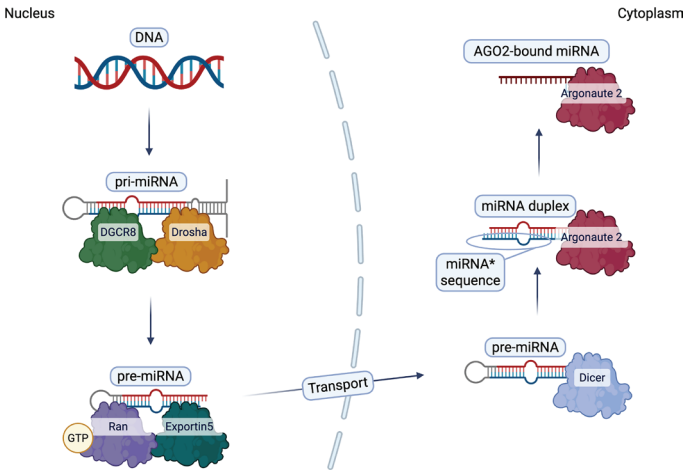

Figure 1. MicroRNA Canonical Biogenesis Pathway. miRNA, microRNA; pri-miRNA, primary microRNA; DGCR8, DiGeorge syndrome critical region 8 protein; Drosha, Drosha protein; pre-miRNA, precursor microRNA; GTP, Guanosine Triphosphate; Ran, Ran proteins; Exprotin5, Exportin5 factor; Dicer, Dicer protein; miRNA* sequence, microRNA duplex passenger strand; Argonaute 2, Argonaute 2 protein; AGO2-bound miRNA, Argonaute 2 protein-bound microRNA. The figure was created in BioRender. Donepudi, A. (2025) https://BioRender.com/3pl1aqa

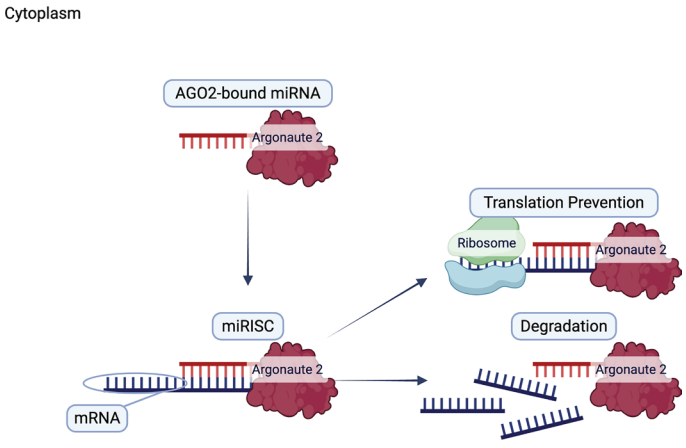

Figure 2. MicroRNA-Induced Silencing Complex. AGO2-bound miRNA, Argonaute 2 protein-bound microRNA; Argonaute 2, Argonaute 2 protein; miRISC, microRNA-induced silencing complex; mRNA, messenger RNA. The figure was created in BioRender. Donepudi, A. (2025) https://BioRender.com/3pl1aqa

decreased therapeutic effectiveness. Overcoming off-target effects is essential to ensuring miRNA efficacy in HD models.

MicroRNA Neuroprotective Effects

MiRNAs that target neurological cells could work to combat symptoms of HD. MiRNAs targeting genes involved in maintaining neuronal cell cycles and function, such as miR-124, were sometimes observed to mitigate symptoms induced by HD. One study used Cy3-labeled miR-124 injections, or miR-124 bound to a fluorescent dye, to measure the effect of miR-124 in R6/2 HD transgenic mice (19). Once injected, R6/2 HD transgenic mice had reduced behavioral symptoms, increased neuron formation (neurogenesis) in the striatum and cortex, and increased BDNF levels. This suggests that miR-124 may prevent symptoms caused by HD by reducing neuronal death and promoting neuronal function. Another study used exosomes to deliver miR-124 (Exo-124) to the striatum of an R6/2 transgenic mouse model; no significant behavioral improvement occurred (13). However, this study served as a proof-of-concept for further testing of similar miRNAs, exosome-based delivery methods, and miR-124 as a potential therapy. Jovicic *et al.,* 2013 measured the overexpression of miR-22, a type of miRNA promoting cell growth, on both wild-type and mutant HTT proteins in striatal and cortical neurons of a rat (20). Htt18Q, a polypeptide chain in wild-type HTT proteins, and Htt82Q, a polypeptide chain in mutant HTT proteins, were both injected using lentiviral vectors to mimic HD. Lentiviral vectors were also used to transport miR-22 to the neurons. miR-22 was seen to reduce neuronal death induced by Htt82Q by decreasing the focal accumulation of HTT proteins and reducing the activation of Caspase enzymes 3 and 7 (enzymes responsible for promoting apoptosis). Although miR-22 does not target the *HTT* gene, this study suggests that miR-22 overexpression can prevent neurodegeneration in HD through its involvement with HTT proteins.

