Treating and Reducing Risk of Developing Alzheimer's Disease with AAV-mediated CRISPR Engineering of the TREM2 Gene in Microglia

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Abstract

Alzheimer's Disease is a neurodegenerative disorder caused by beta-amyloid buildup, associated with loss of memory, judgement, and the ability to function, especially in seniors above the age of 65. Though many genetic factors have been found to influence the development of early-onset Alzheimer's, these factors have not been found to have the same effect in late-onset Alzheimer's disease, leading to greater difficulty in developing general treatments. However, the TREM2 gene has recently been determined to have significant impacts on the development of both early-onset and late-onset Alzheimer's disease, with mutations causing overactivation of microglial cells by beta-amyloid which results in neuroinflammation. As such, this study analyzes the possibility of treating Alzheimer's disease with an adeno-associated virus 6 (AAV6) vector-mediated base-editing approach to target TREM2 variants. This is predicted to result in reduced neuroinflammation and increased beta-amyloid clearance, slowing the progression of Alzheimer's.

Keywords: Alzheimer's disease, beta-amyloid, genetic, early-onset, late-onset, TREM2, mutation, microglia, neuroinflammation, adeno-associated virus, base-editing.

Introduction

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by dementia, a progressive loss of memory, judgement, and the ability to function [1]. 5.8 million Americans above the age of 65 suffer from the disease, comprising about 11 percent of the 43 million Americans above 65 today [2, 3]. In 2018 alone, AD accounted for 122, 019 deaths, making it the sixth leading cause of death in the United States and the fifth leading cause of death among Americans 65 and older [2].

Numerous genetic, pathological, and functional studies have shown that the accumulation and aggregation of amyloid-beta (Aβ) peptides, caused by an imbalance in production and clearance, is strongly associated with the development and progression of AD [4]. This toxic Aβ accumulates in the form of Aβ oligomers, intraneuronal Aβ, and amyloid plaques that all injure synapses and eventually cause neurodegeneration and dementia [4]. The toxicity of Aβ has also been observed to depend on the presence of the hyperphosphorylated neurofibrillary tangles of microtubule-associated tau protein encoded by the *MAPT* gene [4]. Lastly, another neuropathological hallmark of AD is microgliopathy, which describes reactive gliosis, microglia dysfunction, and neuroinflammation [5].

According to the amyloid cascade hypothesis for AD development (Figure 1), Aβ forms upon cleavage of the larger amyloid precursor protein (APP) [4], which is thought to bind to proteins on the cell surface or help cells attach to one another [6]. In the brain, the protein is thought to assist with the processes that direct the movement of neurons during the early stages of development [6]. When the APP proteins are cleaved by enzymes, multiple small fragments

are created, including the Aβ peptide, which is believed to be involved with neural plasticity [6]. When the APP protein is cleaved incorrectly, a variant of the Aβ protein known as Aβ42 is often formed. This variant is stickier than the general forms of Aβ peptide and contributes to the Aβ peptide deposits characteristic of AD.

Because correct Aβ production depends heavily on the proper cleavage of APP, mutations in the *APP, PSEN1,* and *PSEN2* genes are identified as possible key drivers for the development of AD, especially in early-onset AD. Mutations in the *APP* gene, which codes for the amyloid precursor protein, are responsible for less than 10 percent of all early-onset cases of AD [6]. The *PSEN1* gene codes for the presenilin 1 protein, which acts as the cleaving subunit on the gamma- (γ-) secretase complex, which is responsible for cleaving other proteins into smaller peptides via proteolysis [7]. The γ-secretase complex is known best for its involvement in cleaving the amyloid precursor protein into smaller

