

Introduction:

Familial British dementia (FBD) and familial Danish dementia (FDD) are caused by mutations in *ITM2B*. In FBD, a point mutation abolishes the stop codon, extending the C-terminally cleaved peptide by 11 amino acids producing Amyloid-Bri (ABri), an amyloidogenic fragment. In FDD, a 10-nucleotide duplication extends the C-terminus by 11 amino acids to produce Amyloid-Dan (ADan) which aggregates in the brain parenchyma and vasculature. Using patient-derived iPSCs and single cell RNA-seq datasets, we have shown that *ITM2B* expression is enriched in microglia, and cultured microglia produce ABri. Herein, we aimed to generate the first human patient model of FDD, generating iPSCs from two FDD patients and differentiating them into neurons and microglia to assess ADan production and the impact of mutations on microglial biology in vitro.

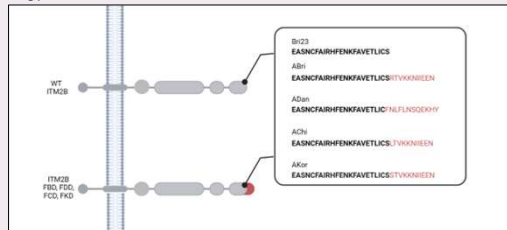


Figure 1: Wild-type ITM2B and C-terminal mutations schematic.

Graphical representation of wild-type ITM2B C-terminal sequence (Bri23) and 4 of the C-terminal mutations described in families with associated dementia. As well as ABri and ADan, a Chinese family have been identified with a mutation predicted to generate the de novo peptide AChi. Likewise, a Korean family harbour a novel mutation, generating the C-terminal fragment AKor.

Methods and Results:

Fibroblasts from two FDD patients were reprogrammed into iPSCs. iPSCs were characterised extensively, using immunocytochemistry (ICC) to confirm expression pluripotency markers SSEA4 and OCT4.

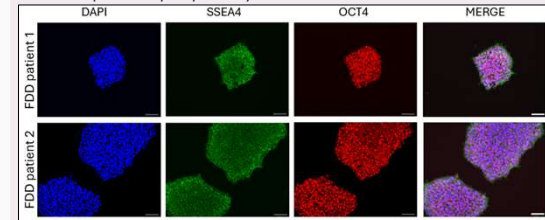


Figure 2: Patient-derived iPSCs express typical pluripotency markers.

ICC images of FDD patient reprogrammed iPSCs for pluripotency markers SSEA4, OCT4 and nuclear marker DAPI. Top image panel: cells from FDD patient 1. Bottom image panel: cells from FDD patient 2. Scale bar represents 50µm.

Microglial differentiation was confirmed by ICC, and qPCR. As we have previously shown (Arber et al., 2024), iPSC-derived microglia are enriched for ITM2B, relative to iPSCs and neurons (figure 3a).

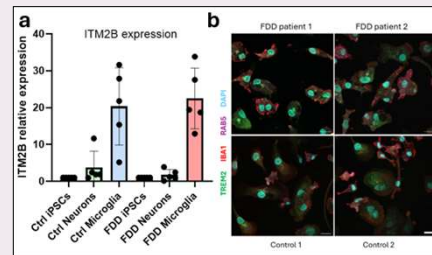


Figure 3: qPCR for ITM2B expression and ICC for microglial markers confirm ITM2B enrichment in microglia and successful microglial differentiation.

(a) ITM2B expression is enriched by qPCR in control and patient microglia relative to iPSCs and neurons. (b) ICC of FDD and control iPSC-derived microglia for microglial markers TREM2, IBA1, and endosomal marker RAB5.

The FDD-associated amyloid peptide, ADan, is produced by FDD microglia but not FDD neurons in vitro. ADan can be detected in samples from FDD microglia conditioned media (below) by western blot for the 4kDa fragment.

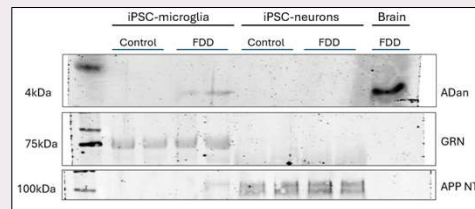


Figure 4: Western blot of conditioned media samples from iPSC-derived microglia and neurons from controls and FDD patients.

Western blot of iPSC-derived microglia and neuron conditioned media using antibodies for ADan, microglial marker Progranulin (GRN), and neuronal soluble APP (APP NTF). FDD patient brain was used as a positive control for ADan expression.

TREM2 expression was measured by qPCR from control and FDD patient iPSC-derived microglia demonstrating no change in expression at the RNA level (figure 5a). TREM2 extracellular domain signal, as measured by FACS, is significantly reduced in FDD microglia when compared to controls (figure 5b).

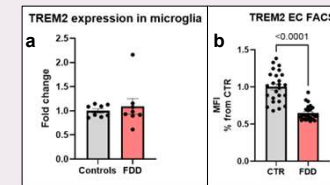


Figure 5: TREM2 expression by qPCR and extracellular domain signal by FACS in control and FDD patient microglia.

(a) qPCR for TREM2 expression in control and FDD microglia plotted as fold change relative to controls. Each data point is a separate microglial induction. (b) TREM2 extracellular domain signal in control and FDD microglia detected by FACS plotted as % change in Median Fluorescence Intensity (MFI) from controls.

At the extended timepoint of 150DIV, soluble APP detected by western blot of conditioned media from WT control, FBD and FDD iPSC-derived neurons shows a differential double band expression (figure 6).

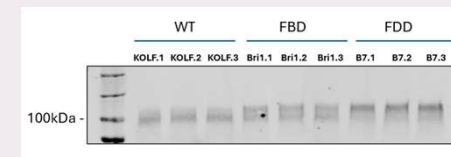


Figure 6: Western blot of conditioned media samples from iPSC-derived neurons from WT control, FBD and FDD.

Each cell line (KOLF, Bri1 and B7) has 3 separate neuronal induction samples. sAPP antibody used is 22C11.

Conclusions:

We have generated the first iPSC model of FDD, demonstrating that FDD patient-derived iPSC-microglia produce ADan. Along with our previously reported FBD model, we can interrogate the molecular mechanisms of *ITM2B* mutations in FBD/FDD. FDD microglia express less TREM2 at the cell surface than control microglia. Further investigation of this finding is ongoing. Efforts to understand the differing soluble APP expression at extended neuronal timepoints is also underway.

A greater understanding of the impact of FBD/FDD mutations on microglial function, including a potential interaction with TREM2, will have broader implications for Alzheimer's Disease.

References:

Arber C, Casey JM, Crawford S, Rambarack N, Yaman U, Wiethoff S, Augustin E, Piers TM, Price M, Rostagno A, Ghiso J, Lewis PA, Revesz T, Hardy J, Pocock JM, Houlden H, Schott JM, Salih DA, Lashley T, Wray S. **Microglia contribute to the production of the amyloidogenic ABri peptide in familial British dementia.** *Acta Neuropathol.* 2024 Nov 15;148(1):65. doi:10.1007/s00401-024-02820-z. PMID: 39546024; PMCID: PMC11568029.

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