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Title of Project: Modelling Alzheimer's disease using patient-specific organoids.

Summary

Alzheimer's disease, the most common type of dementia, is a chronic, progressive disease that leads to the degeneration of neurons, which are key to the function of the brain. Despite enormous research efforts, Alzheimer's disease is still not fully understood. One of the major limitations of understanding Alzheimer's is the technical difficulty in studying the human brain. Additionally, current animal models used to model Alzheimer's do not fully recapitulate the disease. Understanding the mechanisms that result in pathology is crucial for developing treatment strategies to prevent or slow the progression of this disease.

Late-onset Alzheimer's is the most common form of the disease (over 95%), which happens to people age 65 and older. Among these people, those with a particular form of a protein called apolipoprotein E (APOE) might have a higher risk of developing late-onset Alzheimer's disease. The protein APOE can be found in three different versions (APOE2, APOE3 and APOE4). APOE3 is the most common version in Caucasian population and is considered a "healthy" version of the protein. On the other hand, APOE4, is linked to an increased risk of developing Alzheimer's disease, compared to APOE3.Although *APOE4* has been fully recognized as a major risk factor, the mechanisms underlying the role of APOE in the pathology of Alzheimer's disease is still not completely understood because it is difficult to study when other factors such as age and severity of the disease are in place.

Hypothesis vs Findings

Stem cell technology allow us to generate induced pluripotent stem cells from adult tissue samples such as skin biopsies. These stem cells have the capacity to become any cell type of the human body, including brain cells. We use specific methods to create brain tissue (cerebral organoids) in a three-dimensional form that show some level of organisation corresponding to that found in the human brain.

We hypothesised that using the latest gene editing technology, "CRISPR", to modify the *APOE* gene (from high risk APOE4 to lower risk APOE3) from stem cells will allow us to investigate the role of the APOE protein in Alzheimer's disease using cerebral organoids.

In this work we have generated brain tissues from skin biopsies of patients with different forms of APOE protein and genetically modified the allele associated to the highest risk of Alzheimer's diseases (*APOE4*) to the most common and lower risk

allele (*APOE3*) (Figure 1). After 3 months in culture, the cerebral organoids expressed markers for neurons, astrocytes and neural progenitor cells, and showed key features of Alzheimer's disease pathogenesis, including amyloid plaques and Tau phosphorylation (Figure 2), hence demonstrating their validity as a model of Alzheimer's disease. However, 3 months of culture was not sufficient to detect differences between organoids with APOE4 and APOE3 in the amount of beta amyloid and phosphorylated Tau. We have shown that only one third of the cells express APOE protein in the 3-month-old cerebral organoids by single cell transcriptome analysis. Culturing the organoids for longer, up to 6 moths, increases the total level of APOE shown by other groups, and confirmed in our laboratory by western blot analysis.

Unanswered Questions

We are currently culturing cerebral organoids with different APOE genotypes for longer periods (up to 6 months), which we hypothesised would lead to higher expression of total APOE, enabling the comparison of different APOE isoforms on Alzheimer's pathology in the cerebral organoids.

The organoids lack some structures and cells that play an important role in the progression of Alzheimer's disease such as vasculature, blood brain barrier and microglia. Our group, with other collaborators, are currently developing strategies to incorporate these relevant elements into an engineered cerebral organoid.

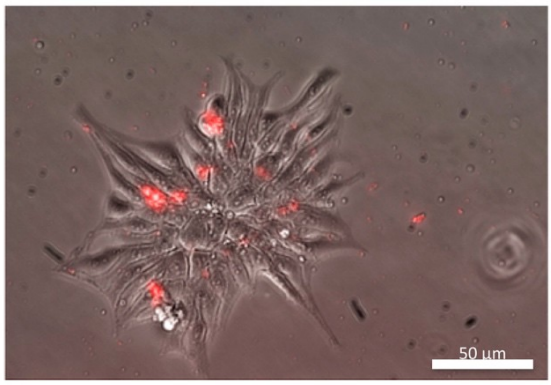
What these research outcomes mean

The development of a human model of Alzheimer's diseases could be key to the generation of novel therapies for dementia, as this model will help researchers better understand Alzheimer's disease and provide a `tool for new drug development.

A

	SNP position
APOE	rs429358
E3	T (Cys112)
E4	C (Arg112)

