Generation and characterisation of a panel of human iPSC-derived neurons and microglia carrying early and late onset relevant mutations for Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common type of dementia, contributing 60-70% of cases, and affecting more than 24 million people worldwide. There are no disease-modifying treatments available and existing drugs only treat the symptoms and not the root cause of the disease.

Development of therapies for AD is hampered because less than 10% of findings derived from preclinical animal models can be translated to humans. Patient-derived induced pluripotent stem cells (iPSCs) enable generation of in vitro models that can recapitulate human disease phenotypes.

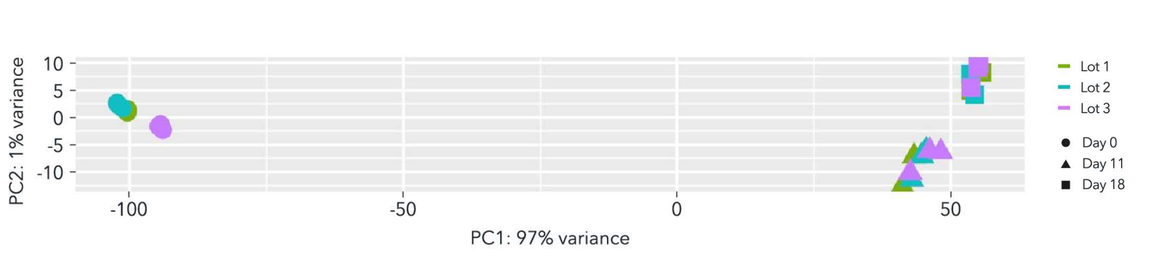
However, conventional human iPSC differentiation protocols are lengthy, inconsistent, and difficult to scale. The lack of genetically matched controls for patient-derived models further complicates the investigation of disease phenotypes.

We have developed opti-ox™, a robust iPSC reprogramming technology that overcomes these limitations and enables generation of mature cell types and disease models, at scale. Our objective was to generate a panel of disease model cells in ioGlutamatergic Neurons™, ioGABAergic Neurons™ and ioMicroglia™ to enable investigation of the most common AD mutations and accelerate drug discovery.

In addition, we provide multiple clones (up to three) per disease model to allow researchers to investigate phenotypes of interest across multiple clones of a specific disease model, originating from the same parental wild-type iPSC line. Studying multiple clones of a specific disease model can lead to a more comprehensive understanding of the impact of the disease-relevant mutation and cellular heterogeneity on associated disease phenotypes.

1. Wild-type ioGlutamatergic Neurons, ioGABAergic Neurons and ioMicroglia demonstrate high lot-to-lot consistency, ideal for the development of reliable disease models for Alzheimer's disease

A



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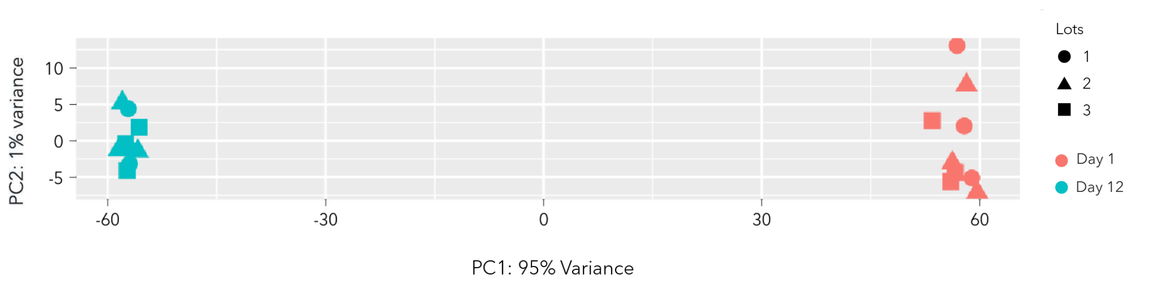

