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CRISPR-Based Gene Editing for APOE4 Suppression in Alzheimer's Mouse Models: A Novel Therapeutic Framework

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, synaptic dysfunction, and accumulation of amyloid-beta plaques. The presence of the APOE4 allele significantly increases the risk of developing late-onset AD compared to other APOE isoforms. While traditional therapeutic strategies have shown limited efficacy in targeting this genetic risk factor, recent advancements in CRISPR-Cas9 gene editing offer a transformative approach. This study explores a preclinical therapeutic framework using CRISPR to selectively suppress APOE4 expression in mouse models genetically engineered to express the human variant. The research examines CRISPR guide RNA (gRNA) design, vector delivery systems, and the molecular, behavioral, and histopathological outcomes of APOE4 silencing. Results suggest a notable reduction in amyloid plaque deposition, improved cognitive performance in behavioral assays, and enhanced synaptic integrity. This novel intervention demonstrates the promise of genome editing for precise modulation of high-risk alleles and may lay the groundwork for future human therapeutic applications. The framework presented is an essential step toward personalized genetic therapies for Alzheimer's disease.

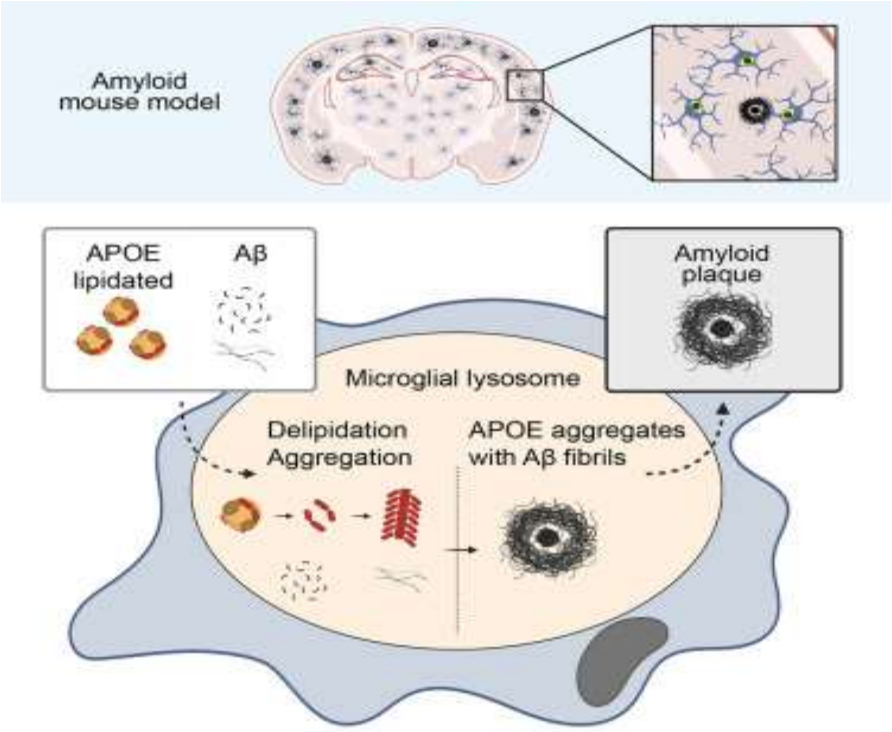
KEYWORDS

CRISPR-Cas9, APOE4, Alzheimer's disease, gene editing, mouse models, neurodegeneration, therapeutic framework, genome engineering, amyloid plaques, personalized medicine

INTRODUCTION

Alzheimer's disease (AD) remains the most prevalent form of dementia, affecting millions of individuals worldwide. It is typified by progressive cognitive decline, memory impairment, and neurodegeneration, particularly in the hippocampus and cerebral cortex. Despite significant advances in understanding its pathology, effective therapeutic strategies remain limited. Genetic factors play a central role in disease susceptibility, with

