Researching Alzheimer's the risk gene, APOE, Using the CRISPR/Cas9 Gene Editing System

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Leading the fight against dementia
Alzheimer's Society

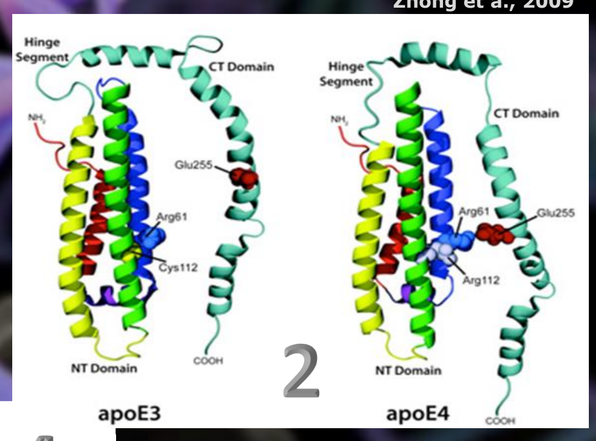
About the APOE Gene

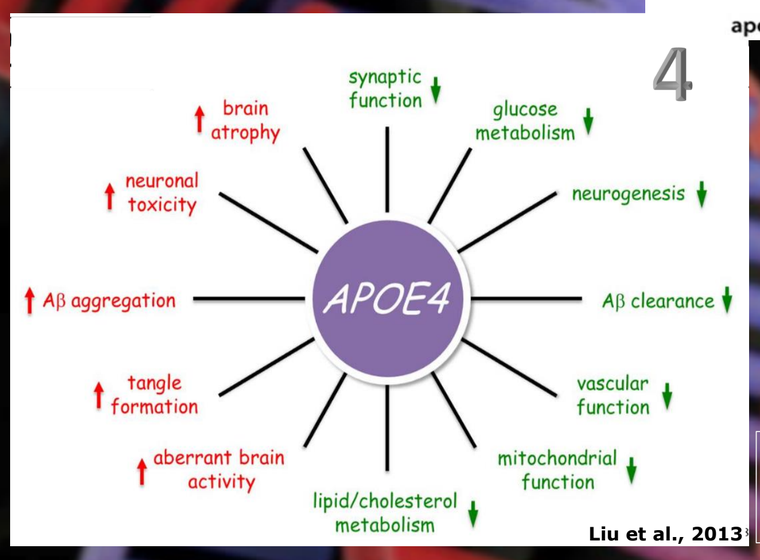
- APOE stands for Apolipoprotein E
- It is a gene of which humans have 2 copies
- A combination of 3 different versions. E2, E3 or E4
- ~25% have 1 (homozygous) or 2 (heterozygous) E4 copies (see fig. 1)
- Different versions → 1 amino acid difference. (SNP (singleNucleotidePolyorphism))
- Amino Acid difference → Different protein structure (see fig. 2)
- Different structure → Altered function
- Altered function → Increase AD risk (see fig. 3)
- E4 affects various biological functions (see fig. 4)

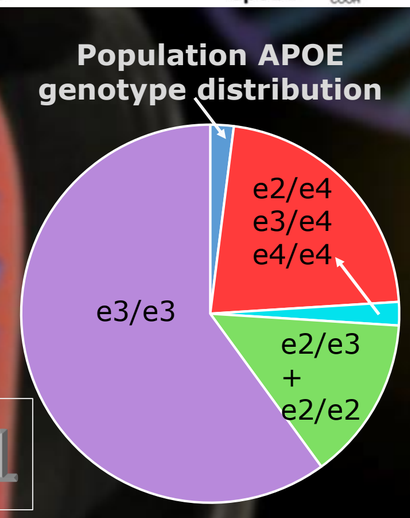
APOE4 is greatest Alzheimer's risk factor beside age

Risk translates to earlier onset and quicker decline

Zhong et a., 2009







Corder et al 1993

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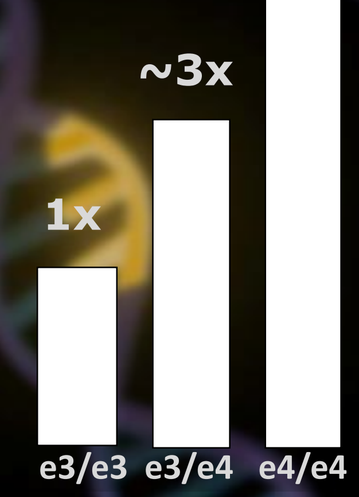
Increase risk for Dementia



The Experiments + What is CRISPR?

About this project

This research was part of the development toward an APOE-switch model (switching from APOE3 into APOE4). 'APOE-switching' in the same organism, at different time points may lead to answers.