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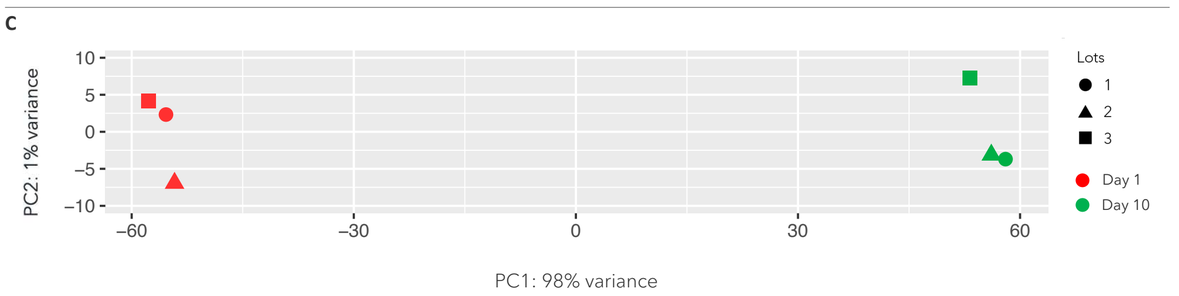
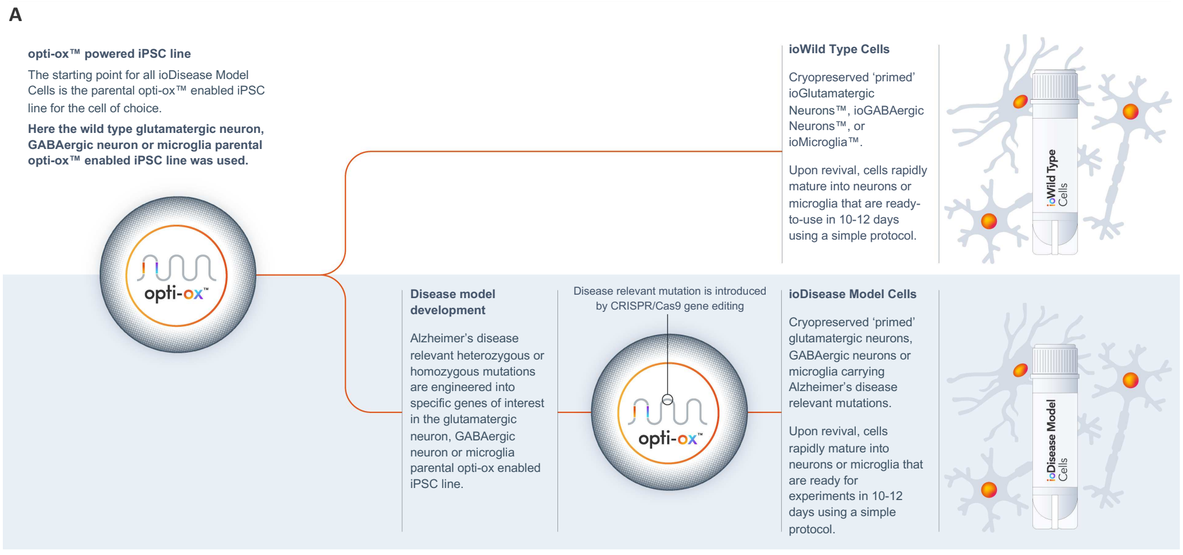


Whole transcriptome analysis demonstrates high lot-to-lot consistency of wild-type ioGlutamatergic Neurons, ioGABAergic Neurons, and ioMicroglia
Bulk RNA-sequencing analysis was performed on three independent lots of (A) ioGlutamatergic Neurons, (B) ioGABAergic Neurons or (C) ioMicroglia at different time points throughout the reprogramming protocol. Principal component analysis (PCA) to assess gene expression variance between three different manufactured lots showed a tight clustering of the samples at each timepoint, demonstrating high consistency between these lots of ioGlutamatergic Neurons, ioGABAergic Neurons, or ioMicroglia. This lot-to-lot consistency demonstrates that populations of ioGlutamatergic Neurons, ioGABAergic Neurons, and ioMicroglia with equivalent expression profiles can be generated consistently from every vial, helping to reduce experimental variation and increasing the reproducibility of experiments.

2. Generation of ioDisease Model Cells™ with Alzheimer's disease-relevant point mutations in the *APP*, *PSEN1*, *APOE* or *TREM2* genes

