NOVEL GENE THERAPY TECHNIQUE TO TREAT HEREDITARY DEAFNESS

by

Myca-Lee Mc Comie

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SUPERVISORY COMMITTEE:

_____

Dr. James K. Wetterer

_____

Dr. Ericca Stamper

_____

Dean Justin Perry, Wilkes Honors College

_____

Date

ABSTRACT

Author:	Myca-Lee Mc Comie
Title:	Novel gene therapy technique to treat hereditary deafness
Institution:	Wilkes Honors College of Florida Atlantic University
Thesis Advisor:	Dr. James K. Wetterer
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Autosomal recessive deafness-9 (DFNB9), caused by mutations in the otoferlin gene (*OTOF*), is the most common form of hereditary deafness, accounting for 2-8% of all cases. Here, I review recent research on using dual adeno-associated virus (AAV) mediated gene therapy to treat DFNB9 in a mouse model system. Dual AAV gene therapy repairs these mutations by injecting pairs of AAV vectors carrying separate fragments of Otoferlin DNA into the round window membrane to the affected cochlea. When these AAV vectors recombine, they produce the expression of the full-length gene and restores hearing. Dual AAV gene therapy provides a biologically regenerative treatment that is faster and less invasive than the cochlear implant currently used to treat DFNB9. This breakthrough will reshape the treatment of genetic diseases.

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Introduction

An estimated 20.3% of Americans suffer from some form of hearing loss (Lin et al. 2013). For my thesis, I reviewed recent research on a therapy used to treat one common form of hearing loss, autosomal recessive 9 deafness (DFNB9).

The process of hearing consists of a series of steps that convert information in sound waves into electrical signals (National Institute of Deafness and Other Communication Disorders, 20202). Sound waves enter the outer ear through to the auditory canal leading to the eardrum. Sound waves cause the eardrum to vibrate, moving three tiny bones in the middle ear: the malleus, incus, and stapes (Fig. 1). These bones transfer a signal to the cochlea, a snail-shaped structure filled with fluid in the inner ear (Fig. 1).

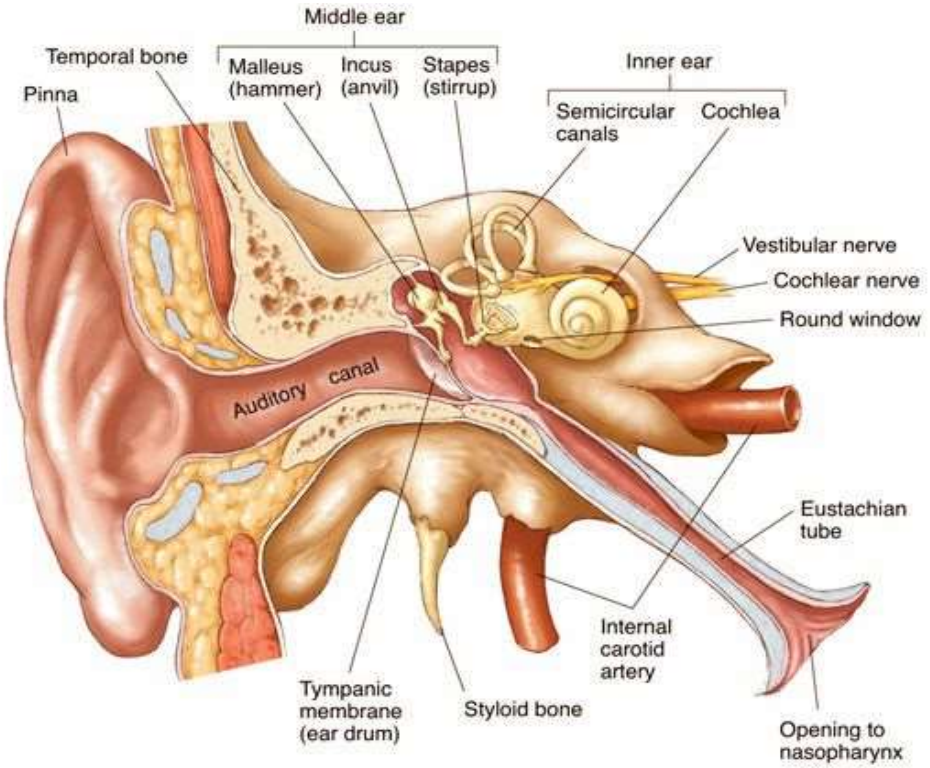

Fig. 1. Anatomy of the human ear (from Amartey, 2018*)*.

