Alzheimer’s disease models in iPSC-derived glutamatergic neurons show increased secretion of pathogenic amyloid beta peptides

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Abstract

Aims. Alzheimer’s disease (AD), a complex, multifactorial neurodegenerative disease, is challenging to study in vitro due to a lack of physiologically relevant models. ioGlutamatergic Neurons are deterministically programmed human iPSC-derived excitatory neurons that provide a consistent and scalable model to study such diseases. A panel of AD models in ioGlutamatergic Neurons was developed and characterised to determine the effects of mutations in PSEN1 and APP on amyloid beta (Aβ) production.

Methods. CRISPR/Cas9 was used to engineer heterozygous and homozygous PSEN1 M146L, APP KM670/671NL or APP V717I mutations in the parental iPSC line of the ioGlutamatergic Neurons, which were subsequently programmed using opti-ox™ technology to generate the disease model cells. The disease models were cultured for 30 days alongside their genetically matched wild type control. Supernatant was collected on days 10, 20 and 30, and concentrations of Aβ38, Aβ40 and Aβ42 were determined using the V-PLEX A-beta Peptide Panel ELISA kit.

Results. ioGlutamatergic Neurons carrying the PSEN1 M146L and APP V717I mutations secreted significantly more Aβ42 compared to their wild type control, showing higher Aβ42:40 ratios at days 20 and 30. Importantly, a clear correlation between genotype and Aβ42:40 ratios was observed, as wild type, heterozygous and homozygous mutants showed a stepwise increase in Aβ42 production relative to Aβ40. ioGlutamatergic Neurons APP KM670/671NL secreted significantly more Aβ38, Aβ40, and Aβ42 than their wild type control, but the Aβ42:40 ratio did not increase, as expected.

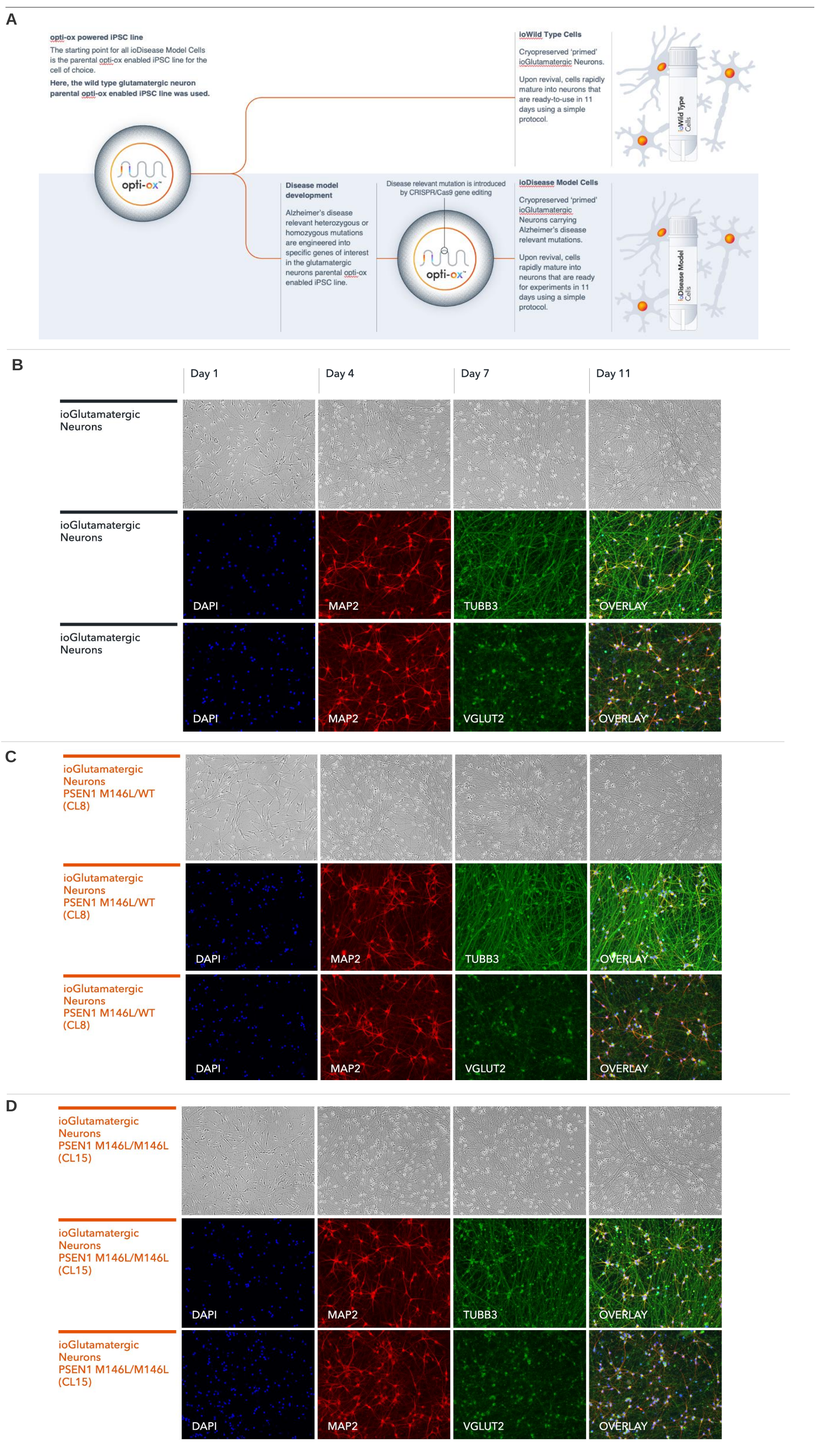
Conclusions. ioGlutamatergic Neurons with mutations in PSEN1 or APP recapitulate the increase in Aβ42 secretion observed in Alzheimer’s patients. This demonstrates their validity as an in vitro model to study AD and for the discovery of drugs targeting the pathogenic Aβ pathway.

