

The Dana Foundation's "Design a Brain Experiment" Competition
2017 First Place

"The Use of CRISPR Technology to Test Gene Therapy as a Treatment to Early-Onset Familial Alzheimer's Disease in Zebrafish"

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Lay Summary (provided by the Dana Foundation):

Early-onset Familial Alzheimer's disease (FAD), which occurs in about five percent of all people with AD, develop the disease sometime between age 30 and 60. Usually FAD is caused by an inherited mutation in one of three genes. An infant born to a parent who carries the genetic mutation has a 50 percent chance of inheriting that mutation; an infant that inherits it has a strong likelihood of developing early-onset FAD. Any of several different single gene mutations on chromosomes 21, 14, and 1 causes abnormal protein formation. A mutation on chromosome 21 results in formation of abnormal amyloid precursor protein (APP), while a mutation on chromosome 14 or 1 produces abnormal presenilin 1 or presenilin 2, respectively. Each contributes to APP breakdown, generating accumulation of harmful amyloid plaques between brain cells that eventually disrupt communication from one cell to another.

Medha's experiment explores the potential of using an exciting new experimental form of gene therapy to treat FAD. To test this potential therapy, she uses the transgenic zebrafish model

which has “ortholog” genes *pzen1*, *pzen2*, and *appa* and *appb* that can be individually manipulated using this experimental gene therapy called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). CRISPR is a unique technology for editing parts of the genome by removing, adding, or altering sections of the DNA sequence. Using in situ hybridization, she is measuring whether CRISPR editing of these genes in the zebrafish has an effect on the accumulation of Amyloid β 42. Specifically, she is seeing whether CRISPR can correct for the gene that codes for the incorrect ϵ -cleavage of the amyloid precursor protein (APP). Based on previous research highlighting the effects of the presenilin 1, presenilin2, and *APP* genes in humans, she would see if the investigated mutations are corrected using CRISPR. If so, the production of $A\beta$ 42 caused by α -secretase and γ -secretase incorrectly cleaving APP should decrease to normal levels, decreasing the likelihood for FAD development.

She notes that if executed, findings regarding the posed question would be profound, as they would indicate whether continued research into gene therapy as a method of treatment for FAD is worth investing in and possibly implementing in humans.

Purpose:

This experiment attempts to explore the use of gene therapy to provide a possible form of treatment to Familial Alzheimer’s Disease (FAD). Using transgenic zebrafish as a model, the ortholog genes *pzen1*, *pzen2*, and *appa* and *appb* can be individually manipulated using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, and the effect on the accumulation of Amyloid β 42 ($A\beta$ 42) can be measured using an in situ hybridization, to determine whether gene therapy is an effective method of treatment for the inherited disease. If executed, findings regarding the posed question would be profound, as they would indicate whether continued research into gene therapy as a method of treatment for Familial Alzheimer's Disease is worth investing in and possibly implementing in humans.

Hypothesis:

The general theory being tested is whether the genetic manipulation of known genes that lead to the development of early-onset Alzheimer’s Disease works to effectively combat the inherited disorder. This theory can be executed by correcting the production of the $A\beta$ 42 by

editing the gene that codes for the incorrect ϵ -cleavage of the amyloid precursor protein (APP). Based on previous research highlighting the effects of the genes *PRESENILIN 1 (PSEN1)*, *PRESENILIN 2 (PSEN2)*, and *AMYLOID BETA A4 PRECURSOR PROTEIN (APP)* in humans, if the investigated mutations are corrected using CRISPR, the production of A β 42 caused by α -secretase and γ -secretase incorrectly cleaving APP should decrease to normal levels, decreasing the likelihood for the development of Alzheimer's Disease. If this proves to be true, this experiment will demonstrate the effectiveness of gene therapy treatment of Familial Alzheimer's Disease.