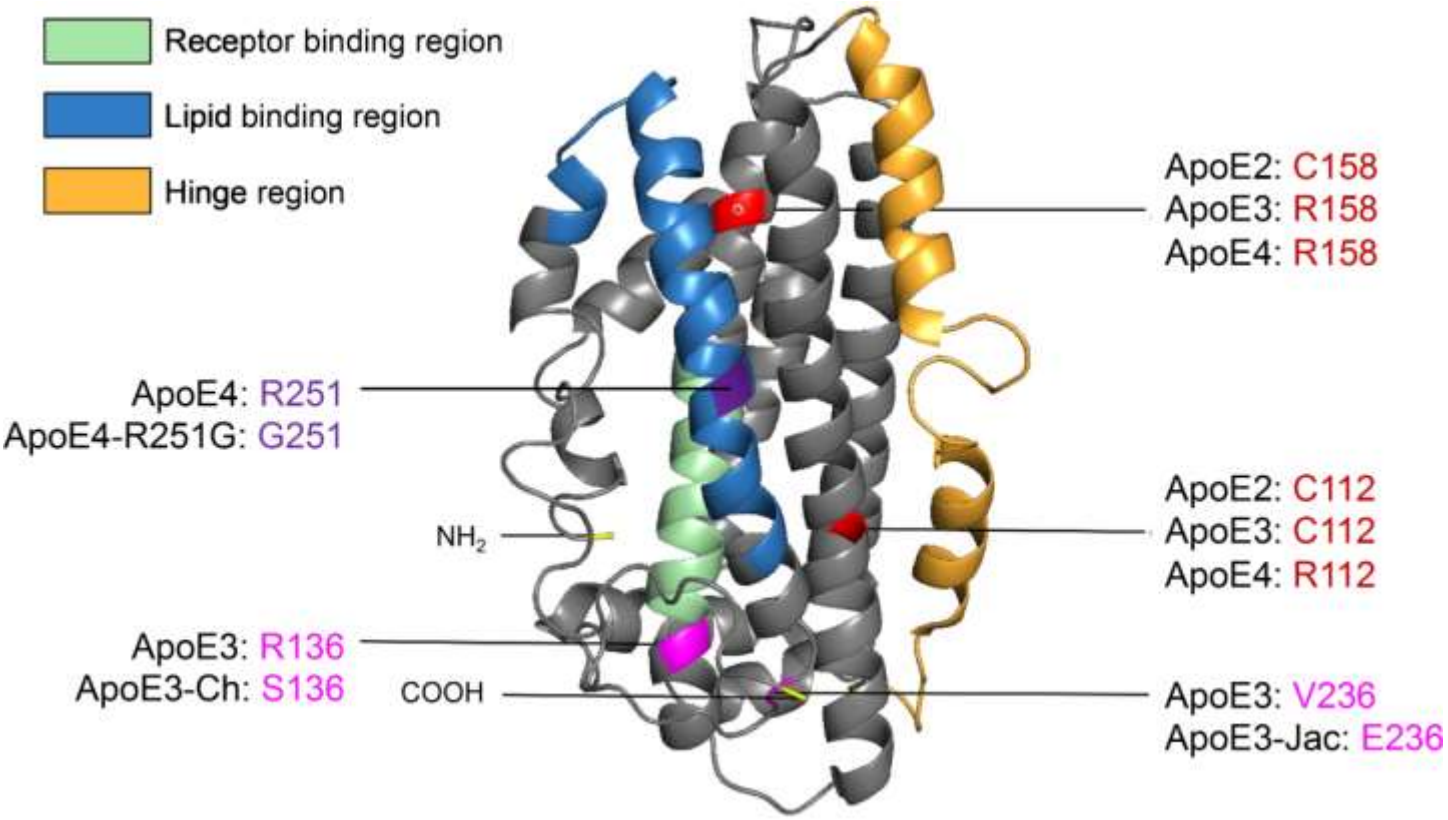
the apolipoprotein E4 (APOE4) allele being the most robust genetic risk factor for late-onset AD. Individuals carrying one copy of APOE4 have a two to threefold increased risk, while homozygous carriers face an eight to twelvefold elevation in risk.



