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Comparing International Gene Therapy Studies in Alzheimer's Disease – A Review

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ABSTRACT

Alzheimer's Disease (AD) is a type of dementia, affecting more than 55 million people across the world. Given that there is no cure for AD, there has been a significant increase in research focused on identifying other possible treatment options. One such treatment option includes gene therapy, which involves the replacement of a missing or defective gene with a normal gene to reduce the development of amyloid plaques and tau proteins in the brain. However, much of this research focuses on treatment in higher-income populations, such as the US and Europe, and there is a need to understand the utility and effectiveness of these treatments in lower-income populations. This is especially critical given that over 60% of AD patients live in low- and middle-income countries (Figure 1). Understanding how gene therapy studies in these communities compare to those in the US has the potential to advance current clinical research for AD patients residing in low- and middle-income countries. In this review, current literature involving gene therapy for AD across different countries will be compared and analyzed to determine which gene therapies can be implemented in lower-middle income countries considering factors such as availability of resources, economic situation, and genetics.

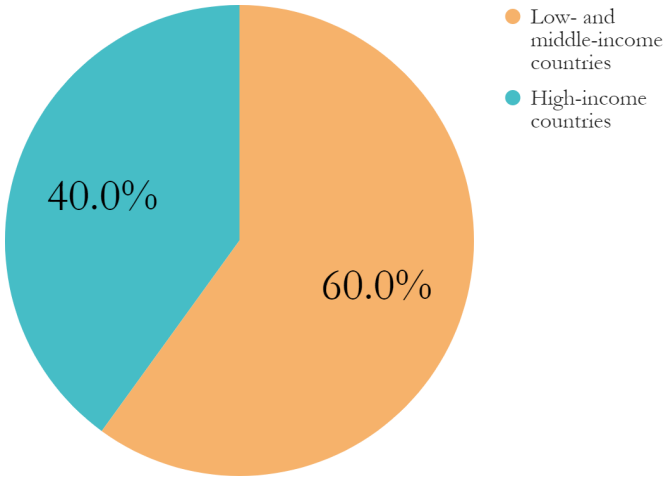

Figure 1. Percent of people with AD in low-middle versus high income countries

Introduction

AD is a neurodegenerative disorder that affects more than 55 million people across the world and has no definite cure. Research suggests that Alzheimer's-related brain changes are caused by abnormal levels of amyloid-beta protein (Aβ) and hyperphosphorylated tau in the neurons. The abnormal amount of Aβ is caused by the breakdown of the amyloid precursor protein (APP) in the brain. The extra Aβ that is produced eventually forms amyloid plaques at the signal receiving end of the neuron (axon) causing a signal blockage in the neuron (Kuznetsov, 2018). Tau is a fiber-like

protein that binds to microtubules in the brain to help stabilize nutrient transport through the microtubules into the neurons. A chemical change to the tau protein causes it to become hyperphosphorylated and detach from the microtubules. The detached tau proteins create neurofibrillary tangles (bundles of the tau protein) within the neurons, blocking signals from passing through the neuron (Kuznetsov, 2018). These protein abnormalities result in the loss of neuronal connections leading to cognitive decline (Rajmohan et al. 2017). Scientists have been studying the pathogenesis of AD and have found that gene therapy is one potential intervention technique to lower the development of these proteins and protect the brain from AD-related brain changes. Through gene therapy techniques, scientists across the world have discovered which genes to introduce or modify in the brain that could significantly slow down cognitive decline caused by accumulation of amyloid plaques and neurofibrillary tangles of tau. These include the administration of nerve growth factor (NGF), knockout of CD33, and use or knockout of apolipoprotein E(APOE). Unfortunately, despite these advances there is a discrepancy about the effectiveness of gene therapy in lower-middle income populations.

Over the last decade Alzheimer's research has been growing more popular and medications have been created to help with slowing down the cognitive decline in the brain. Most medications focus on preserving the neurotransmitters that are affected by AD. Neurotransmitters are molecules that are used to help send signals down a neuron. Since AD causes neuron signals to be blocked, scientists have been focusing on making the signals stronger with medication. Some examples of medication are donepezil, galantamine, and rivastigmine. These three medications are acetylcholinesterase inhibitors. Acetylcholinesterase is an enzyme that breaks down acetylcholine, a neurotransmitter (Yiannopoulou et al. 2020). By inhibiting, or preventing, acetylcholinesterase from breaking down acetylcholine, levels of acetylcholine rise and help slow down cognitive decline. While these medications are helpful towards slowing down cognitive decline, they are not able to completely cure AD. Most treatments focus on keeping the daily life of an AD patient as simple as possible to prevent any confusion. There is no determined treatment that has proven to show curable efforts for AD, but the introduction of gene therapy in AD has made the effort possible (Yiannopoulou et al. 2020).