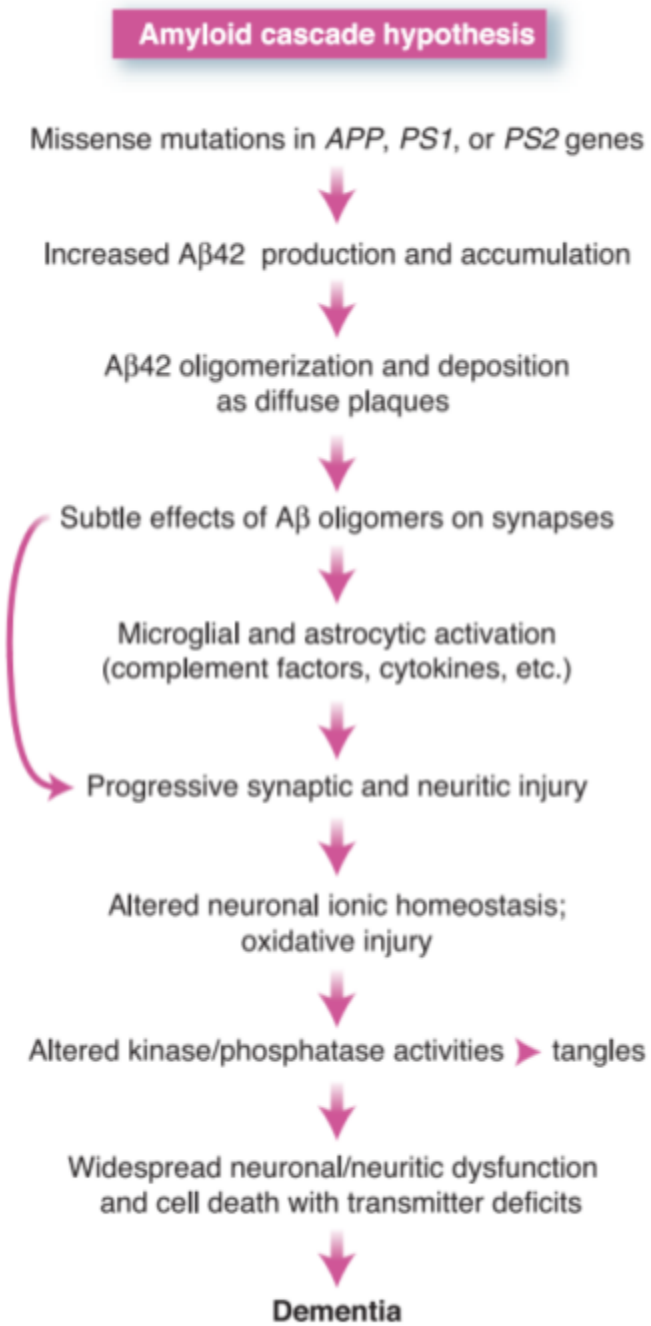

Fig. 1. The sequence of pathogenic events leading to AD proposed by the amyloid cascade hypothesis [7].

peptides including Aβ peptides [8]. Mutations in the *PSEN1* gene are the most common cause of early-onset AD, accounting for over 70 percent of all cases, caused by the improper processing of the APP protein of Aβ leading to the overproduction of a longer, more toxic, and stickier version that aggregate into amyloid plaques [8]. The last key mutation is thought to occur in the *PSEN2* gene, which codes for presenilin 2 [9]. Presenilin 2 combines with other enzymes and helps to process proteins that transmit chemical signals from the cell membrane to the nucleus, including APP [8]. Mutations in the *PSEN2* gene, however, are responsible for less than 5 percent of all early-onset cases of AD [9].

However, these genes have only been linked to early-onset AD. With sporadic, or late-onset AD (LOAD), no clear genetic link has yet been found. However, researchers have found a connection between the *APOE* gene, and the development of AD. *APOE* codes for the apolipoprotein E, a major cholesterol carrier that supports lipid transport and injury repair in the brain [4]. The gene has three main alleles (E2, E3, and E4), of which the E4 allele is the strongest risk factor for both early-onset and LOAD [4]. The frequency of AD is 91% for E4 homozygotes, 47% for E4 heterozygotes, and just 20% in E4 non-carriers, indicating that E4 dramatically increases the risk of developing AD [4]. However, it is important to note that the E4 allele is only linked to an increased risk of developing AD, and does not guarantee the disease itself [10]. Furthermore, this link can only be observed within patients suffering from LOAD and is not as significant in early-onset patients.