The cochlea contains sensory cells called hair cells that detect sound waves. These hair cells have microscopic projections called stereocilia that bend during the movement of fluid in the cochlea. This bending opens pore-like channels at the tips of stereocilia which facilitates the intake of potassium ions that depolarize the cell (Fig. 2). This stimulates voltage-gated calcium channels to open and intake calcium ions to further depolarize the cell (Fig. 2). These calcium ions are detected by otoferlin, a key calcium ion sensor, which stimulates the release of chemical signals from hair cells to an afferent nerve ending which then relays these signals to the auditory nerve (Takago et al., 2019). The auditory nerve sends these signals to the brain where the impulses are translated into sounds that we know and understand.

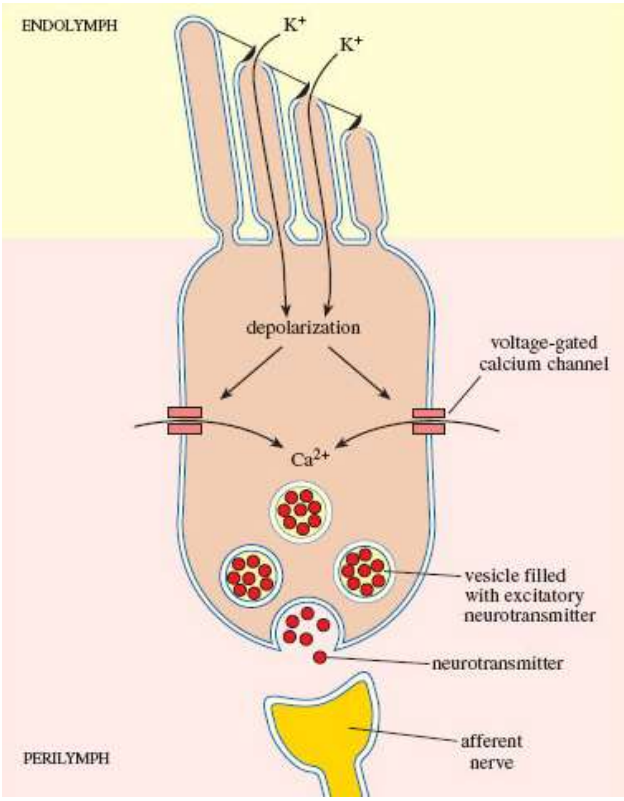

Fig. 2. Depolarization of a hair cell followed by the release of neurotransmitter (from Calleja, 2010).

The Relationship between the Otoferlin Gene and DFNB9 Deafness

The otoferlin gene (*OTOF)* is located on chromosome 2 at locus p23.1 (Meena & Ayub, 2017). This gene encodes for the production of otoferlin, a protein that is predominantly expressed in the inner hair cells of the cochlea. Otoferlin is required for the process of hearing. According to Takago et al. (2019), it is a key calcium ion sensor that is involved in calcium ion-mediated exocytosis from inner hair cells to spiral ganglion neurons, which are cells that connect the inner hair cells with the part of the brain that processes sound, called the brainstem's cochlear nuclei. The presence of otoferlin in inner hair cells facilitates the synaptic transmission between inner hair cells and spiral ganglion neurons. Thus, allowing the frequency and intensity of sounds to be interpreted.

The process of hearing is severely disrupted when *OTOF* is mutated. Loss-of-function mutations in *OTOF* result in Deafness, Autosomal Recessive 9 (DFNB9). This condition is the most common form of hereditary deafness, accounting for 2-8% of all cases (Rodríguez-Ballesteros et al., 2008). According to Iwasa et al. (2019), there are more than 160 reported mutations in *OTOF,* these include missense, nonsense, insertion/deletion, and splicing variants. These variants produce abnormal otoferlin or no otoferlin at all, which affects an individual's ability to hear. With no otoferlin or improper functioning otoferlin, calcium ions are not detected in hair cells, and therefore, no neurotransmitter is released to spiral ganglion neurons for sound to be processed by the brain (Iwasa et al., 2019).

Currently, cochlear implants are the main treatment method for DFNB9. However, a novel gene therapy method that has been tested in mice, the Dual Adeno-Associated Virus (Dual AAV) mediated gene therapy, provides a promising treatment method for those suffering with DFNB9.