For AD treatment around 1700 clinical trials have been conducted, yet only about 17.2% of those trials have been conducted in lower-middle income countries (Llibre-Guerra et al. 2022). The lack of representation of the lower-middle income countries is due to a variety of factors. Primarily, many countries are financially disadvantaged, limiting their access to the high-quality resources used in AD treatment. Some examples include the medications for AD and treatments to prevent worsening symptoms of AD which can be too expensive to obtain and implement. Secondly, low to middle-income countries do not have the same access to healthcare that the other countries have due to lack of educated healthcare professionals as well as funding for medical equipment, which further disadvantages them in treating AD. Gene therapy itself is more expensive than medications because of the technology and materials needed to implement gene therapy. If low to middle-income countries struggle to pay for their basic healthcare, then it would be a challenge to afford gene therapy at all. Expanding gene therapy trials to lower-middle income countries where AD is most prevalent could affect the results of AD treatments by providing an insight on how researchers could lower the cases of AD in the lower-middle income population and the future generations to come. Thus, to be able to continue advances in gene therapy, the cost and scalability of gene therapy must be considered alongside its effectiveness.

Throughout this paper, results of different gene therapies will be summarized to better understand how effective the gene therapies are in general. Then the effectiveness of the therapies in lower to middle-income countries will be discussed as well as the cost and accessibility to resources of the particular gene therapy.

Gene Therapy

Gene therapy was first developed in the early 1960s and has resulted in over 1900 clinical trials worldwide. Gene therapy is the process of modifying genetic code by removing, introducing, or altering a gene to change the function of proteins (Bulcha et al., 2021). There are two primary methods of gene therapy that have been identified for AD research: *Ex vivo* gene therapy involves removing brain cells and modifying them before injecting the cells back into

the brain (Bulcha et al., 2021). *In vivo* gene therapy involves modifying the genes of the brain cell directly, without extracting the cells from the brain (Bulcha et al., 2021). Through both *ex vivo* and *in vivo* gene therapy, new genetic material can be introduced into the cell providing instructions for proteins, which can provide assistance in taking care of the cells as well as the body (Bulcha et al., 2021). New avenues of gene therapy have been introduced including genome editing, which provides a simple method for modifying genes using non-invasive technology tools, such as CRISPR-Cas9. Gene-editing technology has the ability to modify or replace DNA or RNA sequences, and it is able to target specific areas of the brain that require gene editing without affecting the other areas (Duan et al., 2021). Researchers have been improving gene-editing methods since the 1980s and have discovered ways to make it more efficient by using nanoparticle deliveries (like vectors) to be able to deliver the necessary materials for gene modifications within the brain (Duan et al., 2021). Gene-editing has become a crucial part of gene therapy and is continuously developing and improving.

Although gene therapy has shown promising scientific advancements, this technique also has its limitations. One such limitation is that the modified genetic material introduced to the brain cells cannot be delivered directly into the cells. To address this problem carriers, called vectors, were designed to deliver the materials for gene editing. Vectors are viruses that are genetically engineered to target a specific cell within the human body so that the individual that has this virus placed inside of them does not get infected (Bulcha et al., 2021).These vectors are injected into a specific area of the body so that the patient's cells in that area can be infected by the virus (*in vivo)* or a sample of the patient's cells is removed and exposed to the vector, then placed back into the patient (*ex vivo).* If successful, then the gene can make a neuroprotective protein, or the editing molecules can restore protein function or correct a DNA error (Bulcha et al., 2021). In contrast, if the vector is not integrated properly or does not work, it can cause errors that can lead to other complications. In addition, there is a possibility that the vector isn't programmed properly which can trigger the body to unnecessarily activate a dangerous immune response (Bulcha et al., 2021). There are technologies that are being developed and tested to lower these risks, and if successful vectors can provide a possible method for altering different genes found in the AD pathology to decrease Aβ plaque accumulation and tau neurofibrillary tangles.