Another study used medium spiny neuron cell models, neurons that receive input signals from the thalamus and cortex, and send output signals to the striatum (21, 22). In the study, medium spiny neuron cell models were created in a lab from fibroblasts using miR-9/9* and miR-124 to induce neurogenic chromatin remodeling; they were used to test for neurodegenerative correlations. It was found that autophagy activity in the cells of HD patients was correlated with neuronal death. It was also found that the overexpression of miR-29b-3p, a miRNA responsible for neuronal death and mutant HTT protein aggregation, caused decreased autophagy activity. With mutant HTT protein aggregation resulting from the clumping of mutant HTT proteins within neurons due to errors in protein folding, this discovery is key to preventing neuronal death in HD. Although this study does not directly use miRNAs as a therapy for HD, it suggests that miR-9/9* and miR-124 can be used as potential methods for cell remodeling in MSN cell models and that miR-29b-3p should be regulated in order to help patients with HD.

Naturally occurring miRNAs found within brain regions affected by HD may help prevent symptoms of HD. If overexpressed, miRNAs that prevent neurodegeneration and promote neurogenesis or neurological cell function may work effectively to reverse the effects of mutant HTT proteins within the basal ganglia and cortex.

Artificial MicroRNA Efficacy

Artificial MicroRNA Engineering

AmiRNA creation is the engineering of miRNAs to target specific genes or change miRNA function, as illustrated by Figure 3 (10, 23). After the chosen miRNA undergoes the canonical biogenesis pathway or is isolated during a section of the canonical biogenesis pathway, the mature miRNA is separated, along with the miRNA* sequence. Once separated, gene targeting sequences are replaced in both strands, while maintaining previous mismatches found in the original miRNA duplex. Afterwards, promoter and terminator

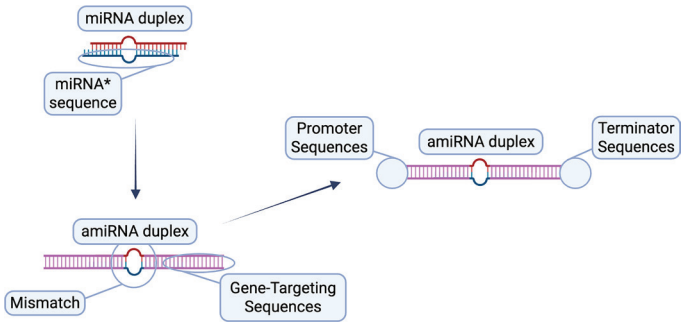

Figure 3. Artificial MicroRNA Engineering. miRNA, microRNA; miRNA duplex, microRNA duplex; miRNA* sequence, miRNA duplex passenger strand; amiRNA duplex, artificial microRNA duplex; Mismatch, Non-coding section of the miRNA and amiRNA duplex. The figure was created in BioRender. Donepudi, A. (2025) https://BioRender.com/3pl1aqa.

sequences are bound to the new amiRNA duplex to ensure the correct mRNA is silenced. By adding promoter and terminator sequences to the amiRNA duplex, RNA Polymerase 2 will allow the correct mRNA to be silenced by the AGO2-bound amiRNA. RNA Polymerase 2 will know where to begin the eventual translation of HTT mRNA and where to end translation to directly impact HTT protein and mRNA levels.

Once created, amiRNA is usually injected into the target organ of mRNA silencing, which varies depending on the type of treatment (24). After injection, amiRNA undergoes the same miRISC as miRNA, eventually binding to AGO2, as illustrated in Figure 2. Once bound, silencing of the target gene happens through degradation or blocking of ribosomal transcription of mRNA.

In HD, amiRNAs are derived from various miRNAs, usually ones found primarily within the brain or miRNAs that can be easily separated (25). Once injected into models, typically within the striatum, amiRNA is processed, and AGO2-bound amiRNA silences the mRNA produced by the *HTT* gene. Some amiRNA is designed to target specific exons or mutant CAG trinucleotide repeats within mRNA produced by the *HTT* gene. Among models of HD, amiRNA targeting the *HTT* gene, such as miHTT, is transported throughout neurological cells, usually within the basal ganglia and cortex (3). Although these amiRNAs undergo the miRISC, they do not have to be processed once injected throughout neurological cells, making for faster results (17, 18). Additionally, since amiRNA can directly target the *HTT* gene, amiRNA is capable of producing more specific advantages in preventing HD progression. However, amiRNA may also target the wrong mRNA, leading to the translation prevention of differing mRNAs. This may cause varying effects, depending on the mRNA that is silenced, as well as decreased therapeutic effect.