Because of the links between the four genes identified above and the development of AD, research has largely focused on determining the functions and effects of these genes, and developing therapeutics to lower their effect. However, another less-studied gene known as

TREM2 has also been linked with AD, specifically contributing to the microgliopathy, and may be a strong therapeutic candidate for treating Alzheimer's.

TREM2 Structure, Function, and Association with Alzheimer's Disease

TREM2 belongs to a family of receptors known as triggering receptors expressed on myeloid cells (TREM), and codes for a 230 amino acid glycoprotein [11]. Members of this class are cell-surface transmembrane glycoproteins with V-immunoglobulin extra-cellular domains and cytoplasmic tails [11]. The gene is located on human chromosome 6p21 and is expressed in a subgroup of myeloid cells including dendritic cells, granulocytes, and macrophages, and in the brain, it is exclusively expressed by the microglial cells [11]. The level of expression in the microglia is dependent on the region of the central nervous system (CNS), with higher expression in the hippocampus, the spinal cord, and the white matter [11]. The expression of the gene is also modulated by inflammation, where the expression of anti-inflammatory molecules enhances *TREM2* while the expression of pro-inflammatory molecules (like TNFα, IL1β, or lipopolysaccharide (LPS)) decreases *TREM2* expression [11]. The TREM2 receptor acts mainly via the intracellular adapter DAP12 and is incapable of initiating intracellular signalling without it [11]. Upon ligation of the receptor, DAP12 undergoes tyrosine phosphorylation within its immunoreceptor tyrosine-based activation motifs (ITAMs) by Src family kinases (usually the Syk kinase), providing a connection site for SH2 domains of molecules that initiate a signalling cascade and immune response [11].

TREM2 recognizes a wide variety of ligands, including LPS or lipoteichoic acids (LTA), lipoproteins (high-density and low-density), and many versions of apolipoproteins, including, most significantly, ApoE and clusterin, both of which are critical genetic risk factors for

LOAD[11]. Additionally, Aβ was also characterized as a ligand for TREM2 and was found to be capable of binding directly to the receptor and activate TREM2 signalling [11]. Upon recognition of this range of ligands, TREM2 carries out several functions in cells, including an increase in phagocytosis [11]. *In vitro* experiments found that inhibition of *TREM2* in microglia and macrophages resulted in decreased phagocytosis of apoptotic neurons, cellular debris, including Aβ, and bacterial products, while *TREM2* expression increases the phagocytotic rates [11]. TREM2 was also found to modulate inflammatory signalling in cells [11]. In microglia, knockdown of TREM2 signalling increased TNFα cytokine and NO synthetase-2 transcription and mediates the switch from a homeostatic to a neurodegenerative microglia phenotype [11]. Lastly, TREM2 has also been shown to modulate myeloid cell number, proliferation, and survival, and has been observed to enhance the proliferation of several myeloid cell populations including microglia [11].

Whole-genome sequencing and genome-wide association studies have identified several rare *TREM2* variants that have been associated with increased risk of AD in European, African American, and Asian populations [5, 11]. The most commonly studied of these variants is R47H (rs75932628), which expresses a single nucleotide polymorphism (SNP) that encodes an arginine-to-histidine missense substitution in amino acid 47, which increases the risk for LOAD 3-fold, comparable to the E4 allele mutations in APOE [11, 12]. Numerous other variants have also been identified, including R62H (rs143332484), D87N (rs142232675), T96K (rs2234353), L211P (rs2234256), and R136Q (rs149622783) [11]. Most studies determined that the SNPs responsible for these variants did not affect the folding, expression, stability, or structure of the TREM2 receptors, but the variants do seem to affect the receptor's affinity for its ligands [11].