Another limitation is the blood-brain barrier (BBB). This is a complex multicellular structure that protects the brain, only letting specific movement into the brain while exporting pathogens, neurotoxins, and inflammatory agents into the bloodstream. The BBB strengthens the membrane to create a microenvironment optimal for brain functioning (Jones et al., 2007). While the BBB is good for the brain, it can work against the results and application of gene therapies, preventing the drugs from crossing into the brain since vectors are read as viruses by the brain. Therefore, transport mechanisms are used to break through this barrier. Receptor-mediated transcytosis (RMT) is a selectively permeable vesicular trafficking system that controls the levels of some substances going inside the brain such as insulin and transferrin molecules through specific ligand-receptor recognition. The ligand-receptors are created when ligands bind to specific receptors on the surface of the plasma membrane allowing for the detection of substances through signaling processes. The signals cause the creation of vesicles to occur, which then engulf ligand-receptor complexes to transport them to the opposite basolateral region which faces the inside of the brain and releases them (Jones et al., 2007). Unlike RMT, adsorptive-mediated transcytosis (AMT) does not require specific interaction, it depends on non-specific interaction of polycationic proteins. Most proteins are usually too big to diffuse through a membrane; however, due to the binding affinity of polycations, they mix with the proteins allowing proteins to diffuse through the BBB. The positively charged half of the polycationic proteins experience electrostatic interactions with negatively charged surface regions triggering adsorptive mediated endocytosis. This means that the cationic net charge of a peptide/protein can be important in the uptake of these molecules within the brain capillary (Jones et al., 2007). The RMT and AMT are the main transports scientists have been using over the past few years to help with the penetration of the BBB. These two transports are used with the genetically engineered ligands to allow for the delivery of biotherapeutic agents into specific brain regions (Jones et al., 2007).

While solutions have been provided for the limitations within the body, solutions for economic limitations and effectiveness of treatments within lower-middle income countries are still to be determined. Gene therapy has

brought in solutions for AD that haven't been seen before, but even its basic components are expensive. For example, *in vivo* and *ex vivo* methods are common methods used for AD gene therapy, but both methods are expensive; yet, *in vivo* tends to be less expensive than *ex vivo* because it does not require as many resources since cells do not need to be extracted. In fact, the *ex vivo* process itself costs about 2 to 3 million dollars for each treatment (Roberts., 2023). Although it is not just about cost; it is also about how well the therapy works on lower to middle-income populations. There are multiple risk factors that come with gene therapy such as it not working properly and possibly leaving a virus in one's body. Because low- to middle-income countries do not have the same access to healthcare that the other countries have, as well as being economically restricted and sometimes genetically restricted, the extent to which gene therapies can be effective is questionable. In this paper, gene therapies that have yielded successful results for reducing AD progression are being looked into, but the focus is the effectiveness of the gene therapies in lower-middle income countries.

NGF

The nerve growth factor (NGF) are neurotrophic factors found within the hippocampus and cerebral cortex of the brain, both involved with memory. These neurotrophic factors are biomolecules that support the development, maintenance, and survival of specific neurons within the brain. The level of NGF in the brain is directly proportional to the levels of acetylcholine (ACh), a neurotransmitter released by cholinergic nerves that play a role in cognition, memory, and perception. When the NGF is not developed efficiently it can cause damage to cholinergic nerves resulting in a decrease of ACh, which is why NGF dysfunction can be found in AD pathology. Although NGF clinical trials that involve delivering NGF into the brain have discovered that the method of NGF gene therapy has proven to be safe and has shown some positive results in slowing the progression of AD, there continues to be some hesitation to pursue additional clinical trials due to NGF gene therapy being a fairly new method. A comparison of a study performed in the US and a study performed in Sweden was done to evaluate the extent of the differences and similarities of the resources used as well as if these resources are available to lower-middle income countries.