Current Treatment for DFNB9

Currently, cochlear Implants are the main treatment method for DFNB9. Cochlear implants are implemented through a process of surgery and extensive therapy (U.S. Department of Health and Human Services, 2020). The implants consist of an external portion that sits behind the ear and an internal portion that sits on the mastoid bone (Fig. 3). Cochlear Implants functions by completely by-passing the nonfunctioning structures in the ear and directly stimulating the auditory nerve (Shearer & Hansen, 2019). The microphone located in the external portion of implant detects sounds from the environment and sends it to the speech processor which translates the acoustic signals into electrical impulses that represent speech (Fig. 3). The transmitter sends these electrical impulses to the internal receiver which converts them to other electrical impulses. The electrode array collects these impulses and sends them to different regions of the auditory nerve which sends sound information to the brain to produce a hearing sensation.

Unfortunately, there are several drawbacks to cochlear implants. Firstly, cochlear implants are an invasive treatment. Due to the internal component on the cochlear implant, surgery is required for this treatment. Secondly, this treatment is time consuming. The type of hearing produced by cochlear implants is extremely different from normal hearing. Therefore, extensive therapy is required to learn or relearn the sense of hearing in patients. Thirdly, cochlear implants do not offer any biological regeneration. Instead of treating the cause of DFNB9, it by-passes the dysfunctional parts of the ear and directly stimulates the auditory nerve.