B



C

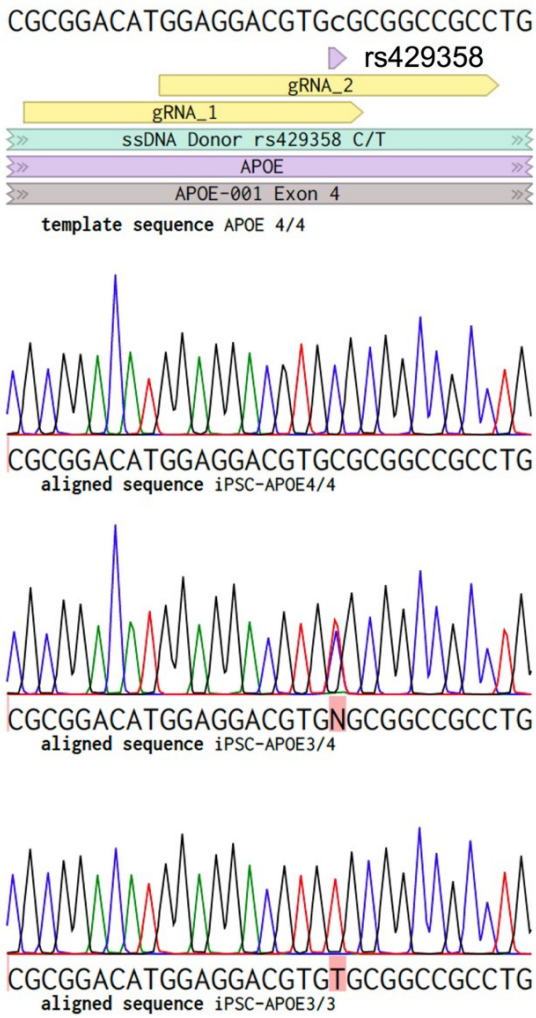


Figure 1. Generation of isogenic induced pluripotent stem cells after genetic modification of genomic DNA with CRISPR-Cas9 system. A) Table showing single nucleotide polymorphism (SNP) in allele *APOE3* (T) and allele *APOE4* (C) that result in different amino acids in position 112. B) Induced pluripotent stem cells after nucleofection of CRISPR-Cas9 system with a fluorescent tracrRNA (red) indicating cells that have being successfully transfected with CRISPR-Cas9. C) Analysis of SNPs by Sanger sequencing of DNA from iPSCs that have being genetically modified with CRISPR-Cas9 system from genotypes with *APOE4* homozygotes (*APOE4/*4) to *APOE3/4* heterozygotes and *APOE3* homozygotes (*APOE3/3*).

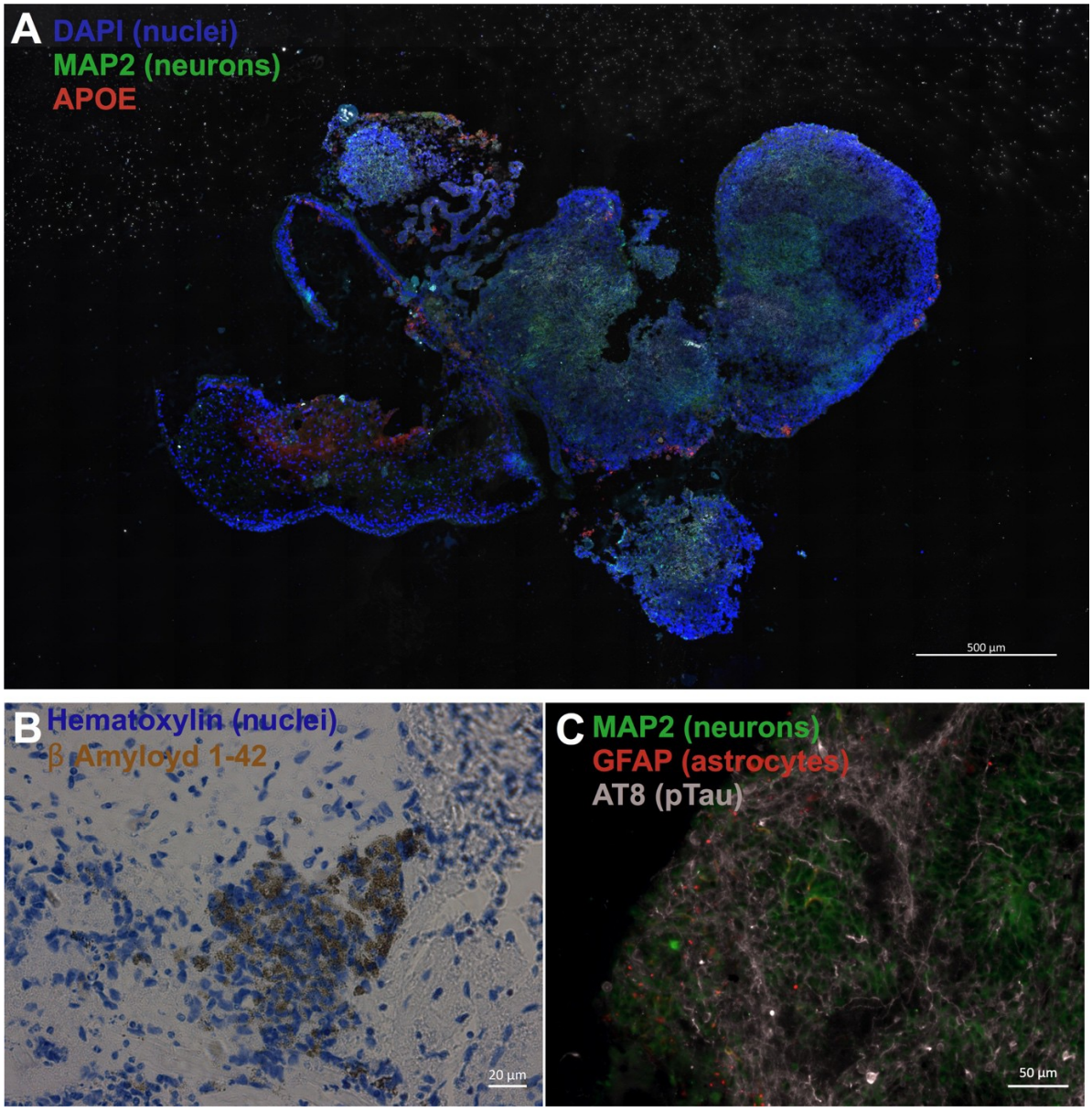


Figure 2. Cerebral organoids derived from induced pluripotent stem cells after 3 months in culture. A) Representative images showing APOE protein and neurons (MAP2 positive cells), B) plaques of beta Amyloid 1-42 and C) tangles of phosphorylated Tau, neurons and astrocytes (GFAP positive cells) in cerebral organoids.