One study done from March 2001 to October 2015 at the University of California, San Diego, Medical Center in La Jolla tested the prevention and reduction of cholinergic neuronal degeneration for AD patients (Tuszynski et al., 2015). In this trial, patients diagnosed with early onset AD (EOAD) underwent NGF gene therapy. Eight patients participated in the first phase of the clinical trial (Phase 1) which used *ex vivo* methods for delivering NGF. Specifically, autologous fibroblasts (i.e., collection of connective tissue cells) from a skin biopsy were converted to express NGF using Moloney leukemia viral (MLV) vectors and placed into the basal forebrain where cholinergic cell bodies can be found. After a 2-year observation period, the results suggested an increase in neuronal activity and a decrease in rates of cognitive decline. Phase 2 used *in vivo* methods for gene delivery. 10 participants had adeno-associated viral vectors (stereotype 2) based NGF (AAV2-NGF) injected into the basal forebrain where the cells of the brain were genetically modified from within. Patients were randomly split into three groups, each receiving a different dose of the vector particles. At the end of the trial, patients exhibited trophic responses to NGF within the brain, and no harmful effects were observed during the trial. While a few positive results were observed, the results did not show an improvement in cognitive function; however, the results suggested that NGF is safe and can be implemented in later trials for a deeper insight into NGF gene therapy (Tuszynski et al., 2015).

A clinical trial done in Sweden implemented intracranial infusion of NGF to a 69-year-old woman with AD. Since administering NGF can be risky for humans, and since it is unable to cross the BBB, it was infused intraventricularly into the patient (Seiger et al., 1993). 6.6 mg of NGF was administered and the patient was monitored for 3 months which resulted in the uptake and binding of nicotine (acetylcholine neurotransmitter imitator that bind to nicotinic receptors and plays a role in memory and learning) in the frontal and temporal cortex and an increase in cortical blood flow; however, similar to the previous study, the results did not show a significant decrease in amyloid plaques or neurofibrillary tangles or any improvement in cognitive function but it was able to detect that NGF is safe to use, suggesting that future clinical trials of NGF in AD are probable (Seiger et al., 1993). With NGF being a new

method there are still improvements to be made to how NGF can be used in gene therapy, but the indication that the method is safe to use is beneficial for future use of NGF to determine if it can truly be a possible solution for AD.

NGF testing trials have been primarily conducted in the United States, but these trials have also had some success in showing a decrease in the rate that cholinergic neurons decline, in turn slowing the rate of cognitive decline of AD patients. The prior clinical trials for NGF have shown results that can be implemented in later gene therapy trials when more research has been done and more information has been found about AD pathology. While NGF proves to be a future method to decrease the progression of AD, few studies have implemented these methods in low- and middle-income countries.

APOE

In 1993, research discovered that Apolipoprotein E (APOE) was associated with the susceptibility of AD. APOE is a protein involved in the metabolism and transport of lipoproteins through the neurons in the brain. It is primarily found within the brain and the liver, playing the role of membrane repair, cell reproduction, and remyelination of new axons within the central nervous system (CNS) as well as controlling cerebrovascular integrity (Raulin et al., 2022). APOE is made up of a long amino acid chain and the N-terminal end binds to APOE receptors at the cell surface; however, a change in the receptor of APOE links to a change in amyloid and tau protein production (Yamazaki et al., 2016). APOE has three isoforms that play different roles in the AD pathology. APOE $\varepsilon 2$, APOE $\varepsilon 3$, and APOE $\varepsilon 4$ differ based on the placement of arginine or cystine on the 112th or 158th amino acids in the chain that makes up APOE. While APOE2 promotes Aβ_{42} (one of the amyloid proteins that are overexpressed in AD) fibrillization, APOE4 over-expresses the amount of Aβ_{42} resulting in Aβ accumulation and neurodegeneration. APOE4 is the main cause of AD out of the 3 isoforms (Kim et al., 2009). In fact, carrying one or two copies of the APOE4 gene can significantly increase the occurrence of late onset AD (LOAD) (Jin-Tai Yu, 2014). On the other hand, APOE2 is considered a protective gene against the disease because it slows down the development of AD, and APOE3 tends to be neutral as it doesn't necessarily speed up or slow down the development of AD (Kim et al., 2009). Unfortunately, APOE2 is the recessive allele compared to APOE4 which is a dominant allele, resulting in the APOE4 allele having a greater chance of being inherited. In addition, while APOE3 is the most common allele within humans, since it is a neutral party between the three, inheriting it with the APOE4 allele only slows the development AD because two APOE4 alleles weren't inherited (Liu et al., 2013) (Figure 2). Using the APOE gene, two studies, one from the US and one from Israel, are evaluated on the success of the technique they used as well as the usefulness of those techniques on lower-middle income populations.