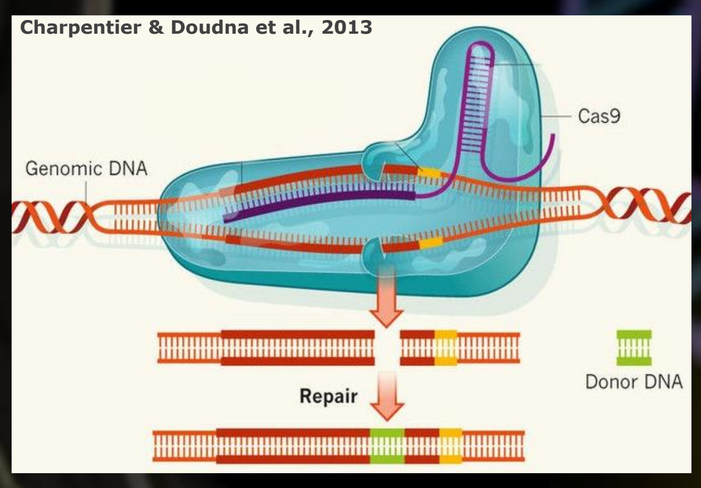
Research on APOE4 in Alzheimer's indicates it to be, not just a very critical, but also a promising therapeutic target.

Why and at what age does APOE4 become damaging or what may protect against this? Current research models are lacking.

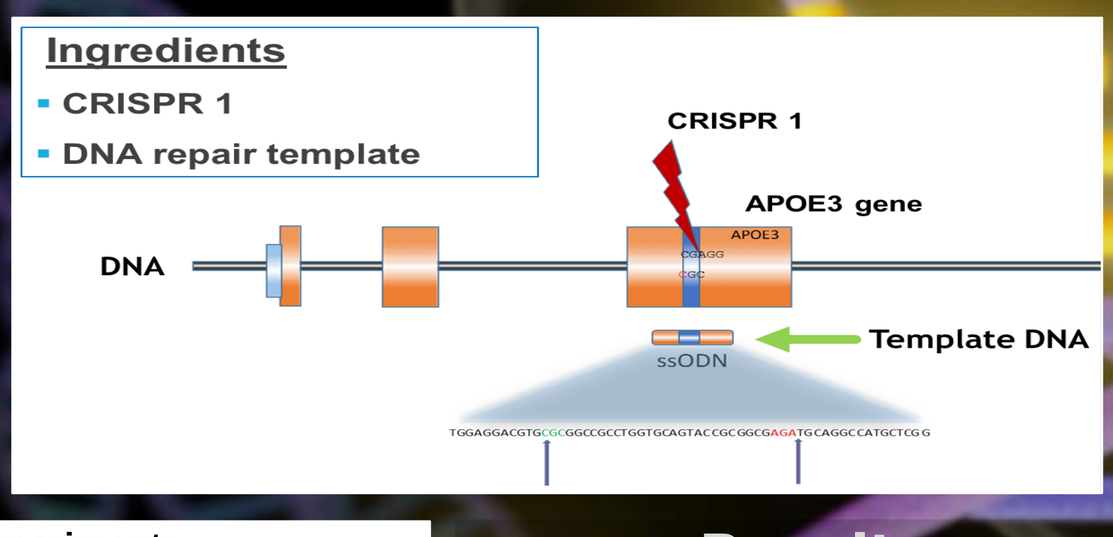
The CRISPR/CAS9 – Gene editing system

How does CRISPR work?

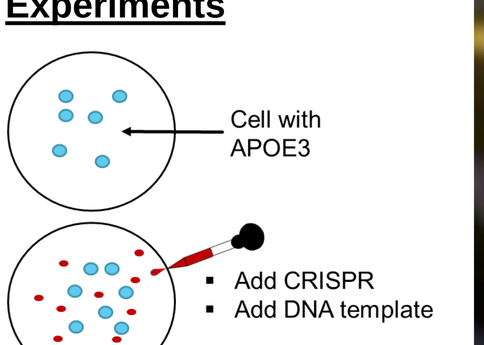
1. Identify gene of interest
2. Design guide-RNA
3. Cas9 finds sequence
4. Cas9 breaks DNA
5. Mutation or directed repair (HR or NHEJ)