Background:

Familial Alzheimer's Disease, also known as early-onset Alzheimer's Disease, is an inherited form of dementia that affects memory retention and develops in humans as early as 30 years of age. Studies have shown that this disease is caused by the inheritance of rare mutations in one of three genes: *PRESENILIN 1 (PSEN1)*, *PRESENILIN 2 (PSEN2)*, and *AMYLOID BETA A4 PRECURSOR PROTEIN (APP)* (Guerreiro and Lahiri). Mutations in these genes result in coding for the incorrect ϵ -cleavage during post-translational modification of the amyloid precursor protein by α -secretase and γ -secretase. This then leads to the overproduction and accumulation of Amyloid β 42 in the brain, a peptide that is toxic to neurons and results in memory failure (Chen). Specifically, the mutations that take place involve a whole gene deletion of exon 9 in *PSEN1*, a missense mutation in *PSEN2*, and a partial or whole gene duplication resulting in varying sequences of exons 16 and 17 in *APP* (Bird).

These mutations can be combated with a growing area of study known as gene therapy. The recent development of CRISPR technology allows for specific genes to be manipulated within a target cell by activating the synthesis of a particular protein and altering or deleting the incorrect base pair sequences during transcription of the mRNA template. Using this method, gene therapy can be performed by correcting the mutations within the *PSEN1*, *PSEN2*, and *APP* genes so that they no longer code for an incorrect cleavage within the APP and incite

overproduction of A β 42. In other words, this genetic manipulation can potentially result in the prevention of Familial Alzheimer's Disease ("CRISPR/Cas9 Guide").

However, before this genetic manipulation can be tested in humans, its competence must be proven elsewhere. Though not the ideal vehicles for mimicking human systems, zebrafish provide an incredible model with which the effect of gene therapy can be tested. These vertebrates contain previously identified genes orthologous to those whose mutations have been determined to result in Familial Alzheimer's Disease (Newman, Ebrahimie, and Lardelli). Using a transgenic line that already contains these similar mutations within the orthologous genes of *psen1*, *psen2*, and *appa* and *appb* as well as *CRISPR*, the mutations can be corrected, and the effects on the accumulation of A β 42 can then be tested using an in situ hybridization with the ³³P-labeled oligonucleotide probe (Abramowski). Because zebrafish are a clear model, they can then be fixed and assayed after about 7 dpf for the amount of A β 42 that appears to be present in the brain (Newman, Ebrahimie, and Lardelli). Overall, the competence of gene therapy to combat inherited Alzheimer's disease can be tested and demonstrated using the zebrafish model.

The scientific research showing that the mutated *PSEN1*, *PSEN2*, and *APA* genes in humans lead to the accumulation of A β 42 provides evidence that using CRISPR technology to correct these mutations in the orthologous genes of zebrafish will result in a decrease in accumulation of A β 42 within the brain, and therefore prevent early-onset Alzheimer's Disease.

Methodology:

1. Obtain three transgenic lines containing mutations in the *psen1*, *psen2*, and *appa* and *appb* genes which are already tagged using green fluorescent protein.
2. Set up crosses between affected female and male fish of each line. Because the mutation is autosomal dominant, about 75% of the offspring will be affected by the mutation.
3. At .2 hpf, the embryos will enter the single cell stage. Divide the offspring from each cross in half, and inject one half of each with the specifically programmed CRISPR to correct the correlating mutation.