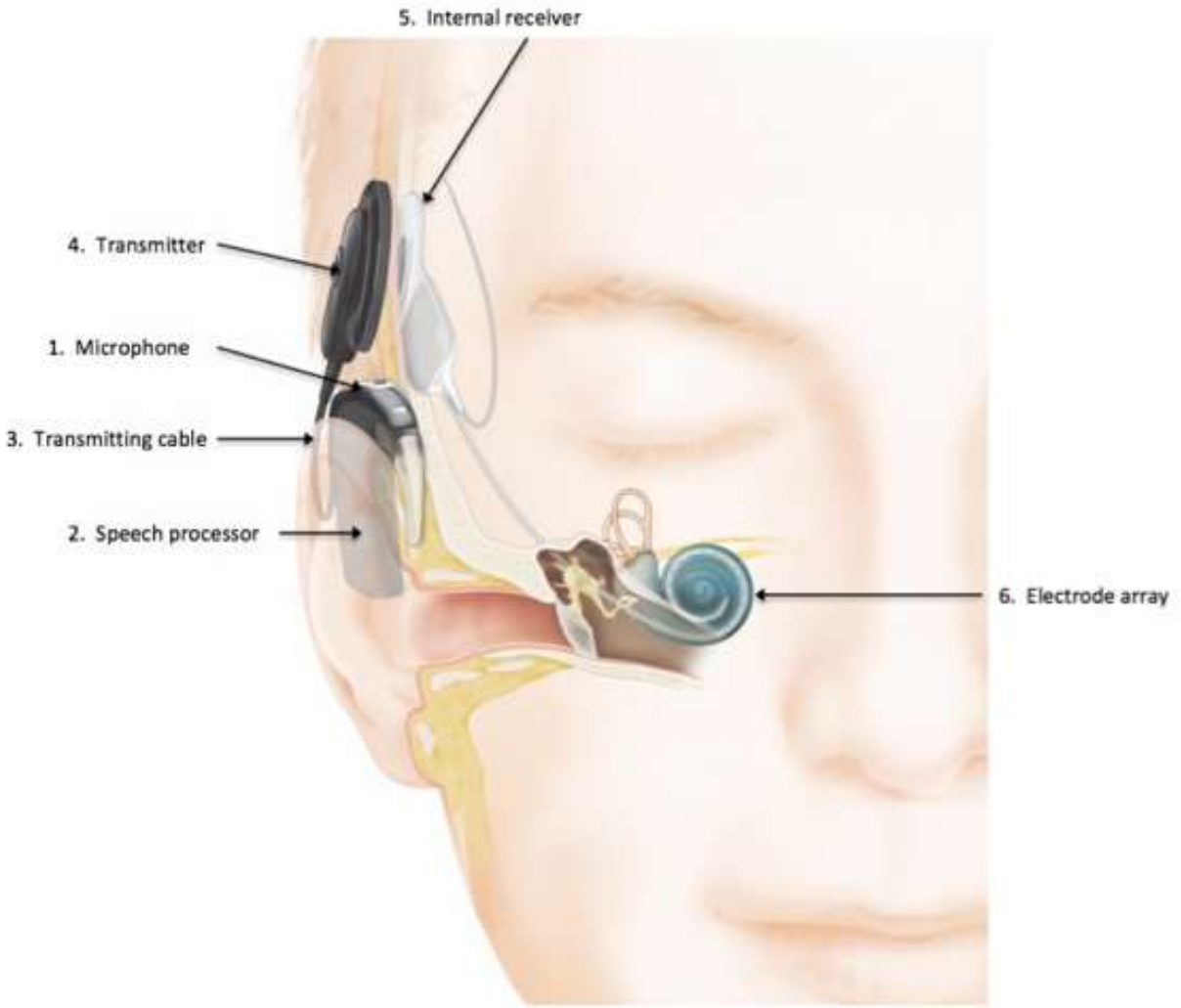

Fig 3: Parts of a cochlear implant in a human ear (from Cochlear Ltd 2019).

The Development of Dual Adeno-Associated Virus Gene Therapy

Adeno-associated virus (AAV) gene therapy has been considered the most promising approach to treat genetic diseases. In 2014, it was utilized to treat Choroideremia, a rare, X-linked retinal disorder resulting from the deletion of the *CHM* gene (Ong, 2019). AAV gene therapy was administered in the right eyes of six human patients to insert the *CHM* gene into retinal pigment epithelium and photoreceptor cells. After 6 months, all right eyes showed stark improvements when compared to the left eye controls, and these results remained consistent after

3.5 years (Ong ,2019). However, the major challenge of this gene therapy method was its limited cargo capacity. AAV vectors were only capable of transporting genes smaller than 5kb (five thousand base pairs). Thus, it was unsuitable for treating genetic diseases caused by mutations in larger genes.

In 2019, Akil et al. (2019) and Al-Moyed et al. (2019) utilized dual adeno-associated virus gene therapy to treat DFNB9 in mice with 6kb (six thousand base pairs) otoferlin cDNA and overcame the main drawback of AAV gene therapy. According to Akil et al. (2019), dual AAV gene therapy begins by obtaining the DNA sequence of *OTOF*. The full-length sequence is divided into two fragments: a 5' fragment and a 3' fragment sequence. These DNA fragments are then synthesized by polymerase chain reaction (PCR) and inserted into separate AAV vectors (Al-Moyed et al. 2019). These AAV vectors (Fig. 4) also contain a splice donor (in the 5' fragment), a splice acceptor (in the 3' fragment), an alkaline phosphatase recombinogenic bridging sequence, and inverted terminal repeats (ITR). These components aid in the recombination of the *OTOF* DNA upon co-infection of both fragments. The virus solution is purified, titered, and then injected through the round window membrane into the affected cochlea. Otoferlin is only expressed when both fragments are administered and recombine (Fig. 4).