EXPERIMENT 1
Switching from APOE3 to APOE4



Experiments

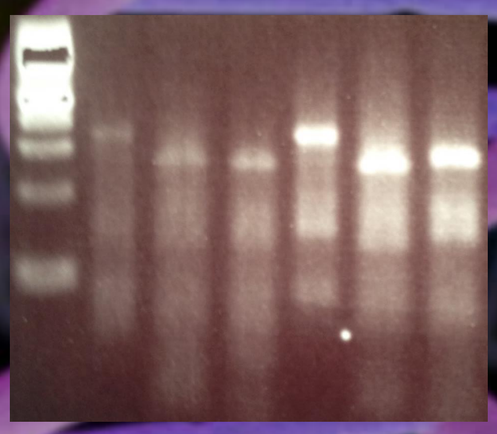


Results

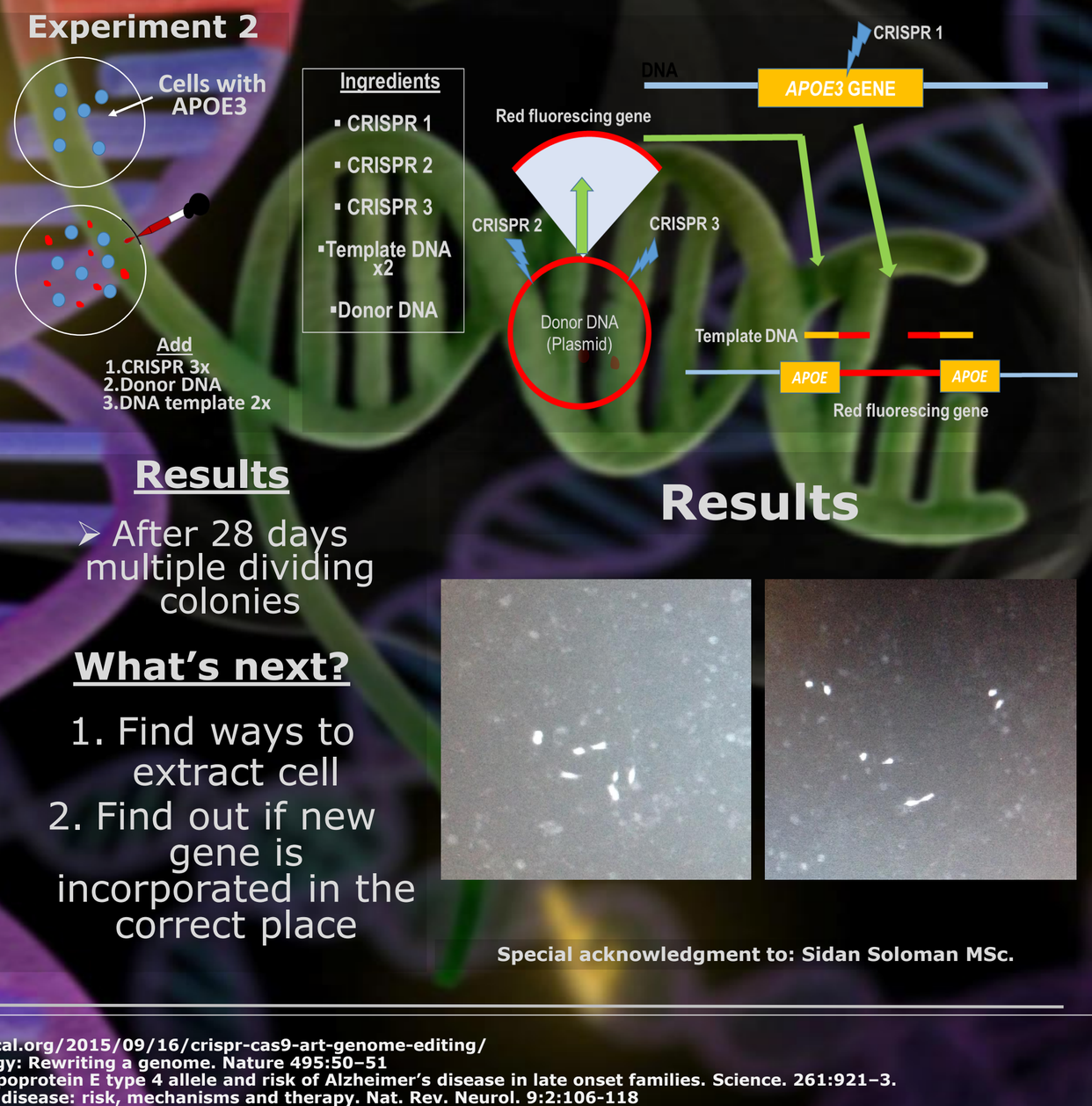
Not enough cells/ DNA?

What is next?

Isolate successful cells



EXPERIMENT 2: disrupt APOE gene by inserting a Red Fluorescing gene



Results

- After 28 days multiple dividing colonies

What's next?

1. Find ways to extract cell
2. Find out if new gene is incorporated in the correct place

Results

Special acknowledgment to: Sidan Soloman MSc.

Background Picture accessed from: http://michelsonmedical.org/2015/09/16/crispr-cas9-art-genome-editing/
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Zhong, N., and Weisgraber, KH (2009). Understanding the association of apolipoprotein E4 with Alzheimer disease: clues from its structure. J. Biol. Chem. 284, 6027–6031.