1. Generation of Alzheimer’s disease models

A. Generation of Alzheimer’s disease models: ioGlutamatergic Neurons APP V717I, APP KM670/671NL, PSEN1 M146L were engineered using CRISPR/Cas9 in the parental iPSC line, while wild-type ioGlutamatergic Neurons (WT) act as a genetically matched control. opti-ox deterministically programmed cells are cryopreserved and mature rapidly upon revival.

Gene	Mutation
APP	V717I/V717I V717I/WT
APP	KM670/671NL / KM670/671NL KM670/671NL/WT
PSEN1	M146L/M146L M146L/WT

Brightfield and immunocytochemistry data for wild-type ioGlutamatergic Neurons (**B**) and representative data for the Alzheimer’s disease models, shown are ioGlutamatergic Neurons PSEN1 M146L/WT (CL8) (**C**), and ioGlutamatergic Neurons PSEN1 M146L/M146L (CL15) (**D**). In each figure, top panels display brightfield images of cells showing rapid maturation and formation of structural neuronal networks over 11 days, with mutant cells highly similar to the genetically matched control. Bottom panels display immunofluorescent staining of WT or PSEN1 mutant cells at day 11 post-revival, showing expression of the pan-neuronal marker MAP2 (red), TUBB3 (middle panel, green), VGLUT2 (lower panel, green), and the DAPI counterstain (blue).



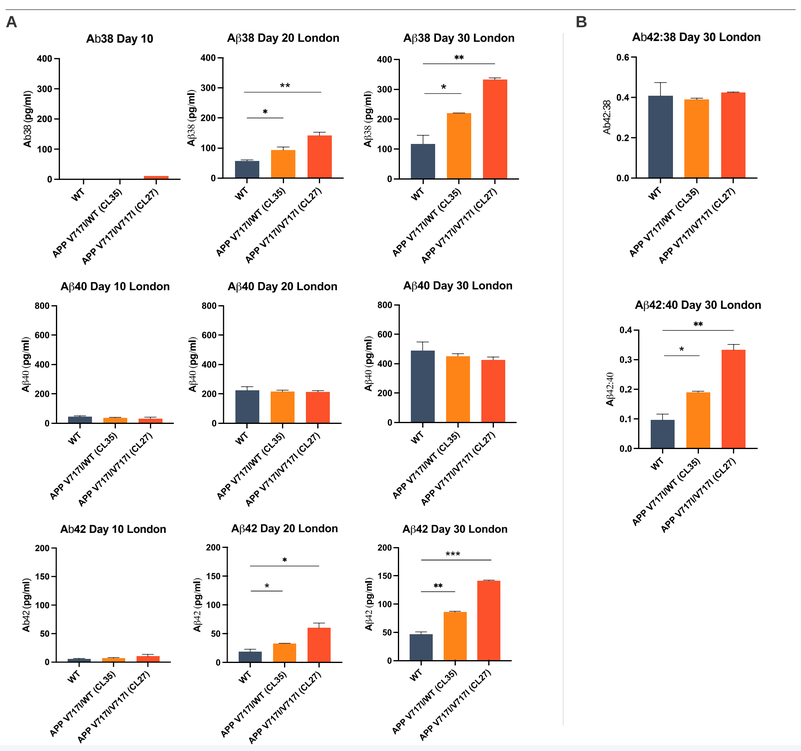
2. ioGlutamatergic Neurons APP V717I show an increase in Aβ38 and Aβ42 secretion and the Aβ42:Aβ40 ratio