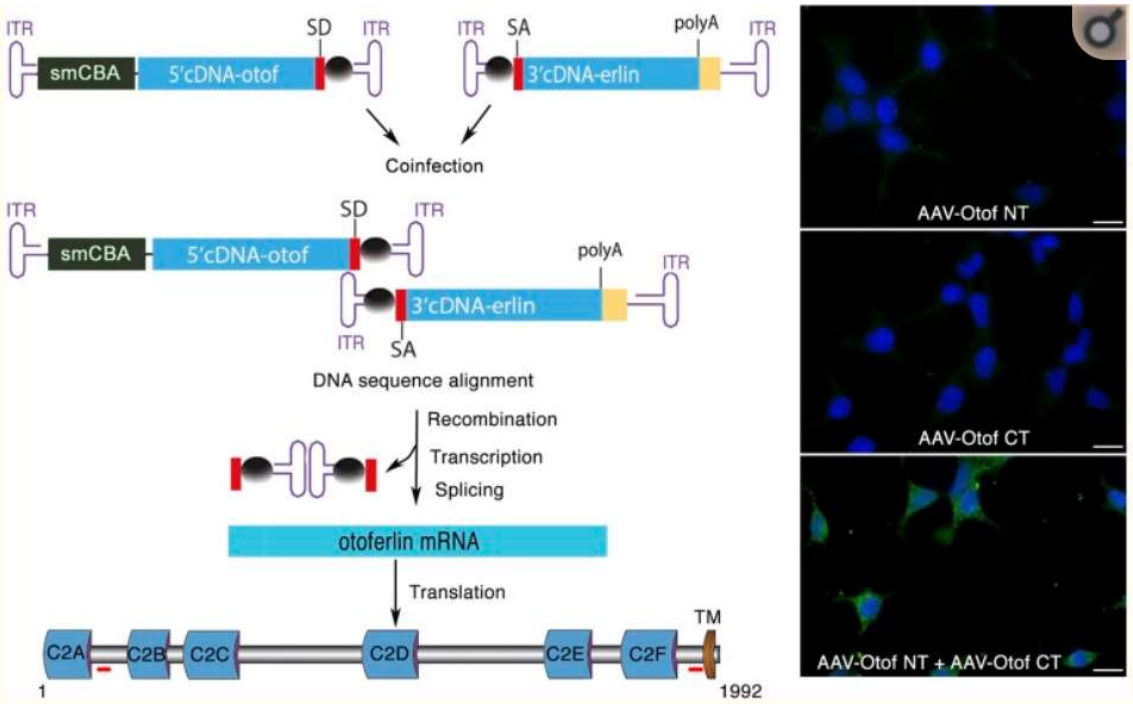

Fig 4: The schematic representation of the dual AAV-vector pair used in this study, and the expression of otoferlin in HEK293 cells after coinfection (from Akil, O. et al., 2019).

The experiments used 24 mice with DFNB9 deafness divided into 3 groups: the control, immature hearing organs (P10), and mature hearing organs (P17 and P30) (Akil et al., 2019). The group with immature hearing were injected with the virus solution 10 days after their birth when their hearing organs were not yet fully developed. Hearing restoration was observed in these mice 4 weeks after the injection was administered. After 30 weeks post-injection, 6 of 8 treated mice retained hearing thresholds withing 10dB (decibel) (Akil et al., 2019). Thus, providing evidence that dual AAV gene therapy prior to hearing onset prevents deafness in DFNB9 mice. The group with mature hearing were injected with the virus solution 17 days and 30 days after their birth. Hearing recovery in these mice were observed 3-4 weeks after the injection. Experiments carried out 20 weeks post-injection revealed that all 8 mice retained their hearing ability (Akil et al., 2019). The control group of DFNB9 mice was split into 2 groups.

One group that received only one AAV vector and another that received no injection. No changes were observed in these control groups.

Evaluation of Dual Adeno-Associated Virus Gene Therapy

The success with this method in mouse models suggests that dual AAV gene therapy has the potential to become a specific, noninvasive, fast, and corrective treatment for DFNB9 in humans.

Dual AAV gene therapy provides a specific treatment that transduces large genes (Akil et al., 2019). In the early stages of gene therapy, single AAV vectors were used to transduce the desired gene. However, this method was only capable of transferring genes that were smaller than 5kb (five thousand base pairs) (McClements and MacLaren, 2017). This made treating diseases caused by mutations in large genes such as DFNB9 impossible. These limitations were overcome with the implementation of the dual AAV method. Since it involved transducing the correct sequence of DNA via 2 separate fragments in 2 separate vectors, dual AAV increased the carrying capacity to 10 kb (ten thousand base pairs) which is twice the capacity of regular AAV gene therapy. Therefore, dual AAV gene therapy has provided an opportunity for the integration of larger transgenes into individuals with genetics conditions such as DFNB9.

Additionally, dual AAV allows for the regeneration of the wild-type *OTOF* sequence. By transducing *OTOF* DNA into affected mice, the ability to express the otoferlin protein in the cochlea is restored. Since otoferlin is a key factor in synaptic transmission between inner hair cells and spiral ganglion neurons, its expression stimulates the transmission of chemical signals which encodes the frequency and intensity of sound. Thus, permanently restoring the sense of hearing (Al-Moyed et al., 2019).

Moreover, dual AAV is administered via a single injection. This greatly reduced the treatment time when compared to years of therapy required for cochlear implants. In the experiments, a single injection was administered through the round window membrane of the cochlea in mice. After 3-4 weeks, hearing was restored in these mice and was retained as evident in experiments carried out 20-30 weeks post-injection (Akil et al., 2019).

Furthermore, dual AAV gene therapy is highly specific with the cells that it targets. Immunostaining experiments carried out by Al-Moyed et al. (2019) and Akil et al. (2019) revealed that otoferlin was only integrated into the hair cells in the cochlea (Fig. 5). This is extremely beneficial because potential off-target expressions of otoferlin in non-sensory cells are avoided. Thus, reducing the risk for cellular mutations.