The Percentage of Occurrence of Each APOE Isoform in Inherited Genes

APOE Type	Occurrence
APOE 2	5-10%
APOE 3	10-15%
APOE 4	70-80%

Researchers at Weill Cornell Medical College investigated how APOE2 can serve as a protective gene for AD and whether there is a particular route for the delivery of APOE2 into the CNS that is the safest for the brain and maximized APOE2 distribution within the CNS. Adeno-associated virus rhesus isolate 10(A AAVrh.10) stereotype coding for an HA-tagged human APOE2 DNA sequence(AAVrh.10hAPOE2-HA) was created-a type of virus that can be coded to deliver APOE2 to the CNS (Rosenberg et al., 2018). This vector was used throughout the experiment and was administered through intraparenchymal (within the tissue), intracisternal (within cisterna, enlarged pockets of cerebrospinal fluid), and intraventricular routes to the CNS. The subjects were evaluated over 2 months and it was

determined that intracisternal delivery of the vector was the safest and led to a greater distribution of APOE2 over the CNS, compared to the other two routes. The authors concluded this study can be used in further gene therapy trials and research that includes the use of APOE2 to balance out the APOE4 gene (Rosenberg et al., 2018).

A team of researchers at Tel Aviv University wanted to knock out the APOE4 gene completely so they designed a novel "clustered regularly interspaced short palindromic repeats"(CRISPR) based system that targets the APOE4 gene (Rabinowitz et al., 2019). The procedure was done on mice astrocytes that exhibited both the APOE3 and APOE4 traits, but there was only a significant decline in the APOE4 gene while the APOE3 gene was unchanged. This meant that the CRISPR system created did successfully target the APOE4 gene and was able to decrease the levels of the gene by about 70% allowing for the possibility of slowing down AD progression through decrease of APOE4 (Rabinowitz et al., 2019).

Of the available studies, one was focused on the safety and efficiency of the administration of APOE2 (protective gene) while the other investigated whether the knockout of APOE4 (dangerous gene) was possible; both focusing on how populations exhibiting an APOE gene were affected from the gene therapy methods used. Both methods had successful results, but it still is just the beginning of discoveries involving the APOE gene. APOE4 remains the strongest genetic risk factor for AD while APOE2 is the strongest protective factor and implementing these strategies of knockout of APOE4 or administration of APOE2 could prove to be an effective method in gene therapy for AD (Raulin et al., 2022). In addition to APOE's involvement in AD pathology, it is considered useful in gene therapies across the world as it is a common gene found in humans. Specifically, APOE3 is the most frequently occurring within humans out of all genes related to the AD pathology, especially in ethnicities that have a history in agricultural economies. APOE4 is an ancestral allele and is more frequent in smaller populations that still participate in ways of foraging (Corbo et al., 2003). Examples include Pygmies, Khoi, aborigines of Malaysia and Australia, Papuans, some Native Americans, and Lapps. The APOE2 allele is the least frequent among the three alleles and is absent in Native Americans. APOE is not an in-depth gene that has been tested and researched, but it is a gene that can provide future success in the prevention of AD (Corbo et al., 2003). The different APOE gene's presence among varying ethnicities provides information for the types of gene therapies that need to be implemented among those communities. Although one type of gene therapy may work for a certain ethnicity, it may not have the same effects on another ethnicity due to differences in genetic backgrounds. For example, the administration of APOE2 gene could work for the Malaysia population because it can act as a protective agent against APOE4, but it may not work for the Native Americans because there is no prior APOE2 gene present within their genetic background, so the effects of the APOE2 gene may not have the intended outcome. The information of the genetic background of an ethnic group is important to consider to ensure gene therapies have the best outcome for AD patients from diverse backgrounds.