Off-target risks of miHTT may be more damaging than off-target risks of miRNAs, as reduced miHTT effectiveness directly supports HD progression. This is because amiRNA, such as miHTT, directly impacts mutant HTT protein and mRNA levels.

AmiRNA Allele-Specific Effects of Neuroprotection

MiHTT is a type of amiRNA that specifically targets the human *HTT* gene and mRNA created by the gene (11). AGO2-bound amiRNAs that are designed to target the *HTT* gene, such as miHTT, were observed to prevent the translation of mutant HTT proteins. For example, one study used adeno-associated serotype 5 viral vectors to deliver miHTT (AAV5-miHTT) in a homozygous Q175FDN mouse model of HD (11).

Intra-striatal injections of the AAV5-miHTT were seen to reduce mutant HTT protein levels by 65.8% in the striatum and 29.8% in the cortex, as measured through RNA sequencing. Although this study did not separate mutant HTT proteins from wild-type, it still suggests that miRNAs can directly impact mutant HTT protein levels, possibly preventing neurodegeneration within the basal ganglia, the region of the brain most impacted by HD, as well as the cortex. Sogorb-Gonzalez *et al.* 2024 tested the efficacy of intrastriatal injections of AAV5-miHTT designed to target the *HTT* exon 1, a segment of the *HTT* gene, in heterozygous zQ175 KI mice with expanded CAG trinucleotide repeats (26). At the highest dose of the AAV5-miHTT treatment, there was a 45% and 28% reduction of mutant HTT exon 1 protein levels in the striatum and frontal cortex, respectively, at 2 months post-treatment. They also measured the effectiveness of the AAV5-miHTT treatment in a humanized Hu128 mouse model, a mouse designed to have expanded CAG repeats resembling a human with HD. In this model, there was a reduction of both wild-type HTT proteins and mutant full-length HTT proteins (FL-HTT) up to 90% in the striatum and 60% in the cortex. It was also observed that the miHTT decreased striatal volume loss while promoting neuron function.

With the reduction of both wild-type and mutant HTT protein levels in the Hu128 mouse model, further research on miHTT amiRNA as a therapy is necessary. However, this study still gives insight into miHTT function within *in vivo* systems. Another study measured the effect of an AAV5-miHTT treatment on neurological cell cultures and astrocytes in HD patients, using human-induced pluripotent stem cell (iPSC)-derived neuronal cultures (27). The AAV5-miHTT treatment was observed to reduce mutant HTT mRNA by approximately 57% and mutant HTT protein levels by 68%. The AAV5-miHTT was also observed to produce minimal off-target effects. Miniarikova *et al.,* 2017 measured the effectiveness of multiple AAV5 and Lentiviral-bound miHTTs in the striatum and cortex of Hu128/21 HD mice through injections (28). The most effective miHTTs tested were the AAV5-miHTT-451 and AAV5-miSNP50T-451 therapies, where mutant HTT mRNA levels were reduced by 81 ± 4.3% by the AAV5-miSNP50T-451 treatment, and there

were almost no mutant HTT protein aggregates found. Mutant HTT protein aggregates, or protein clumps caused by errors in protein folding of the mutant HTT protein, are a common sign of HD. With the reduction of these aggregates and mutant HTT protein levels, this study suggests that AAV5-miHTT-451 and AAV5-miSNP50T-451 may be effective at preventing HD within heterozygous individuals and models due to their ability to target specific alleles. Additionally, Evers *et al.,* 2018 measured the efficacy of AAV5-miHTT at preventing neurodegeneration in a transgenic HD minipig model (29). The highest dose of AAV5-miHTT was observed to reduce mutant HTT mRNA by 47.5 ± 21.8% in the putamen, 44.2 ± 34.8% in the caudate nucleus, 42.7 ± 25.4% in the cortex, and 72.8 ± 13.4% in the thalamus. With these reductions, the minipig is considered an effective method to test miRNAs targeting the *HTT* gene *in vivo*. As a bigger model, with a brain similar in size to a human brain, the minipig model may be helpful for future exploration of HD progression and treatment. Another study also tested AAV5-miHTT intrastriatal injections as a potential therapy for HD in heterozygous Q175 mice and R6/2 mice models (30). In Q175 mice, reduction of mutant HTT protein levels was 39% in the striatum and 13% in the cortex 12 months post-injection. During a motor test, the highest-dose-treated R6/2 mice also exhibited a 47% reduction in latency to fall. This study explains that AAV5-miHTT may prevent the loss of motor skills in HD patients.