A. ioGlutamatergic Neurons APP V717I (London) heterozygous (HET) and homozygous (HOM) disease models show increased production of Aβ38 and Aβ42 peptides, involved in the amyloidogenic pathway, with no increase seen for Aβ40 compared to the WT control.

B. The Aβ42:Aβ40 ratio was significantly increased in both HET and HOM clones compared to the WT control at day 30, with no change observed for the Aβ42:Aβ38 ratio.

Data were obtained from two independent experiments and are shown as mean ± SEM.

Data were analysed statistically (at days 20 and 30) using Student’s t-tests comparing each clone to the WT control.
* p<0.05, **p<0.01, *** p<0.001.



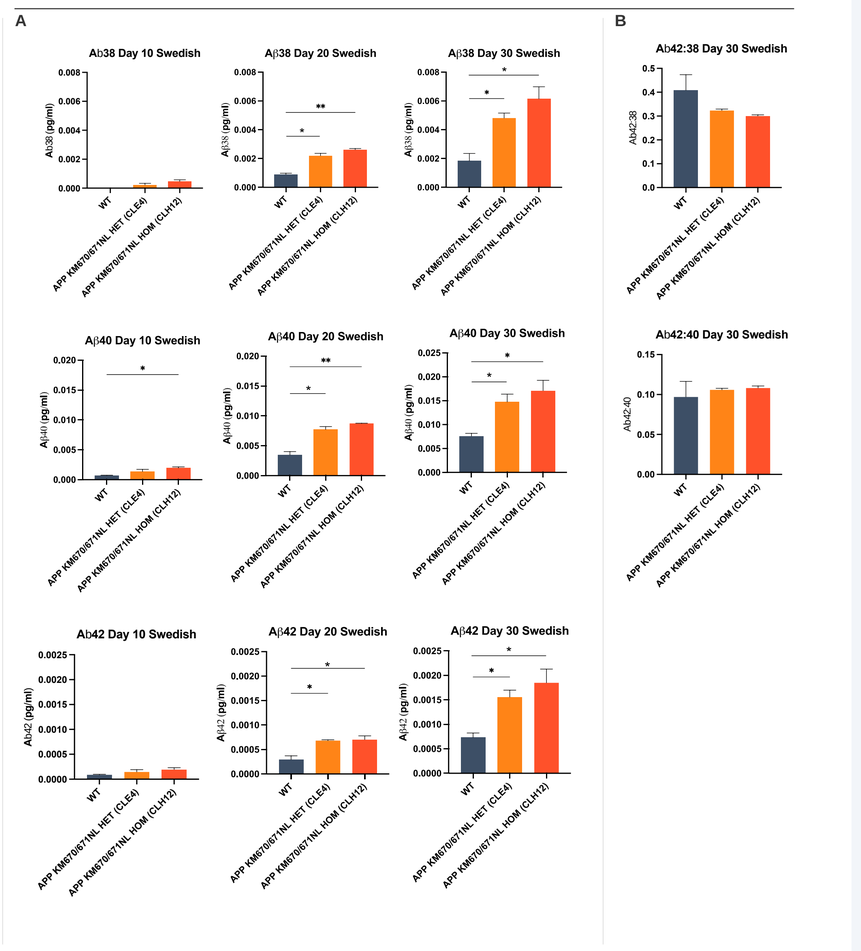
3. ioGlutamatergic Neurons APP KM670/671NL show increased secretion of all Aβ peptides

A. ioGlutamatergic Neurons APP KM670/671NL (Swedish) heterozygous (HET) and homozygous (HOM) disease models show increased production of all Aβ peptides in supernatant compared to the WT control at all time points.

