# Gene Therapy in Alzheimer's Disease: Novel Therapies and Ethical Aspects of Somatic Gene Editing

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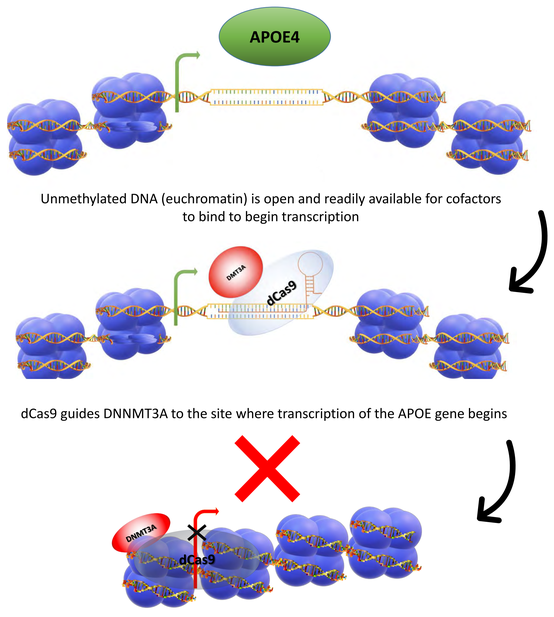
## Blocking APOE reduces risk of Alzheimer's

- Gene therapy can be delivered using viral vectors.
- APOE4 is the strongest, most reproducible risk factor associated with development of late-onset Alzheimer's Disease (LOAD).
- Overexpression of APOE4 protein leads to LOAD. By limiting gene expression via **transcription** (protein production), we hope to mitigate disease risk.
- DNMT3A (enzyme) acts as a repressor to block transcription.




## Adding DNMT3A increases methylation

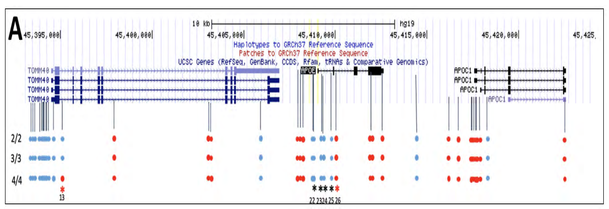



**Figure 1**. The APOE promoter has low levels of methylation, making DNMT3A a logical choice for an effective repressor.

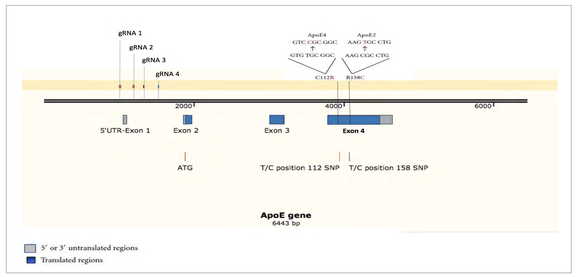



**Figure 2**. Plasmid design with small guide RNAs (sgRNAs) targeting different regions of the promoter, in order to see which is most effective in reducing protein production.

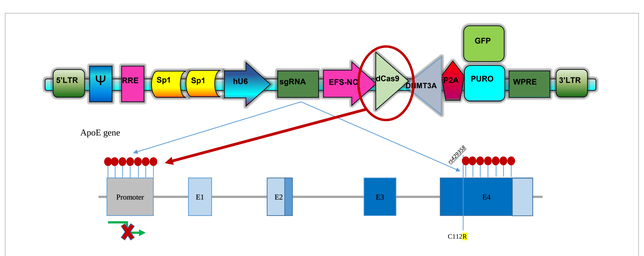



**Figure 3.** Lentirival vector with DNMT3A methylates the promoter, which stops production of APOE4.

## Successful downregulation blocks APOE4 production

**Figures 4-8**. Preliminary results from experiments display levels of APOE4 mRNA and protein in cells containing lentivirus with either active (red) or inactive (gray) DNMT3A, relative to unaltered HEPG2 cells (blue), without any virus. HEPG2 (liver) cells yield lots of APOE4 and are a cheap, simple model for proof of concept.