When AAV9 is bound to Green Fluorescent Protein (GFP), the AAV9-GFP delivery method can transfer amiRNA while staining neurons to make it easier to track HTT protein levels. A study testing AAV9-GFP-based delivery of amiRNA in a Dorset sheep model with HD has shown a 50-80% decrease in mutant HTT mRNA within the *HTT* gene in the striatum (31). The study also showed no significant loss of neurons. This study suggests that in sheep, an animal with a large systemic structure similar to humans, amiRNA may effectively prevent neurodegeneration caused by HD, while maintaining overall neuronal activity. Another study measured the efficacy of intrastriatal injections of an AAV5-based delivery of amiR136-A2, a CAG trinucleotide-repeating amiRNA, on HD YAC128 mice models (32). By measuring polyglutamine amino acid aggregates – a common sign of HD – 20 weeks post-injection, it was observed that mutant HTT protein levels were reduced by about 50%. A different study tested the efficacy of mi2.4, another type of amiRNA designed to target exon 2 of both human and mouse HTT mRNA transcripts (33).

The AAV1-mi2.4 was observed to reduce levels of human mutant HTT and wild-type mouse HTT mRNA in HD-N171-82Q mice. This study then compared AAV1-mi2.4 to a short-hairpin RNA therapy, AAV1-sh2.4. Compared to AAV1-sh2.4, AAV1-mi2.4 was observed to reduce HTT mRNA levels at a reduced toxicity level. Although AAV1-mi2.4 is not allele-specific and also degrades wild-type HTT mRNA, this study serves to explain the possible efficacy of mi2.4 as a gene therapy for HD. Wang *et al.,* 2023 studied AAV-based deliveries of primary amiRNAs (pri-amiRNAs) in YAC128 mice and non-human primates, both *in vitro* and *in vivo*, through intrastriatal injections (24). The pri-amiRNAs were designed to target HTT mRNA, modified from mi2.4. The AAV vectors were bound to CBA promoters to lower mutant HTT mRNA levels. The 16 pre-candidate pri-amiRNAs were observed to reduce mutant HTT by approximately 20-80% *in vitro*. Of the 7 pre-candidate pri-amiRNAs observed to reduce mutant HTT *in vivo*, the most effective were seen to reduce mutant HTT by approximately 55%. This suggests that even pri-amiRNAs designed to target mutant HTT mRNA and/or mutant HTT proteins may be effective at preventing neurodegeneration in individuals with HD.

Since amiRNA can identify changes in DNA sequences like differing CAG trinucleotide repeats in the *HTT* gene, AGO2-bound amiRNA, if designed and delivered correctly, can differentiate between mRNA that can be produced and mRNA that needs to be degraded (11). This has been seen to reduce mutant HTT protein levels while potentially allowing the production of wild-type HTT proteins to remain constant in heterozygous and homozygous models with HD. While heterozygous individuals with HD may benefit from amiRNA-based therapies, amiRNA is still being researched as a potential therapy for homozygous individuals with HD, as removing HTT protein production entirely is not ethical and may not be beneficial.

Delivery Vector Performance

Adeno-Associated Virus Vector Efficacy

An adeno-associated virus (AAV) is a type of virus that can be used to deliver gene therapies to humans due to the low immune response it elicits in human bodies (12). Without a lipid bilayer, AAVs are considered non-enveloped viruses, and because of this, they can be

easily designed to hold and transport genetic therapies, such as miRNA and amiRNA. AAV vectors are transport mechanisms engineered from AAVs. Different types of AAV vectors include AAV2, which targets neurons, and AAV5, which targets astrocytes (34). AAV1, AAV8, and AAV9 are also commonly used to transport genetic material throughout the brain. AAV5 has been greatly studied as a transport mechanism for miRNA and amiRNA within HD models due to its ability to transfer gene-based therapies throughout the brain without compromising neuronal function or disrupting neuronal activity, while preventing neurodegeneration.

In miRNA and amiRNA-based therapies for HD, AAV vectors carry miRNA and amiRNA duplexes directly to astrocytes or other neurological cells (34). As observed by studies measuring the efficacy of AAV-bound amiRNA within HD models, AAV vectors are considered effective as a transport mechanism for amiRNA in HD models. Among studies testing the efficacy of AAV5 as a transport method of amiRNA, each study was shown to be effective (11, 26-30, 32). Even studies testing AAV1 and AAV9, less-used AAV vectors in HD models, were seen to be effective as well (24, 31, 33). This effectiveness is shown through a consistent pattern of mutant HTT protein and mRNA reduction and no signs of harmful immune responses across studies (11, 24, 26-33). Additionally, with AAV5's ability to target astrocytes and AAV9's ability to pass the blood-brain barrier, AAV vectors may be engineered to effectively target astrocytes without the need for direct neural injections. However, high AAV vector transportation can induce harmful immune responses. Astrocytes and microglia may be activated in response to AAV vectors. A study testing an AAV1 vector-based delivery of miHTT observed the activation of microglia and overall immunoreactivity (33). Although these effects are not commonly observed in the transport of AAV5 vectors, it is not impossible (11, 26-30, 32). AAV vectors may be effective as a delivery method for miRNA and amiRNA in other HD models or potential humans, however, continual editing and development of vectors must occur to eliminate risks of harmful immune responses (35).