Parhizkar et al. determined that the absence of functional TREM2 increasing amyloid seeding, accompanied by decreased microglial clustering around newly seeded plaques, suggesting that early amyloidogenesis is accelerated because of reduced phagocytic clearance of amyloid deposits [13]. The lack of microglial clustering around plaques was associated with defects in plaque compaction, microglia proliferation, and increased levels of dystrophic neurons, while microglia lacking TREM2 exhibited strong metabolic defects, including low ATP levels and elevated stress markers such as autophagic vesicles [11]. This was identified to be caused by defective mammalian Target of Rapamycin (mTOR) signalling, suggesting that TREM2 provides trophic support to microglial cells during stress [11]. Lee et al. demonstrated that both soluble and insoluble $A\beta_{42}$ were significantly decreased in mice expressing the human TREM2 (hTREM2) transcripts, and showed that *TREM2* expression can ameliorate Aβ pathology in 5xFAD mice [14]. Thus, the *TREM2* variants that have been identified present a significant risk for the development of AD, and must be targeted to decrease susceptibility to LOAD and slow the development of AD.

Proposal

Upon analyzing the effects of TREM2 variants on the progression of AD, and the effects of increasing TREM2 expression in $A\beta_{42}$ deposition, it can be inferred that editing the *TREM2* gene to express the normal variant can significantly reduce the risk of developing AD. To achieve this, a CRISPR-Cas9 base-editing experiment can be carried out to replace the non-functioning variant with a functioning variant.

CRISPR editing - Base Editing

The clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) system, initially a bacterial defense mechanism against lysogenic bacteriophage infections and plasmid transfer, has been repurposed and utilized as a versatile RNA-guided DNA targeting platform, used for genome engineering, epigenetic modulation, and genome imaging [15]. Base editing is a gene-editing approach that uses components from the CRISPR system with other enzymes to edit point mutations in DNA, using donor templates or relying on cellular homology-directed repair (HDR) [16]. Instead, DNA base editors (BEs) comprise fusions between a catalytically impaired Cas nuclease and a base modification enzyme that operates on single-stranded DNA (ssDNA) [16]. Upon binding to the target gene locus, base pairing between the guide RNA (gRNA) molecule and DNA leads to a small displaced segment of ssDNA in an R-loop, where DNA bases are modified by the deaminase enzyme [16]. To further improve efficiency of the edit, the Cas enzyme also induces a nick in the non-edited strand to induce DNA repair on the non-edited strand using the edited strand as a template [16]. Two main classes of DNA base editors have been developed: cytosine base editors (CBEs), which convert a C-G pair into a T-A pair, and adenine base editors (ABEs), which convert an A-T pair into a G-C pair. CBEs do so by deaminating the exocyclic amine of the target cytosine nucleotide to generate a uracil nucleotide, which is read as a T by polymerases [16]. Similarly, ABEs deaminate adenosine, forming inosine in its place, which is read as a G by polymerases [16]. This methodology can be utilized to correct mutations in the *TREM2* gene from variants that increase susceptibility to the normal, functioning variants.

AAV-Vectors

Recombinant adeno-associated viruses (rAAV) are safe gene therapy vectors for both preclinical and clinical usage and have been widely used in gene therapy applications in central nervous system disorders like Parkinson's disease and Huntington's disease [17]. Studies have found that scCBA-GFP AAV6 vectors with the Y731F/Y705F/T492V (or the triple-mutant AAV6 or TM6) mutated capsid most efficiently transduced microglial cells [17]. Intracerebroventricular injections of the AAV6-TM6 vectors was observed to lead to extensive brain transduction and transgene expression [17]. Similarly, the base-editing machinery, along with the donor template DNA for functional *TREM2*, can be introduced into microglial cells with intracerebroventricular injections of the AAV-TM6 vectors.

Mouse Models of Alzheimer's Disease

In order to test the efficacy of the treatment, the experiments must be carried out on transgenic mouse models of AD with various *TREM2* nonfunctioning variants, including R47H. To observe effects of the treatment on the neuroinflammation, immunohistochemical (IHC) analysis can be performed on the mice. Furthermore, the enzyme-linked immunosorbent assay (ELISA) can be used to observe or measure beta-amyloid levels in the brain [18].

Expected Results

It is hypothesized that the base-editing approach to altering the *TREM2* variants from non-functioning versions to a functioning variant should limit neuroinflammation as a result of reduced microglial overactivation, while allowing for increased beta-amyloid clearance by the microglia, reducing neurodegeneration and slowing the onset of Alzheimer's Disease.

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