CD33

AD is still considered a fairly new research topic in the medical world. As more research is done and more clinical trials are performed, the AD pathology continues to grow. Recently, insights from multiple genome-wide association studies (GWAS) have led to the discovery of a new factor related to the AD pathology. Cluster of differentiation 33 (CD33) is a transmembrane protein involved in cell-to-cell interaction within the body. CD33 is mainly associated with leukemia but has made an appearance within the AD pathology (Jiang et al., 2014). CD33 has been found on microglial cells (immune cells of the central nervous system) as a transmembrane protein. Microglial cells are involved with Aβ clearance within the brain, however, CD33 inhibits this function of the cells, causing constant production of Aβ leading to Aβ plaques. This new evidence reveals that the inability to clear out Aβ rather than overproducing Aβ is causing Aβ plaque production. Because CD33 is mainly associated with LOAD (late-onset AD), the genome of LOAD was researched (Jiang et al., 2014). Within LOAD are single-nucleotide polymorphisms (SNP) which are a type of genetic variation where one nucleotide can replace another nucleotide in a DNA strand. SNPs often occur in every 1,000 nucleotides in a strand of DNA and each variation is classified in 1% of the population. These variants act as biomarkers for genes that play a role in a certain disease. With the variation, scientists can determine how likely

a patient is to develop a disease, the genetic risk of developing a disease, an individual's response to certain medications, and a few other health-related factors. SNPs are commonly studied in connection to LOAD because of their genetic risk. Two SNP types (rs3865444 and rs3826656) have been detected as markers of LOAD and are associated with the increase and decrease of CD33 in microglial cells (Jiang et al., 2014).

These two SNPs have been found to have different effects on populations with different ethnic backgrounds. For rs3865444, the minor (T) allele of this SNP has been identified within Caucasian populations and has shown protective effects against AD while in the Han Chinese population, the minor (C) allele proves to be a risk factor for AD. In fact, the minor (T) allele is found in 30% of the Caucasian population while only being found in 17% of the Han Chinese population. On the other hand, rs3826656's minor allele has been found to decrease the risk of AD in the Han Chinese population but increase its risk in the Caucasian population (Jiang et al., 2014). In addition to the two SNPs discovered, there has also been another SNP discovered which is found within African American populations. The SNP rs114282264 is found within the CD33 protein itself and is significantly associated with African Americans with AD. These studies show how there are genetic variations between populations which can affect how a treatment works for each population (Jiang et al., 2014). Scientists trying to find a treatment for CD33 have to consider these conditions for a safer and more effective outcome for CD33 genetic therapies.

CD33 is different from the other genes discussed in this passage considering how NGF and APOE correlate with excessive Aβ production causing the creation of Aβ plaques while CD33 inhibits degradation of Aβ. A study conducted where CD33 was inhibited showed the levels of Aβ being produced from the APP(amyloid precursor protein) did not change compared to when CD33 was active; rather the breakdown of Aβ increased. This allowed scientists to conclude that CD33 was not causing excess production of Aβ, but it was specifically preventing microglial cells from breaking down Aβ, so Aβ was being produced continuously creating Aβ plaques (Jiang et al., 2014). The fact that CD33 inhibits the breakdown of Aβ changes how scientists approach gene therapies involving CD33. They can not only focus on the removal of CD33, but also need to focus on making sure excessive Aβ accumulation does not occur after CD33 is removed (Jiang et al., 2014). The two studies discussed in this section both focus on the knockout of CD33 but with different methods. While CD33 is still a new gene being tested in the AD pathology, it has opened up new avenues for finding more solutions for AD.

In a study performed in the Department of Neurology in Massachusetts General Hospital, an adeno-associated virus(AAV) was used to inject an artificial microRNA (amiR) to target and knockout CD33 to see if there would be a decrease in Aβ accumulation. AAVs have started to become a common *in vivo* method for gene therapies as it is able to pass the blood-brain barrier. However, in this study, the scientists decided to use exosome-enveloped AAV (exo-AAV) because the exosomes allowed for a less risky integration of the virus, without it being attacked by antibodies (Griciuc et al., 2020). The amiR was encoded into the exo-AAV and injected into APP/PS1 mice(specific mice used for AD gene therapies). When the exo-AAV was injected, astrocytes and neurons were transduced into the brain as well as some microglial cells. The CD33 amiR was likely introduced into the brain by the transduced neurons which was then engulfed by the microglial cells where it targeted CD33. The results of the study showed a 30-36% reduction in CD33 within the brain of mice which relates to the decrease of Aβ accumulation. This suggests that the knockout of CD33 can help with decreasing Aβ plaques being produced (Griciuc et al., 2020)