Lentiviral Vector Efficacy

Lentiviral vectors, derived from lentiviral viruses, serve as a potential method of delivery for miRNA and amiRNA. Lentiviral viruses are enveloped viruses, meaning they have a lipid bilayer surrounding them (36, 37). Although they are harder to manipulate compared to AAV viruses because of this, they are less likely to fail in the transport of gene-based therapies. Additionally, since lentiviral viruses are larger than AAV viruses, lentiviral vectors can carry larger capacities of gene-based therapies (38).

As few studies explore lentiviral vectors as transport mechanisms for miRNA and amiRNA, further testing is necessary to prove their efficacy. Two studies using lentiviral vectors as transport mechanisms for miRNA and amiRNA were observed to be effective, as indicated by their ability to reduce focal accumulation of HTT proteins, activation of Caspase enzymes 3, and mutant HTT protein and mRNA levels (20, 28). Lentiviral vectors may serve as a potential transport mechanism due to their low toxicity and high carrying capacity. Studies showed low lentiviral toxicity, however, this was due to a low influx of lentiviral vectors transported. Lentiviral vectors, when used to transport miRNA and amiRNA therapies may still cause the activation of astrocytes or microglia. Also, as most lentiviral vectors are unable to cross the blood-brain barrier, lentiviral efficacy depends on direct injection into brain regions (39). This is not ethical for individuals with HD, as direct neural injections into humans are currently not used in medicine and may cause more harm to humans. Further manipulation and testing of lentiviral vectors may propose a solution for this disadvantage.

Exosome Efficacy

Exosomes are naturally occurring vesicles secreted by cells that transport necessary bodily amino acids between cells. When modified, exosomes can carry miRNA and amiRNA to models. They are capable of holding amounts of gene-based therapies similar to AAV vectors (40).

A study measuring an exosomal-based delivery of miR-124 was not successful, as measured by no behavioral change post-treatment (13). However, this may have been caused by the miRNA, and not the exosome. Also, the exosomes were still able to reach target neurons and travel throughout the target region of the brain. Another advantage of exosomes is their ability to pass the blood-brain barrier, an essential milestone for the use of exosomes to carry miRNA and amiRNA within HD patients both ethically and effectively (41). The immune response posed by exosomes carrying miRNA or amiRNA is still being studied, as exosomes are still emerging as a mechanism for carrying gene-based therapies (42).

Although the immune response of Exo-124 wasn't explicitly stated, it can be deduced that exosomes can result in astocyte and microglia activation due to their ability to facilitate movement between neurological cells easily (13, 40). Few studies observe the efficacy of exosome-based deliveries of miRNA and amiRNA in HD models; further manipulation and observations of exosome-based deliveries may be required to prove the efficacy of exosomes as transport mechanisms for miRNA and amiRNA in HD models.

The evaluation of microRNAs throughout models of the nervous system establishes that the amiRNA, miHTT, demonstrates the most robust and consistent efficacy in reducing both mutant HTT protein and mRNA levels across *in vitro* and *in vivo* models, as shown in Table 1. This direct gene silencing strategy of the HTT gene showed stronger results of preventing neurodegeneration when compared to neuroprotective mechanisms employed by neuronal-targeting microRNAs, specifically miR-124 and miR-22. MiHTT's therapeutic advantage derives from its ability to achieve greater mutant HTT reduction while maintaining the production of wild-type HTT in models. Specifically, studies consistently proved that AAV5-miHTT achieved significant depletion of mutant HTT mRNA and protein levels in patient-derived neuronal cultures, rat models, and R6/2 and Q175 mouse models (11, 26-28, 30). When testing the ability of AAV5-miHTT to improve motor coordination among *in vivo* systems, studies reported that the AAV5-miHTT treatment relieved motor deficits and improved overall motor performance (11, 30). These observations suggest that miHTT's ability to reduce neurodegeneration also prevents motor changes associated with HD.