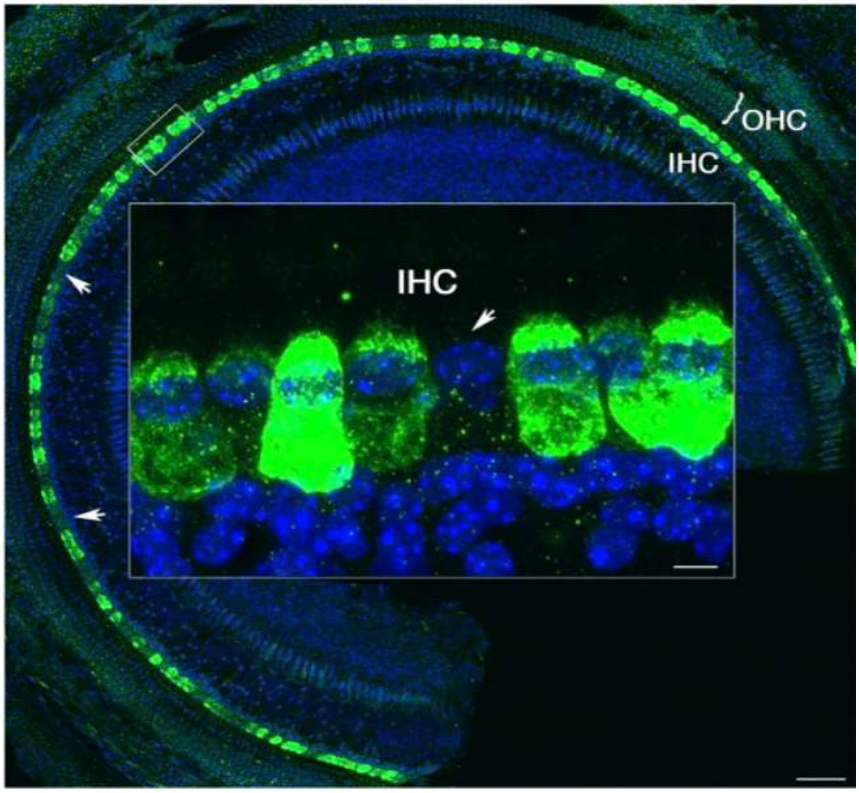

Fig 5: Restored otoferlin expression after dual AAV-mediated gene therapy in mice with DFNB9 deafness (Akil, O. et al., 2019).

Future Implications

Dual AAV-mediated gene therapy for DFNB9 is a very recent breakthrough in genetics that occurred in 2019. Therefore, there are several future implications for this therapy.

Firstly, a major implication is the optimization of this technique for use by humans with DFNB9. While AAV gene therapy has been utilized in humans, dual AAV gene therapy has only been employed on the mouse model. However, mice are very similar to humans in terms of anatomy, physiology, and genetics. As such, mouse models provide useful insights into the human diseases and treatments. Consequently, focusing on aspects such as refining split-AAV vectors, testing which AAV serotypes are most suitable for human application, and efficiency of the injection procedure will shape future research on dual AAV gene therapy and develop it to become effective in treating DFNB9 in humans (Al-Moyed et al. 2019).

Secondly, the dual AAV technique will facilitate the development of gene therapy for many other diseases. The ability to transduce large genes to correct mutations has opened up several opportunities to treat not only other types of deafness but other types of genetic diseases as well.

Thirdly, like all methods of gene therapy, there is an ethical argument on whether these treatments should be administered at all. Some argue that researchers are playing God by altering genes and tampering with the natural order of things. They believe that any form of gene therapy is completely immoral and should not be practiced. However, on the opposite pole are those that support gene therapy. These individuals argue that people with genetic diseases deserve a higher quality of life and so withholding these treatments would be completely immoral.

Conclusion

In conclusion, Dual AAV mediated gene therapy has the potential to provide a safe, fast, and less invasive treatment for DFNB9 deafness. This method administered via a single injection poses no opportunity for off-target expressions and has the ability to prevent as well as reverse the phenotype of deafness. Thus, making it more effective than cochlea implants used to treat DFNB9 deafness. The aim of this paper is to highlight the effectiveness of Dual AAV gene therapy in treating DFNB9 deafness and promote awareness of its existence. Currently, dual AAV gene therapy has only been tested utilizing the mouse model. As such, future research should be shaped to establish the treatment of humans. Dual AAV gene therapy has the ability to dominate the treatment of not only DFNB9 deafness, but other genetic diseases as well.

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