Advanced Alzheimer's Disease Models Using iPSC-derived Microglia and Neurons Harboring Disease-related Gene Variants

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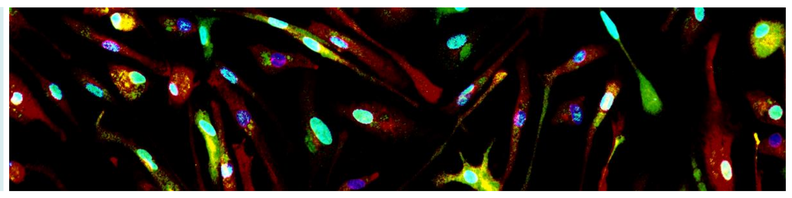

Introduction

Alzheimer's disease (AD) is a major cause of death in people over 65, and as the global population ages, the need for effective drugs against AD is crucial. Current animal models lack key human disease traits, highlighting the need for relevant human cell models for drug screening. Recent advances in induced pluripotent stem cell (iPSC) technologies have enabled the efficient generation of functional human brain cells, providing valuable tools to model these complex diseases *in vitro*. Microglia, the brain's innate immune cells, play a key role in AD pathogenesis. Genetic mutations or variations in microglia-specific genes, such as *TREM2, APOE* and *GRN* have been linked to increased risk for dementia, making them promising therapeutic targets.

We have developed and validated robust protocols to generate iPSC-derived microglia carrying AD-related genetic variants in monoculture and co-culture with neurons. We have also evaluated their response to key factors contributing to AD pathology such as amyloid-beta (Aβ).

Differentiation of Microglia from iPSCs

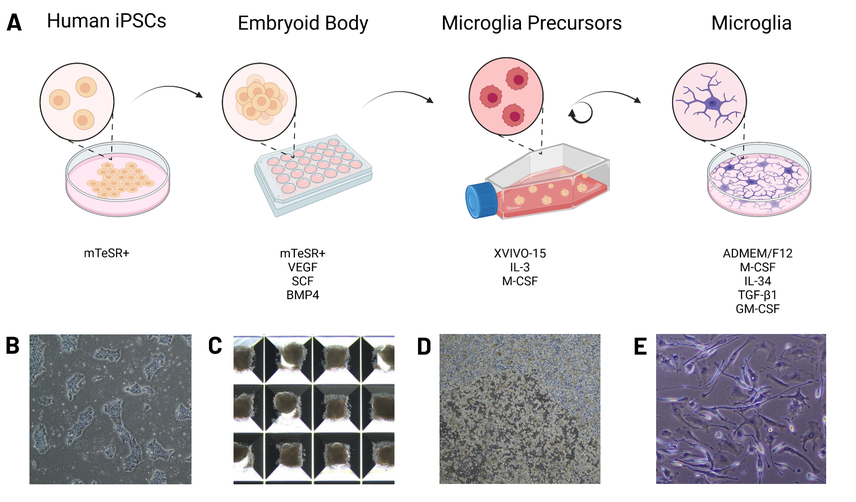

Figure 1. Differentiation of human iPSCs into microglia. (A) Graphical illustration describing the method for generating microglia and the growth factors included[1,2]. **(B)** iPSCs. **(C)** Embryoid bodies in a AggreWell™ 200 plate. **(D)** Pre-microglia, ready to be harvested from the factory flask. **(E)** Fully differentiated microglia.