A



B

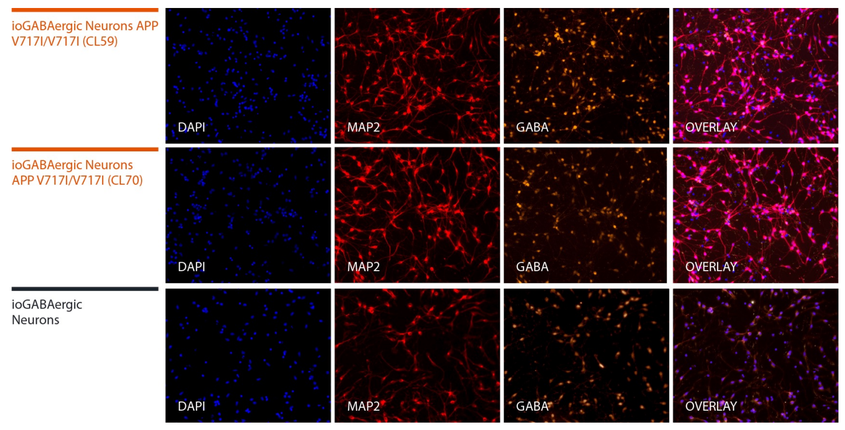
Gene	Mutation	ioCell background	Disease relevance
APP	V717I	ioGlutamatergic Neurons	Early onset AD
APP	V717I	ioGABAergic Neurons	Early onset AD
APP	KM670/671NL	ioGlutamatergic Neurons	Early onset AD
PSEN1	M146L	ioGlutamatergic Neurons	Early onset AD
APOE	C112R	ioMicroglia	Late onset AD
TREM2	R47H	ioMicroglia	Late onset AD

(A) Schematic diagram illustrating the development of ioDisease Model Cells for early and late onset Alzheimer's disease
Alzheimer's disease relevant heterozygous or homozygous mutations were engineered into specific genes of interest, as shown in **(B)** the table above, in the glutamatergic neuron, GABAergic neuron or microglia parental opti-ox enabled iPSC line, to generate genetically matched disease models. CRISPR/Cas-9 gene editing was used to generate iPSC-derived glutamatergic and GABAergic neurons carrying the APP V717I, APP KM670/671NL and PSEN1 M146L mutations, and microglia carrying the APOE C112R (converts the wild-type APOE3 allele to APOE4) and TREM2 R47H mutations.

3. Characterisation of ioDisease Model Cells for early and late onset Alzheimer's disease

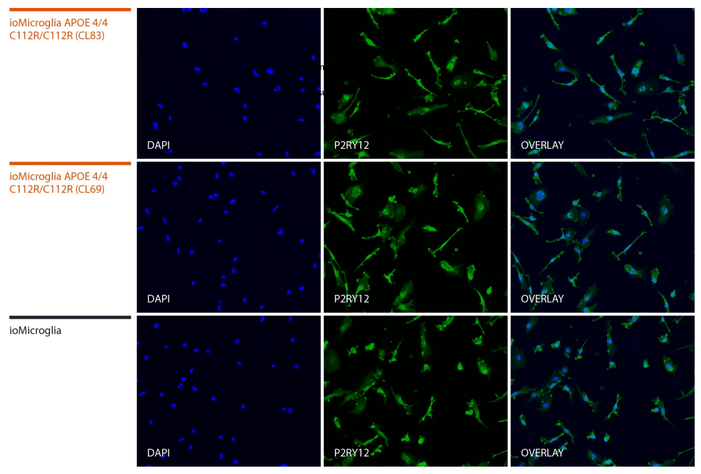
A

ioGABAergic Neurons APP V717I homozygous disease model cells express key GABAergic neuron-specific markers comparably to the genetically matched wild-type control
Immunofluorescent staining on day 12 post-revival demonstrates similar homogenous expression of the pan-neuronal marker, MAP2 and GABAergic neuron-specific marker, GABA in both disease model clones compared to the genetically matched wild-type control. 100X magnification.



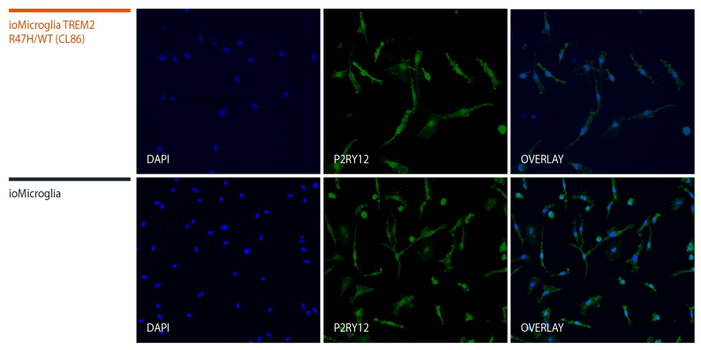
B

ioMicroglia APOE 4/4 disease model cells express key microglia marker comparably to the genetically matched wild-type control
Immunofluorescent staining on day 10 post-revival demonstrates similar homogenous expression of the key microglia marker P2RY12 and ramified morphology in both disease model clones compared to the genetically matched wild-type control. 100X magnification.