4. At 24 hpf, the expression of the genes will be present, and the mutated genes will result in an expression that should be tagged with fluorescence. Look for fluorescence in both the genetically manipulated and unmanipulated zebrafish. If fluorescence is found in a majority of the manipulated fish, this indicates that the genetic manipulation did not work.
5. Using Mendelian genetics, count the number of non-fluorescent, unmanipulated fish and predict the number of manipulated fish that were not originally mutated. Account for this in the final assay of the fish.
6. Allow for the fish to grow to about 7 dpf monitoring growth daily and recording the number of fish lost in the process. In addition, observe and compare early brain development specifically paying attention to the developing hindbrain where the accumulation of A β 42 is likely to occur. Record any differences between the manipulated and unmanipulated fish as well as the mutated and non-mutated fish from the half of each cross that did not undergo genetic manipulation.
7. At 7 dpf, fix the fish from each cross - both manipulated and unmanipulated.
8. Perform an in situ hybridization using the ³³P-labeled oligonucleotide probe.
9. Perform a brain dissection of each fish, and analyze the amount of A β 42 present. This should be marked by the stain of the in situ hybridization.
10. Image each dissection and compare the amount of A β 42 present within the non-mutated, mutated but unmanipulated, and genetically manipulated fish of each cross.

Results:

Comparing the in situ hybridization results of the brains of the non-mutated, non-mutated but unmanipulated, and genetically manipulated fish, the effect of the gene therapy on the amount of A β 42 in the zebrafish brain can be assessed. As it is hypothesized that the genetic manipulation using CRISPR will return the production of A β 42 to normal rates, these results would show that the gene therapy worked effectively. If the assay of the in situ hybridization yields an expression of A β 42 similar to the non-mutated fish, the hypothesis will

not be rejected. However, if the in situ hybridization yields an expression of A β 42 similar to the mutated fish, the hypothesis will be rejected.

Conclusion:

If the hypothesis is proven correct, this would result in numerous implications, including that growing research in the field of gene therapy should continue to be explored. Most importantly, however, it would imply that genetic manipulation is effective in combating inherited neurological disorders. To date, gene therapy has only been significantly experimented on in regards to combating immune deficiencies and inherited blindness. However, if gene therapy worked to combat Familial Alzheimer's Disease, genetic manipulation in regards to other inherited neurological disorders could be further explored and eventually put into practice.

Several neurological conditions are known to be hereditary, such as epilepsy and Parkinson's disease. However the specific genes that link these disorders between generations have not been accurately identified, and the likelihood that several mutations lead to these diseases has prevented conclusive results. With the knowledge of how these neurological conditions are inherited, experiments to combat them using gene therapy can also be performed, and further research can be developed in this area.

In addition, if the hypothesis is not rejected, further research in the use of gene therapy to treat Familial Alzheimer's Disease would need to be executed before any solid conclusion could be drawn. And if the method of treatment was determined to be effective, the research would eventually be moved to a mouse model to test for competence in mammalian organisms before being tested on humans.

However, if the hypothesis is rejected, this may indicate that either Familial Alzheimer's Disease does not cohere with gene therapy or that gene therapy does not cohere with inherited neurological disorders. If the latter is true, this may imply that the development of inherited neurological disorders, such as Familial Alzheimer's Disease, is not affected by genetics alone. The brain is known to be affected by both environmental and genetic factors, and behavior can

be a result of both acquired and inherited traits. While the hereditary effects of this disease on memory retention cannot be disputed, perhaps there are other unconsidered factors that distinguish when and how the disease will initially come into effect. Therefore, if the treatment of gene therapy on neurological disorders is rejected, this may indicate that the development of even those neurological disorders, even those that are inherited, is impacted by environmental factors.

Furthermore, if this experiment is put into action, and development in the study of gene therapy is executed and proven effective, there would still be several ethical implications behind moving to human trials. Because of the negative stigmatism surrounding mental illness and neurological disorders, using gene therapy to treat these conditions needs to be handled carefully if put into effect. While any method of treatment behind these illnesses is important, genetic manipulation comes with the ethical consequences of its use for purposes other than the combat of disease, such as eugenics. A moral controversy, eugenics must be avoided because the inherent human nature to discriminate combined with the ability to edit the human genome could potentially lead to the attempt to create a superior race. In early stages however, gene therapy and its coherence with neurological disorders is worth exploring, especially as biotechnology developments allow for more specific ways to edit genomes, and combat inherited diseases; this area of research may provide hope for many.

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