iPSC-derived Microglia Cytokine Immune Responses

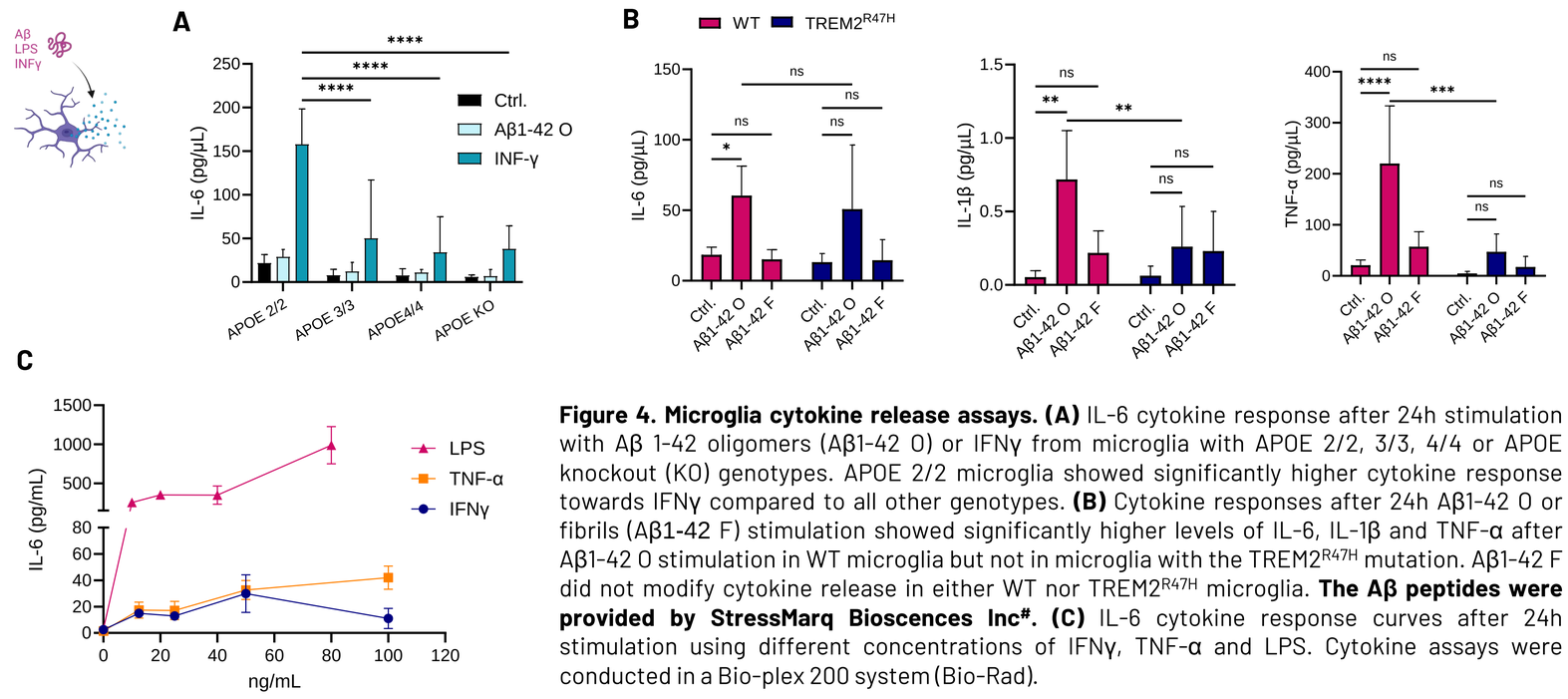

Figure 4. Microglia cytokine release assays. (A) IL-6 cytokine response after 24h stimulation with Aβ 1-42 oligomers (Aβ1-42 O) or IFNγ from microglia with APOE 2/2, 3/3, 4/4 or APOE knockout (KO) genotypes. APOE 2/2 microglia showed significantly higher cytokine response towards IFNγ compared to all other genotypes. **(B)** Cytokine responses after 24h Aβ1-42 O or fibrils (Aβ1-42 F) stimulation showed significantly higher levels of IL-6, IL-1β and TNF-α after Aβ1-42 O stimulation in WT microglia but not in microglia with the TREM2^{R47H} mutation. Aβ1-42 F did not modify cytokine release in either WT nor TREM2^{R47H} microglia. **The Aβ peptides were provided by StressMarq Biosciences Inc#. (C)** IL-6 cytokine response curves after 24h stimulation using different concentrations of IFNγ, TNF-α and LPS. Cytokine assays were conducted in a Bio-plex 200 system (Bio-Rad).