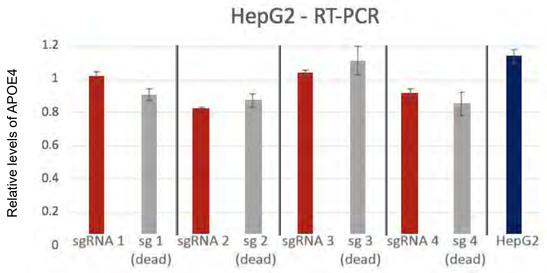
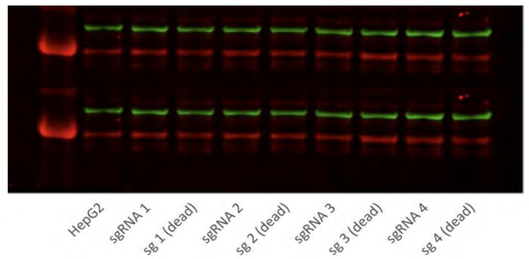
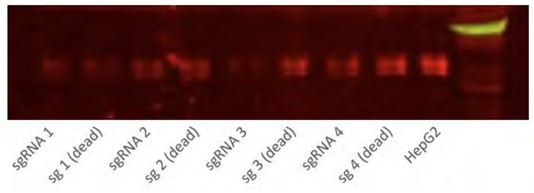
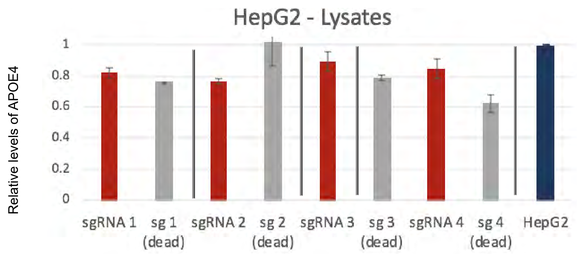
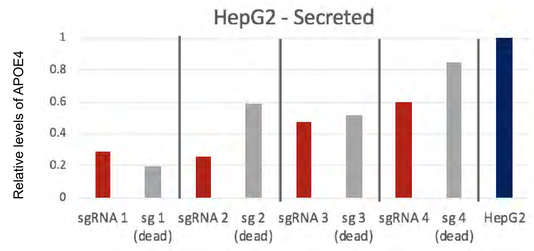



**Figure 4**. First, levels of mRNA (a protein intermediate) were measured to determine how much APOE4 was still being made, relative to unaltered HEPG2 cells, among cells containing one of four small guide RNAs (sgRNA) and DNMT3A. Active (red) and inactive (gray) forms of the enzyme were measured within each set of sgRNAs (separated by gray lines) to determine the effect of DNMT3A on transcription.




**Figures 5 and 6.** (Above) Western Blot shows APOE4 protein and HEPG2 in red. Green bands are a positive control (Actin) to measure relative protein production. (Below) After mixed results from the RT-PCR data, levels of APOE4 protein lysates (internal component) were compared against positive control HEPG2 cells across the same sets of sgRNAs.

**Figures 7 and 8.** (Above) Western Blot of secreted APOE4 in red. (Below) The experiment in Figure 6 was repeated with secreted APOE4. These results confirm our hypothesis and demonstrate successful downregulation APOE4 using DNMT3A. Cells carrying active forms of the repressing enzyme displayed lower levels of protein overall, limiting expression.

## Future Directions

- Assess extent of DNMT3A on repression → other factors may be at play (e.g. steric hindrance – the size of dCas9 alone may block cofactors from binding)
- Transition existing system to another method of delivery that is smaller and safer for human use (e.g. adeno-associated viral vectors [AAV])
- Test different ways to limit production of APOE4 aside from DNMT3A
- Repeat experiments using more complex model systems (near future -neural progenitor cells, long-term – neurons in culture)

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