In Bonn, Germany at Bonn Medical Faculty and University found evidence that the knockout of CD33 could decrease Aβ accumulation but also showed that the knockout of CD33 could result in negative effects of oxidative bursts(can cause damage to tissue in the brain) and inflammation in the brain. To obtain evidence of this, the scientists performing the study used THP1 cells for the knockout of CD33. THP1 cells imitate monocytes, which then can be differentiated into macrophages (Wißfeld et al., 2021). The THP1 cells generated by the Cas9 genome editing system were integrated into exon 3 of CD33, which is where part of the CD33 protein coding is located. TPH1 deleted 4 of the nucleotides within exon 3, resulting in the knockout of CD33. The results provided evidence that knocking out CD33 could lead to dangerous oxidative bursts and inflammation. This evidence shows that knockout CD33, while helpful, still needs to be tested more thoroughly to help develop ways to knockout the protein without resulting in damage to the brain (Wißfeld et al., 2021).

Both experiments showed that the knockout of CD33 helped with decreasing production of Aβ plaques which is closer than the other discussed gene therapies have gotten. While it could have negative effects, this could lead to more experiments on making the knockout of CD33 safer. Since CD33 is a protein that can also be found in multiple ethnicities as well, it can be helpful in treating AD in low-middle income countries. All types of gene therapies are expensive, but they are not all an extensive process. CD33 knockout uses an *in vivo* method, so the removal of tissues isn't necessary to be altered. Since CD33 is a newer discovery it can use previous methods in other gene therapies to be implemented which it already has. Using AAV to deliver an amiR against CD33 was similar to how a study for APOE used AAV methods to deliver APOE2 past the blood-brain barrier. The clinical studies involving knockout of CD33 have shown promising results and have opened up opportunities of further research for solutions for AD in the future.

Discussion

This review of clinical application of different gene therapies can impact how gene therapy for AD in low- and middle-income countries can be implemented. There are not many studies that can be found on AD in lower income countries that can be used to see what therapies may be effective; however, by understanding the limitations of gene therapy within lower to middle-income countries and effects of gene therapies in higher-income countries, these therapies could be made effective in lower to middle-income countries. The purpose of this paper was to evaluate therapies for their monetary value and resource costs as well as their worldwide relevance. So far, gene therapies that involve the APOE gene being knocked-out of or integrated in the brain have shown to be the most relevant universally since APOE is the most common gene found among humans. In addition, the gene is especially relevant for lower to middle-income countries since populations in these countries tend to express this gene more than the NGF or CD33 genes. Since APOE is such a common gene, using methods from recent, successful gene therapies can make these therapies more accessible and useful to lower to middle-income countries. While APOE is the most common gene, knockout of CD33 and integration of NGF are still promising pathways for gene therapy. Both these genes can be found in a decent amount of humans worldwide and can help when APOE is not as prominent in certain populations such as Native Americans or Han Chinese. The use of gene therapy depends on a region's accessibility to resources as well as the prevalence of a certain gene, and in low to middle-income countries these factors vary greatly among different populations. This is why expanding gene therapy treatments to lower-middle income countries allows researchers to see how to slow down AD development in different ethnic backgrounds and will force them to find lower cost AD treatment options. Being able to accommodate these limitations and factors can take AD gene therapies one step closer to decreasing AD worldwide.

This article provides a summary of studies done in different countries worldwide. It shows the different resources used to perform the gene therapy as well as ways that gene therapy can be done. The factors of AD discussed affect different ethnicities differently, which adds to the limitations of how the gene therapy is performed. Gene therapy has evolved significantly since it was first developed, and these advances came with new resources to make the process easier. However, these newer resources, such as CRISPR-Cas9, tend to be expensive and affect how they can be implemented into low- and middle-income countries. Yet, as gene therapy develops further, new avenues can be tested to see what may work within the limitations that these low- and middle-income countries have. In general, gene therapy can provide a positive effect for AD patients. While AD cannot be tracked in a person when they are younger, genetic history and identification of certain genes within a young individual's brain can help prepare for supposed development of AD.

Now that multiple genes have been discovered that play a part in the AD pathology, more research has to be done on the alteration of those genes to see how they can first slow down AD development. Completely preventing AD has a low chance, considering the genetic strength of it, but if gene therapies can be developed to slow down AD, then that can lead to helping completely knockout genes related to AD to prevent the genetic map from continuing.

Including limitations that low- and middle-income countries have in these gene therapies can also help with reaching a solution for the majority of the AD population who are located within these countries.

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