iPSC-derived Microglia Phagocytosis of Aβ and *E.coli*

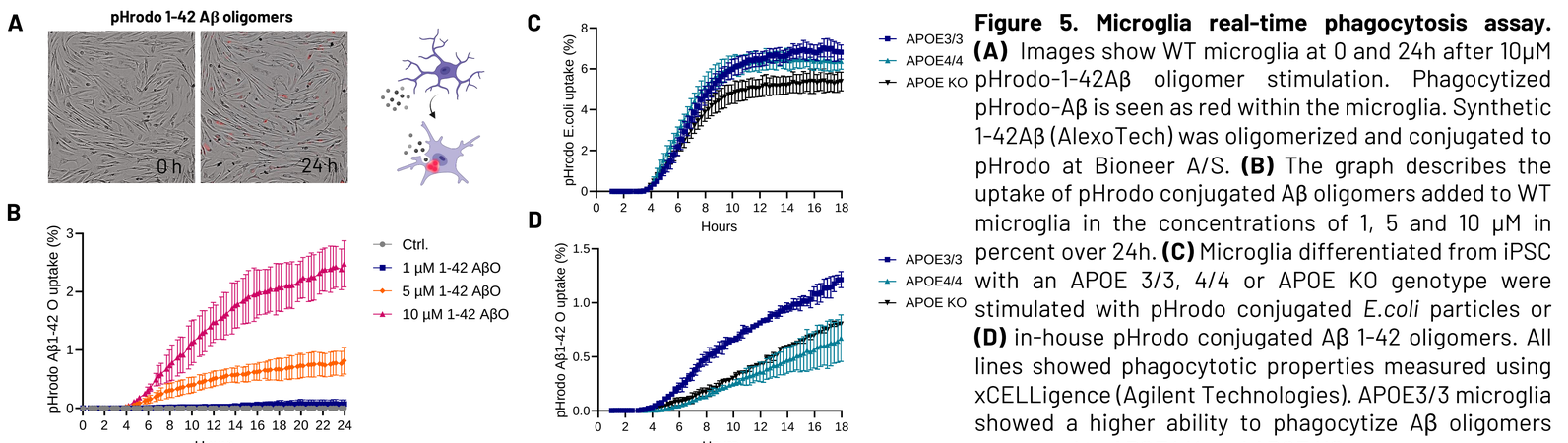

Figure 5. Microglia real-time phagocytosis assay. (A) Images show WT microglia at 0 and 24h after 10µM pHrodo-1-42Aβ oligomer stimulation. Phagocytized pHrodo-Aβ is seen as red within the microglia. Synthetic 1-42Aβ (AlexoTech) was oligomerized and conjugated to pHrodo at Bioneer A/S. **(B)** The graph describes the uptake of pHrodo conjugated Aβ oligomers added to WT microglia in the concentrations of 1, 5 and 10 µM in percent over 24h. **(C)** Microglia differentiated from iPSC with an APOE 3/3, 4/4 or APOE KO genotype were stimulated with pHrodo conjugated *E.coli* particles or **(D)** in-house pHrodo conjugated Aβ 1-42 oligomers. All lines showed phagocytotic properties measured using xCELLigence (Agilent Technologies). APOE3/3 microglia showed a higher ability to phagocytize Aβ oligomers compared to APOE4/4 and APOE KO.