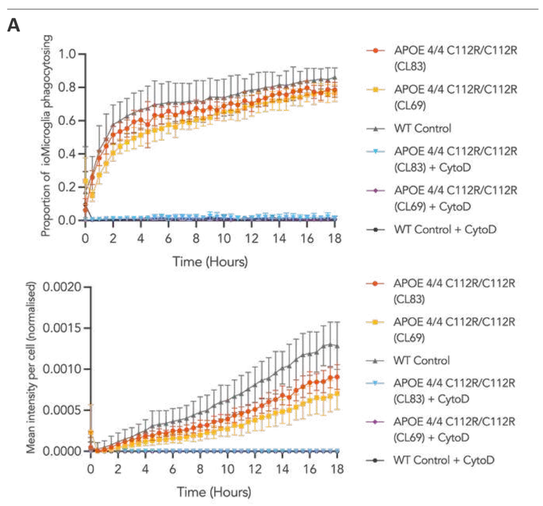
C

ioMicroglia TREM2 R47H homozygous disease model cells express key microglia marker comparably to the genetically matched wild-type control
Immunofluorescent staining on day 10 post-revival demonstrates similar homogenous expression of key microglia marker P2RY12 and ramified morphology in the disease model clone compared to the genetically matched wild-type control. 100X magnification.



4. ioMicroglia APOE 4/4 and TREM2 R47H Het disease models show reduced phagocytic activity

A



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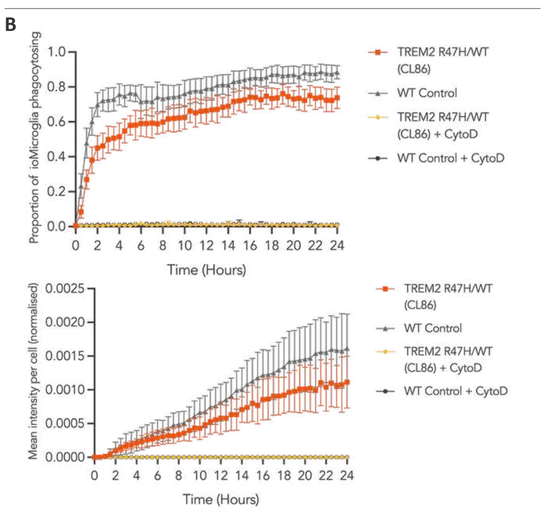

Disease model cells show a reduced proportion and degree of phagocytosis of E. coli particles compared to the genetically matched wild-type (WT) control
Phagocytosis was analysed at day 10 post-revival after incubation with 1 µg/0.33 cm^2 pHrodo™ RED labelled E. coli particles for 24 hours +/- cytochalasin D control. The top graphs display the proportion of cells phagocytosing E. coli particles over 24 hours and shows that all disease model clones display a reduced proportion of phagocytosis compared to the WT control. The bottom graphs display the fluorescence intensity per cell displaying degree of phagocytosis per cell and shows that all disease model clones display a reduced degree of phagocytosis per cell compared to the WT control. Images were acquired every 30 mins on the Incucyte® looking at red fluorescence and phase contrast. Three technical replicates were performed per experiment.

Summary & conclusions

ioGlutamatergic Neurons, ioGABAergic Neurons and ioMicroglia have been precision reprogrammed from human iPSCs into consistent, mature, functional neurons or microglia showing a high level of transcriptomic similarity between lots.

A panel of disease model cells carrying Alzheimer's disease-relevant mutations within the APP, PSEN1, APOE & TREM2 genes were generated from the wild type iPSC lines using CRISPR/Cas9 gene engineering.

The disease model cells showed similar protein expression of key neuronal or microglia markers to the genetically matched wild-type control, ensuring biological comparability of the disease models.

The ioMicroglia APOE 4/4 and TREM2 R47H Het disease models also show reduced phagocytic activity compared to the genetically matched wild-type control.

Using opti-ox technology and CRISPR/Cas9 gene engineering, we have produced a panel of hiPSC-derived Alzheimer's disease model cells for research and drug discovery.

The panel offers an accessible, consistent, and functional system for investigating the impact of AD-relevant mutations in human neuronal and glial cells enabling research into molecular mechanisms and treatments for AD.