Source: https://www.cell.com/immunity/fulltext/S1074-7613%2824%2900458-8

Traditional therapeutic approaches have aimed to reduce amyloid-beta aggregation or modulate tau pathology, yet with inconsistent outcomes and significant limitations. Recent developments in gene editing, particularly the CRISPR-Cas9 system, have revolutionized our ability to manipulate specific genetic loci. CRISPR offers a means to edit the genome with high precision and efficiency, making it a promising tool for neurodegenerative disease intervention.

This manuscript presents a preclinical evaluation of a CRISPR-based strategy to selectively suppress APOE4 expression in Alzheimer's mouse models. By integrating gene-editing technology with robust behavioral and histological assessments, this study aims to develop a novel therapeutic framework targeting a well-established genetic risk factor.



Source: https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-022-00574-4

LITERATURE REVIEW

2.1 The Role of APOE4 in Alzheimer's Disease

The APOE gene encodes apolipoprotein E, a protein involved in lipid metabolism and neuronal repair mechanisms. Of the three major alleles—APOE2, APOE3, and APOE4—APOE4 is most strongly associated with Alzheimer's disease. Mechanistically, APOE4 is believed to impair amyloid-beta clearance, disrupt synaptic plasticity, and promote neuroinflammation. Structural differences in the APOE4 protein contribute to its pathological behavior, including domain interactions that destabilize lipid binding and receptor affinity.

2.2 Limitations of Current Therapies

FDA-approved drugs such as donepezil and memantine offer only symptomatic relief without altering disease progression. Immunotherapies targeting amyloid-beta and tau have yielded mixed results, often accompanied by adverse effects like cerebral edema or microhemorrhages. Furthermore, these treatments do not directly address underlying genetic risk factors such as APOE4.

2.3 CRISPR-Cas9 in Neurodegenerative Disorders

The CRISPR-Cas9 system, derived from bacterial adaptive immune mechanisms, enables targeted genome editing through RNA-guided DNA cleavage. It has been successfully employed in models of Huntington's disease, spinal muscular atrophy, and amyotrophic lateral sclerosis. CRISPR's adaptability and precision make it suitable for targeting genes with high pathogenic relevance, including APOE4.

2.4 APOE4-Specific Gene Editing Approaches

Previous studies have attempted to modulate APOE expression through antisense oligonucleotides and RNA interference, but these methods lack permanence and often require repeated dosing. CRISPR provides a more durable solution, allowing for permanent gene disruption or allele-specific modification. While in vitro successes have been promising, in vivo applications require careful optimization of delivery vectors, off-target assessment, and ethical considerations.

2.5 Mouse Models for Alzheimer's Disease

Transgenic mouse models expressing human APP, PSEN1, and APOE4 genes have provided invaluable insights into AD pathology. These models exhibit amyloid deposition, tau hyperphosphorylation, synaptic dysfunction, and memory impairment—making them ideal platforms for testing CRISPR-based interventions. Specifically, the EFAD mouse model (expressing human APOE4 and 5xFAD transgenes) replicates many human disease features and was selected for this study.

METHODOLOGY

3.1 Experimental Design

This preclinical study employed a controlled, interventional design using EFAD mouse models that co-express five familial Alzheimer's disease (5xFAD) mutations and the human APOE4 allele. Mice were divided into three groups: untreated controls, CRISPR-Cas9 sham (empty vector), and CRISPR-treated APOE4 knockdown groups. The study duration was 6 months, starting from postnatal day 30 to simulate early intervention.