Microglia Cell-line Validation

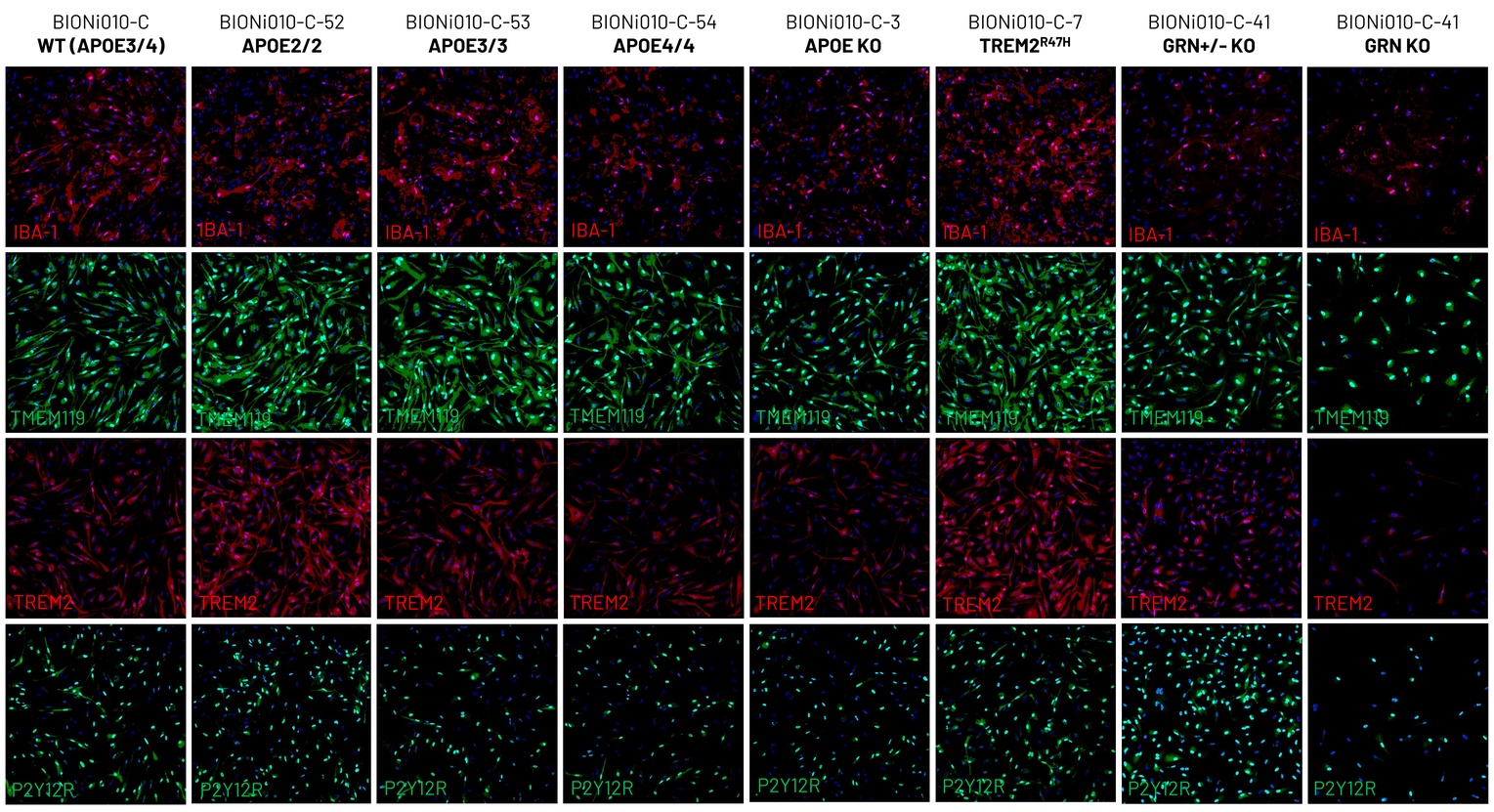

Figure 2. Validation of the key microglia markers IBA-1, TMEM119, TREM2 and P2Y12R in iPSC derived microglia. Seven different iPSC lines generated from BIONi010-C (WT) using CRISPR/CAS-9 technology, for deletion or introduction of AD or dementia-associated risk genes including WT, were differentiated into microglia. All lines express the key microglia markers IBA-1 (red), TMEM119 (green), TREM2 (red) and P2Y12R (green). Imaging was performed using x20 objective with ImageXpress (Molecular Devices).

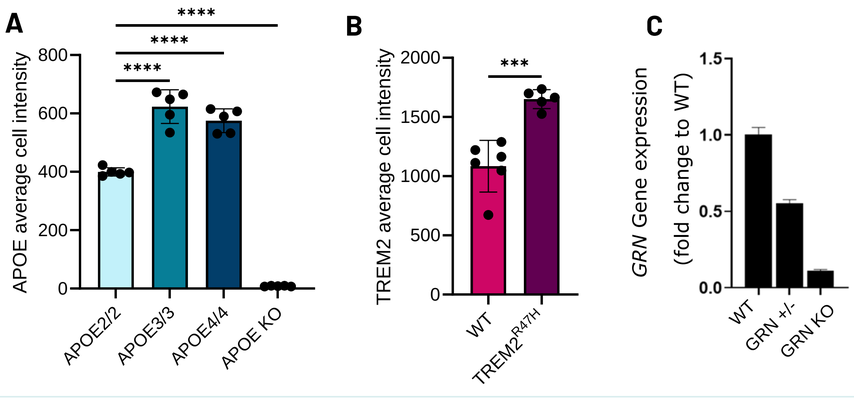

Figure 3. Expression of APOE, TREM2 and GRN in gene-edited lines. (A) APOE 2/2, 3/3, 4/4 and APOE knockout (KO) microglia show significant differences in APOE expression between the lines, where APOE 3/3 expresses the most and APOE 2/2 the least. There is no expression of APOE in APOE KO cells. **(B)** Expression of TREM2 is significantly higher in microglia with TREM2^{R47H} mutation compared to WT. **(C)** qPCR gene expression of iPSC derived neurons show a 50% reduction of *GRN* in *GRN*+/- KO cells compared to WT. There minimum expression of *GRN* in *GRN*-KO, can be a background signal from the matrix.