Table 1. Study Comparison Chart

Study Reference	MiRNA/ AmiRNA Type	Delivery Method	Model	Study Aim	Outcome
19	MiR-124	Direct intrastriatal injections	R6/2 transgenic mouse model of HD	Test if miR-124 could promote neurogenesis, therefore slowing HD progression.	Increased neurogenesis and BDNF levels slowed the progression of HD, as shown through motor tests.
13	MiR-124	Direct intrastriatal injections of Exosome encasing miR-124	R6/2 transgenic mouse model of HD	Test if miR-124 could downregulate the *REST* gene to determine therapeutic potential in HD.	Although reduced *REST* gene expression was observed, no prominent improvements in the motor behavior of the mice was found.
20	MiR-22	Lentiviral vectors	Primary striatal and cortical neuron cultures exposed to Htt18Q and Htt82Q	Test whether the overexpression of miR-22 protected against neurodegeneration in HD.	Reduced neuronal death because of less HTT protein focal accumulation along with less Caspase 3 and 7 enzyme reaction.
22	MiR-9/9* and MiR-124	Editing fibroblasts to become medium spiny neurons	Medium spiny neurons derived from fibroblasts found in individuals with and without HD	Uncover how aging contributes to HD onset by analyzing neuronal activity and survival.	The overexpression of miR-29b-3p in HD models caused decreased autophagy activity. This resulted in mutant HTT aggregation and neuronal death.
11	MiHTT	Direct intrastriatal injections of AAV5 vectors	zQ175 mouse model of HD	Test if AAV5-miHTT could lower mutant HTT expression and promote neuronal survival.	The AAV5-miHTT treatment lowered mutant HTT mRNA and protein levels, reduced striatal atrophy, and prevented behavioral movement loss.

Continued Table 1. Study Comparison Chart

Study Reference	MiRNA/ AmiRNA Type	Delivery Method	Model	Study Aim	Outcome
26	MiHTT targeting *HTT* exon 1	Direct intrastriatal injections of AAV5 vectors	zQ175 KI mice and Hu128 mice models of HD	Test whether the overexpression of miHTT targeting *HTT* exon 1 would reduce mutant HTT protein levels.	Reductions of mutant exon 1 proteins in the zQ175 KI mice models and reductions of both mutant and wild-type protein levels in the Hu128 mice models.
27	MiHTT	AAV5 vectors transduced through cultured cells	Patient-derived neuronal cultures and astrocytes	Test whether AAV5-miHTT could reduce mutant HTT mRNA and protein levels without unintended consequences.	Significant reductions of both mutant HTT mRNA and protein levels without any unintended consequences.
28	MiHTT	Direct bilateral intrastriatal injections of AAV5 vectors	Transgenic Hu128/21 mouse model of HD	Test if the AAV5-miHTT treatment reduced mutant HTT protein aggregation and neuronal dysfunction.	The AAV5-miHTT treatment reduced mutant HTT protein aggregation while promoting neuronal function.
29	MiHTT	Direct intrastriatal injections of AAV5 vectors	Transgenic minipig model of HD	Test if the AAV5-miHTT treatment was able to be distributed throughout the brain to promote reduced mutant HTT mRNA levels.	The broad distribution of the AAV5-miHTT treatment in the Central Nervous System, along with the reduction of mutant HTT mRNA levels across affected brain regions.
30	MiHTT	Direct bilateral intrastriatal injections of AAV5 vectors	Transgenic R6/2 and Q175 knock-in mice models of HD	Determine the long-term effects of the AAV5-miHTT treatment on mouse models.	The AAV5-miHTT treatment resulted in sustained mutant HTT protein level reduction accompanied by improved motor performance.
31	MiHTT	Direct intracranial injections of AAV9-GFP vectors into the striatum	Transgenic Dorset sheep model of HD	Test the broad distribution of the AAV9-GFP-miHTT in the striatum, along with its ability to lower mutant HTT mRNA in a larger model.	The widespread appearance of the AAV9-GFP-miHTT in the striatum and the significant reduction of mutant HTT mRNA levels.
32	MiHTT	Direct bilateral intrastriatal injections of AAV5 vectors	Transgenic YAC128 mouse model of HD	Test the AAV5-miHTT treatment to reduce mutant HTT protein levels without compromising the production of wild-type HTT proteins.	Significant reduction in mutant HTT protein levels and the stabilization of wild-type HTT protein levels.