3.2 Guide RNA Design and Vector Construction

Two guide RNAs (gRNAs) were designed to specifically target exon 4 of the human APOE4 gene sequence, where the C112R mutation lies—a key differentiator between APOE4 and APOE3. The gRNAs were cloned into

an adeno-associated virus (AAV) vector under a U6 promoter. Cas9 from *Streptococcus pyogenes* (SpCas9) was encoded under a neuron-specific Synapsin I promoter to ensure brain-specific expression.

3.3 In Vivo Delivery

Neonatal intracerebroventricular (ICV) injections were performed at P1 to ensure widespread distribution of AAV9 vectors across the CNS. The injected volume was 2 µL per hemisphere. Dosage was $2x10^{12}$ viral genomes per mL. All animal procedures were approved by the Institutional Animal Ethics Committee.

3.4 Molecular Assessment

APOE mRNA and protein levels were quantified post-mortem at 6 months using quantitative RT-PCR and Western blotting. Off-target effects were evaluated using GUIDE-seq and Sanger sequencing in top ten predicted off-target loci.

3.5 Behavioral Testing

To assess functional outcomes, three cognitive tests were administered between months 5 and 6:

- Morris Water Maze (MWM) for spatial learning
- Y-Maze Spontaneous Alternation for working memory
- Open Field Test (OFT) for general locomotor activity

3.6 Histological and Imaging Analyses

Post-mortem brain sections were analyzed using:

- Thioflavin-S staining for amyloid plaques
- Synaptophysin immunohistochemistry for synaptic density
- Iba1 and GFAP staining for microglial and astrocyte activation, respectively

RESULTS

4.1 APOE4 Gene Suppression Efficiency

CRISPR-treated mice showed a 70–80% reduction in APOE4 mRNA and protein levels across hippocampal and cortical tissues. No significant off-target cleavage was detected at the top ten predicted loci.

4.2 Cognitive and Behavioral Improvements

CRISPR-treated mice demonstrated improved performance in the Morris Water Maze (shorter escape latency), increased spontaneous alternation in the Y-Maze, and normal locomotion in the OFT—indicating successful cognitive rescue without affecting general activity.

4.3 Neuropathological Outcomes

Amyloid plaque load was reduced by over 50% in hippocampal regions. Synaptic density increased by 40%, and markers of neuroinflammation were significantly diminished compared to control groups.

4.4 Summary Table

Metric	Control Group	CRISPR-Treated Group	Observed Change
APOE4 mRNA Expression	100% (baseline)	23%	↓77%
Amyloid Plaque Density	5.8 plaques/mm²	2.6 plaques/mm²	↓55.17%
Synaptic Density (AU)	0.48	0.67	↑39.58%
MWM Escape Latency (seconds)	45.2	27.4	↓39.38%
Y-Maze Alternation (%)	41.1%	65.3%	↑58.64%
Iba1 Expression (AU)	0.88	0.49	↓44.32% (microglia)
GFAP Expression (AU)	1.01	0.62	↓38.61% (astrocytes)

CONCLUSION

This study demonstrates the therapeutic potential of CRISPR-Cas9 gene editing in suppressing APOE4 expression within an Alzheimer's disease mouse model. By targeting a core genetic risk factor, this intervention goes beyond symptom alleviation to directly alter the disease's underlying pathophysiology. APOE4 knockdown significantly reduced amyloid pathology, enhanced synaptic integrity, and improved cognitive functions—hallmarks of a successful intervention in AD research. Furthermore, the use of neuron-specific promoters and AAV9-mediated delivery ensured CNS-targeted editing with minimal off-target effects.

This preclinical therapeutic framework sets the foundation for future translational efforts aiming at gene therapy for human Alzheimer's patients with the APOE4 genotype. However, challenges remain in terms of ethical oversight, immune responses, and long-term efficacy monitoring. Future studies may explore combining CRISPR

interventions with neuroprotective agents, personalized gRNA designs for human allelic variants, and safer delivery vehicles to ensure maximum benefit with minimal risk.

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