B. No change was seen in the ratios of the Aβ peptides.

Data were obtained from two independent experiments and are shown as mean ± SEM. Raw ELISA data were normalised to total cell number/well as quantified at day 30.

Data were analysed statistically (at days 20 and 30) using Student’s t-tests comparing each disease model to the WT control.
* p<0.05, ** p<0.01.



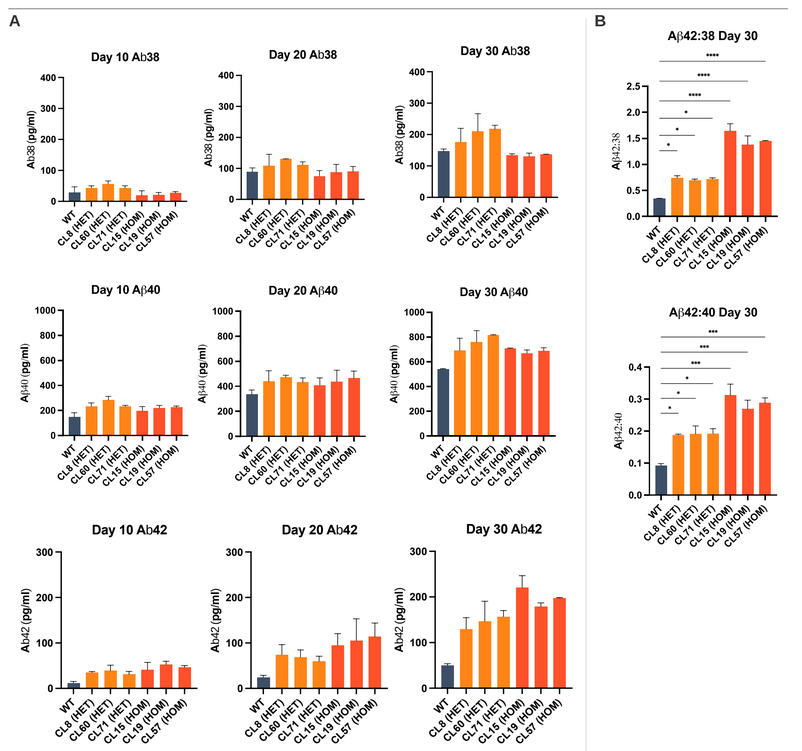
4. ioGlutamatergic Neurons PSEN1 M146L show an increase in Aβ42:38 and Aβ42:40 ratios

A. Quantification of Aβ38, Aβ40 and Aβ42 peptides at days 10, 20 and 30. Aβ42 levels were increased ~2-fold when compared to the WT control at all time points tested. The highest increase was seen at day 30, when a clear difference in Aβ42 levels can also be observed between the HET and HOM clones.

B. The Aβ42:Aβ38 and Aβ42:Aβ40 ratios are higher in the disease model cells compared to WT at day 30.

Data were obtained from two independent experiments and are shown as mean ± SEM.

Data were analysed statistically using one-way ANOVA with Tukey’s post-hoc analysis.
* p<0.05, *** p<0.001, **** p<0.0001.



Summary & conclusions

ioGlutamatergic Neurons heterozygous (HET) and homozygous (HOM) for the PSEN1 M146L, APP V717I (London) or APP KM670/671NL (Swedish) mutations were characterised as an Alzheimer’s disease model compared to their wild type, genetically matched control.

ioGlutamatergic Neurons carrying the APP V717I mutation show increased secretion of Aβ peptides in the amyloidogenic pathway, resulting in a significantly higher Aβ42:40 ratio.

ioGlutamatergic Neurons carrying the APP KM670/671NL mutation show increased production of all Aβ peptides at all time points tested.

ioGlutamatergic Neurons carrying the PSEN1 M146L mutation show higher Aβ42:38 and Aβ42:40 ratios (~2-fold increase for HET clones and ~3-fold increase for the HOM clones).

ioGlutamatergic Neurons with mutations in APP or PSEN1 recapitulate the changes in Aβ ratios observed in Alzheimer’s disease patients.