Continued Table 1. Study Comparison Chart

Study Reference	MiRNA/ AmiRNA Type	Delivery Method	Model	Study Aim	Outcome
33	MiHTT targeting *HTT* exon 2 in humans and in mice	Direct bilateral striatal injections of AAV1 vectors	Trangenic N171-82Q mice model of HD	Test the efficacy of non-allele-specific reduction of HTT mRNA through amiRNA and short-hairpin RNA therapies.	Reduction of both mutant and wild-type HTT mRNA levels.
24	Primary amiRNA targeting the *HTT* gene	Direct bilateral intrastriatal injections of AAV1 and AAV2 vectors	Transgenic YAC128 mice models and nonhuman primates (*in vitro* and *in vivo*)	Examine if pri-amiRNA could effectively be used to lower mutant HTT mRNA levels in understudied models of HD.	Reduction of mutant HTT mRNA was present both *in vitro* and *in vivo*. No unintended consequences were observed in the understudied models.

A comparative analysis of the effects of microRNA and artificial microRNA overexpression and their methods of delivery among *in vitro* and *in vivo* systems genetically engineered to mimic Huntington's disease.
miRNA, microRNA; amiRNA, artificial microRNA; miR-124, microRNA-124; HD, Huntington's disease; BDNF, Brain-derived neurotrophic factor; miR-22, microRNA-22; HTT, Huntingtin; Htt18Q, a wild-type Huntingtin protein containing 18 glutamine repeats; Htt82Q, a mutant Huntingtin protein containing 82 glutamine repeats; miR-9/9*, microRNA-9/9*; miR-29b-3p, microRNA-29b-3p; miHTT, an artificial microRNA engineered to target the *Huntingtin* gene; AAV5 vectors, adeno-associated viral vector serotype 5; mRNA, messenger RNA; AAV9-GFP, adeno-associated viral vector serotype 9 engineered to express green fluorescent protein; AAV1 vectors, adeno associated viral vector serotype 1; AAV2 vectors, adeno associated viral vector serotype 2; pri-amiRNA, primary artificial microRNA.

When comparing the delivery methods used to transport these microRNA-based therapies, AAV vectors, specifically AAV5 vectors, were commonly used in studies where a significant reduction of mutant HTT protein and mRNA levels was prominent (11, 26-28, 30). When these AAV vectors were administered to *in vivo* systems through direct intrastriatal injections, mutant HTT protein and mRNA were significantly reduced among brain regions such as the striatum and cortex. These regions are the areas of the brain most affected by HD progression, suggesting the potential of direct AAV5-intrastriatal injections to yield beneficial results when compared to other delivery methods (3).

DISCUSSION

Determining the efficacy of miRNA and amiRNA-based therapies for treating HD depends on symptom reduction, mutant HTT protein and mRNA reduction, and the decreased possibility of potential risks.

MiRNA was observed to sometimes reduce behavioral symptoms associated with HD through mechanisms promoting neurogenesis and neuronal function rather than through reducing mutant HTT proteins and mRNA (19, 20). Two studies showed evidence of reduced neurodegeneration within models. However, with a lack of studies observing the effect of miRNA overexpression in HD models, further testing may be required to prove the efficacy of miRNA overexpression as a therapy for HD. Future research should test for neurogenesis and neuronal function *in vitro* and the reduction of behavioral symptoms within structured models *in vivo*. AAV vectors, lentiviral vectors, and exosomes may continue to be altered to more effectively transport miRNA throughout neurological cells both *in vitro* and *in vivo*.

Although not direct proof of the efficacy of miRNA treatment in HD models, other studies observed the overexpression of neuronal miRNAs to induce cell remodeling in order to track miRNA pathways within a medium spiny neuron cell model (22). This may further develop current research on the efficacy of miRNA overexpression in HD by allowing the creation of *in vitro* cell models more effectively. As observed in the study, the regulation of miR-29b-3p, a miRNA responsible for neuronal death and mutant HTT protein

aggregation, may reduce neurodegeneration induced by HD. Future research may explore the regulation of miR-29b-3p in HD models through miRNA-based therapies, potentially using miRNA-induced cell remodeling to provide an accurate observation of miRNA travel within the brain. Another study also tested cell reprogramming to understand the efficacy of AAV5-miHTT treatments in human-derived fibroblasts to create medium spiny neuron and astrocyte culture models (27). This study also served as proof-of-concept for tests using cell reprogramming to test the effects of miRNA and amiRNA overexpression in HD models. This may allow for further testing of miRNA and amiRNA overexpression in HD models using cell remodeling.

AmiRNA overexpression in HD models was seen to effectively reduce mutant HTT protein and mRNA levels, simultaneously reducing harmful behavioral symptoms induced by neurodegeneration caused by HD (11, 24, 26-33). Studies observing the overexpression of amiRNAs engineered to target HTT mRNA, such as miHTT and mi2.4, found consistent mutant HTT protein and mRNA reduction. This is unlike studies observing the overexpression of miRNAs, as miRNAs promote neurogenesis and neuronal function rather than impacting mutant HTT mRNA and protein levels. Another study that specifically tested the behavioral extent of HD saw a reduced latency to fall due to HD in a mouse model (30). These breakthroughs suggest further research on miHTT is likely, which may yield a potential treatment of HD due to the efficacy of amiRNA overexpression in preventing neurodegeneration in HD models.