Microglia and NGN2 neuronal Co-culture

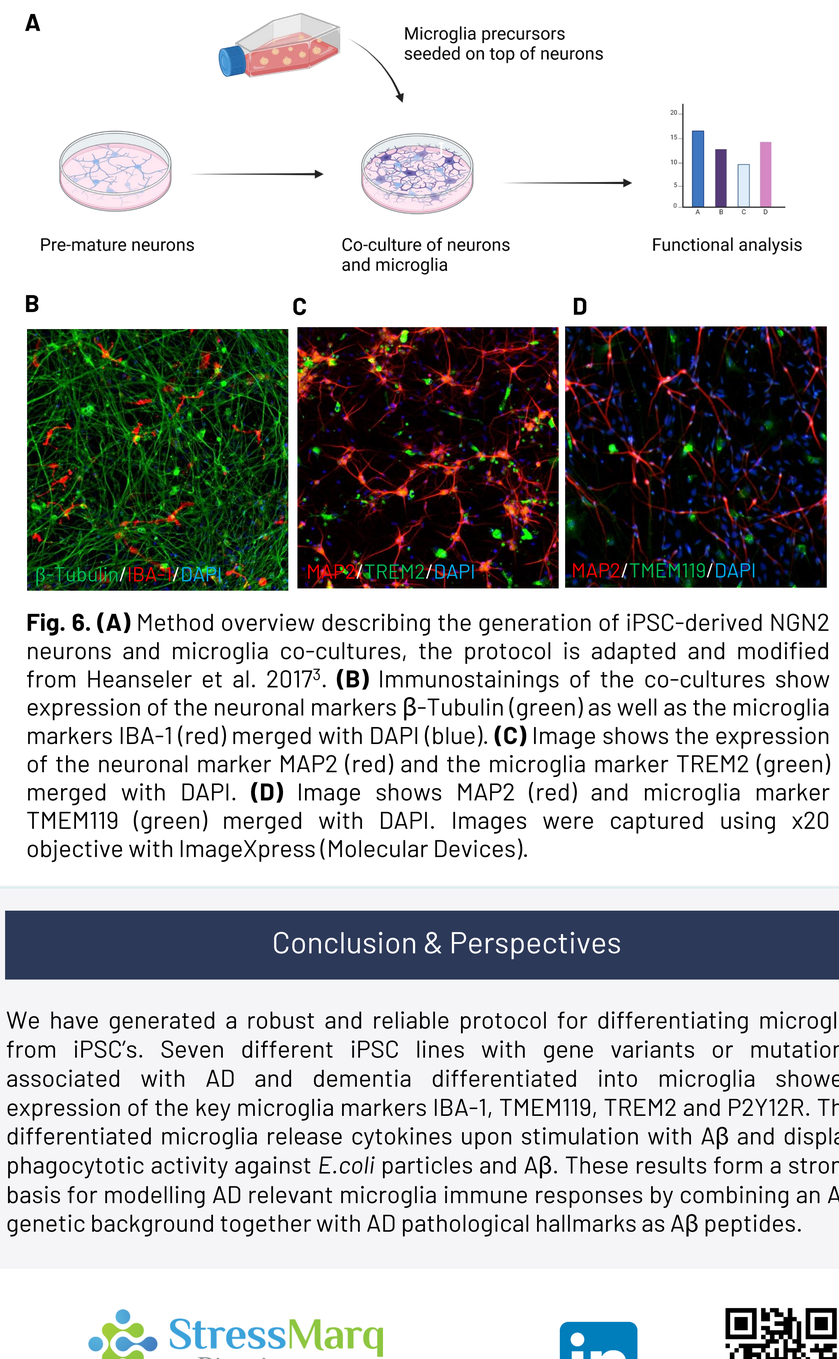

Fig. 6. (A) Method overview describing the generation of iPSC-derived NGN2 neurons and microglia co-cultures, the protocol is adapted and modified from Heanseler et al. 2017[3]. **(B)** Immunostainings of the co-cultures show expression of the neuronal markers β-Tubulin (green) as well as the microglia markers IBA-1 (red) merged with DAPI (blue). **(C)** Image shows the expression of the neuronal marker MAP2 (red) and the microglia marker TREM2 (green) merged with DAPI. **(D)** Image shows MAP2 (red) and microglia marker TMEM119 (green) merged with DAPI. Images were captured using x20 objective with ImageXpress (Molecular Devices).

Conclusion & Perspectives

We have generated a robust and reliable protocol for differentiating microglia from iPSC's. Seven different iPSC lines with gene variants or mutations associated with AD and dementia differentiated into microglia showed expression of the key microglia markers IBA-1, TMEM119, TREM2 and P2Y12R. The differentiated microglia release cytokines upon stimulation with Aβ and display phagocytotic activity against *E.coli* particles and Aβ. These results form a strong basis for modelling AD relevant microglia immune responses by combining an AD genetic background together with AD pathological hallmarks as Aβ peptides.

References

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