Within these studies, new, previously underdeveloped methods of miRNA overexpression were observed. Evers *et al.,* 2018 observed amiRNA overexpression in a minipig model of HD (29). With the reduction of mutant HTT mRNA in the minipig, the minipig may be considered an effective *in vivo* system to continue observing HD. A different study observed AAV9-GFP-bound miHTT in an HD Dorset sheep model (31).

The Dorset sheep model is an understudied model concerning amiRNA overexpression in HD. Also, AAV9-GFP was used in this study, a strong method of delivery due to the vector's ability to pass the blood-brain barrier while simultaneously tracking amiRNA effectively within neurological cells *in vivo*. With the observed reduction of mutant HTT mRNA in the study, the Dorset sheep model and AAV9-GFP-based delivery system may be effective for future testing of amiRNA overexpression for an HD treatment. Future observation of amiRNA overexpression in the Dorset sheep model and/or AAV9-GFP-based delivery systems has the potential to ethically reduce the harmful behavioral symptoms of HD in humans.

Most studies involving miRNA and amiRNA overexpression in HD models used AAV5 to deliver treatments (11, 26-30, 32). This was found to be effective, as seen by the ability of the miRNA and amiRNA treatments within neurological cells to combat neurodegeneration and/or mutant HTT accumulation. Other delivery methods for miRNA and amiRNA treatments, including AAV1 vectors, AAV9 vectors, lentiviral vectors, and exosomes, were sometimes found to effectively transport the genetic therapies (13, 20, 24, 31, 33). These delivery methods of miRNA and amiRNA were sometimes observed to induce immune responses. Immune responses in the nervous system can be defined as astrocyte and/or microglia activation in response to vector delivery throughout neurological cells. AAV vectors, lentiviral vectors, and exosomes were observed to cause both low toxicity and high toxicity dependent on vector amount and vector type. AAV1 vectors were observed to cause high toxicity through microglia activation, while AAV5 vectors presented low toxicity and no significant immune responses (11, 20, 26-30, 32, 33). Lentiviral vectors also presented low toxicity and immune response in low influx. Further testing may confirm their efficacy as a delivery method for miRNA and amiRNA within HD models, as harmful immune responses should be prevented.

Once injected, a high influx of miRNA may result in decreased miRNA efficacy through off-targeting of differing mRNAs (17, 18, 27). Although symptoms depend on the mRNA that is being targeted, toxic effects may occur. Similarly, amiRNAs such as miHTT may create off-target risks as well. Although usually not as common as in miRNA-based therapies, as amiRNAs are created to exactly target specific strands, it is not impossible. Further testing and therapy development of miRNAs and amiRNAs should explore the reduction of off-targeting potential. Direct intrastriatal injections, although having been used as a delivery method among several *in vivo* systems listed, are currently considered unethical for human use due to the high risk associated with potential error. Any minor error with direct intrastriatal injections can result in severe harm. Although emerging transport mechanisms, such as AAV9 vectors, aim to alleviate potential risks that arise from the transport of miRNAs and amiRNAs,

further development and creation of delivery systems are required to eliminate potential hazards.

CONCLUSION

The overexpression of miRNA and amiRNA via injections serves as a strong, potential treatment to help patients living with HD (11, 13, 19, 20, 24, 26-33). This is because miRNA can target neurons and promote neuronal function, and amiRNA can prevent the production of specific proteins. Both of these, when applied to HD models, have been seen to prevent harmful symptoms related to HD, a therapy that those with HD can greatly benefit from. Specifically, the AAV5-miHTT treatment showed the greatest amount of mutant HTT protein and mRNA reduction across *in vivo* and *in vitro* systems. Studies using miRNA treatments showed less impact on mutant HTT protein and mRNA amount. However, to ensure a therapeutic outcome, miRNAs, amiRNAs, and delivery methods of miRNAs and amiRNAs must continue to be developed. Potentially harmful effects of off-targeting and direct intrastriatal injections are present among current proposed therapies for HD. With the prominent use of small *in vivo* systems to understand the therapeutic potential of miRNA and amiRNA therapies, larger systems must also be used to understand the therapeutic potential of miRNA and amiRNA therapies for HD patients. Also, immune responses of vector-based-delivery systems must continue to be explored to prevent harm in HD patients. By continuing to explore the overexpression of miRNAs and amiRNAs, a treatment for HD may arise and truly benefit those who continue to live their everyday lives impacted by the disease.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest regarding the publication of this article.

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