PSEN1, but not PSEN2, mutation in familial form of Alzheimer’s disease is associated with impaired barrier phenotype in a stem cell-based model of the blood-barrier

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Abstract

Background: Alzheimer’s disease (AD) is the most common form of neurodegenerative disease. It is an irreversible condition marked by irreversible cognitive loss, commonly attributed by the loss of hippocampal neurons due to the formation of senile plaques and neurofibrillary tangles. Although the sporadic form is the most prevalent, the presence of familial form (involving several genes such as APP, PSEN1 and PSEN2) of the disease is commonly used as a model for understanding the pathophysiology of the disease. The aim of this study is to investigate the effect of mutation on PSEN1 and PSEN2 genes on the BBB function using induced pluripotent stem cells.

Methods: iPSC lines from patients harboring mutations in PSEN1 or PSEN2 were used in this study and compared to a control iPSC line. Cells were differentiated into BMECs following existing protocols. Barrier function was assessed by measuring TEER and fluorescein permeability, drug transporters activity was assessed by uptake assay, glucose uptake and metabolism assessed by cell flux analyzer, mitochondrial potential by JC-1 and lysosomal acidification by acridine orange.

Results: PSEN1-BMECs, but not PSEN2-BMECs, showed impaired barrier function compared to control group. Such impaired barrier function correlated with poor tight junction complexes and reduced drug efflux pump activity. In addition, both PSEN1 and PSEN2 displayed reduced glucose uptake and glycolysis, as well as impaired mitochondrial membrane potential and lysosomal acidification.

Conclusion: Our study reports evidence that PSEN1 and PSEN2 mutations, two genes commonly associated with familial form of Alzheimer’s disease, can impair the development and the maintenance of the BBB, both by an impairment of the barrier function, vesicle trafficking and bioenergetics. Therefore, assessing the contribution of genetic mutations associated with Alzheimer’s disease will allow us to better understand
the contribution of the BBB in dementia, but also in other neurodegenerative diseases.

Background

Alzheimer’s disease (AD) is the most common neurodegenerative disorder accounting as the 6th cause of death in the United States. It is a fatal form of dementia that is progressive, irreversible, and a growing public health concern. It is estimated that over 10% of the US senior population is diagnosed with AD (1). It is characterized by the formation of senile plaques (2–5).

Despite the important effort aimed to find a cure for such disease, there is still no cure for the disease (6), with the translation from pre-clinical models to clinically relevant candidates being an important pitfall. Therefore, a shift of paradigm from a neuron-centric to non-neuronal components is necessary to identify novel therapeutic targets.

The contribution of AD at the BBB remains anecdotal. It is well-known that cerebral amyloid angiopathy (a type of dementia resulting in the formation of amyloid plaques around the brain vasculature) can induce a disruption of the BBB. Several studies also reported the presence of an “amyloid clearance” mechanism by which the BBB may contribute to the “brain-to-blood clearance” of amyloid beta (Aβ) peptides (7–15). Notably, there is also evidence that alteration in glucose homeostasis peripherally (e.g. Type2 diabetes) or centrally (glucose uptake and metabolism at the CNS) may contribute to the onset of dementia (16–27).

Yet, the use of post-mortem tissue from AD patients strictly limits the ability to document dysfunctional signaling pathways at the BBB, whereas current in vitro studies using Aβ to assess its neurotoxicity at the BBB requires the use of amount not reflective of concentrations observed in vivo. Thus, assessing the contribution of genes associated with AD could provide a better understanding of AD pathophysiology at the neurovascular unit. Induced pluripotent stem cells (iPSCs) derived from patients suffering from familial form of
AD (FAD) have been incremental in the literature standpoint to understand their contribution in the pathophysiology of the disease. Yet, the function of such genes at the BBB, and their possible implication in the AD pathophysiology.

The aim of this study is to document the effect of mutations in PSEN1 and PSEN2 genes on the barrier function using iPSCs derived from patients suffering from FAD (28–30). Using the differentiation protocol initially developed by Shusta and colleagues (31, 32), this study investigated the effect of mutations on PSEN1 and PSEN2 genes on the barrier phenotype in iPSC-derived brain microvascular cells (BMECs).

Materials And Methods

Cell culture and iPSC differentiation

Control (CS06iCTR), PSEN1 (CS40iFAD) and PSEN2 (CS08iFAD) iPSC lines used in this study were acquired from the Cedars-Sinai iPSC core (Los Angeles, CA). The PSEN1 iPSC line was isolated from a 56-year old Caucasian male diagnosed with memory impairment and harbors a Ala246Glu mutation. The PSEN2 iPSC line was isolated from a 81-year old Caucasian female diagnosed with progressive dementia and harbors a Asn141Ile mutation. Undifferentiated iPSCs were maintained in hESC-grade Matrigel® (Corning, Corning, NY) in presence of Essential 8 (E8) medium (Life Technologies, Thermofisher, Waltham, MA) as previously described (33). iPSC differentiation into BMECs occurred following the differentiation protocol previously published by our lab (33). Briefly, cells were maintained in E8 for 5 days prior to differentiation, followed by 6 days in unconditioned medium [UM: Dulbecco’s modified Eagle’s medium/F12 with 15 mM HEPES (ThermoFisher), 20% knockout serum replacement (Thermofisher), 1% non-essential amino acids (Thermofisher), 0.5% Glutamax (Thermofisher), and 0.1 mM β-mercaptoethanol (SigmaAldrich, St. Louis, MO, USA)] and 2 days 2 days in EC+/- [EC medium
(Thermofisher) supplemented with 1% platelet-poor derived serum (PDS, Alfa-Aesar, Thermofisher, Haverhill, MA, USA), 20 ng/mL human recombinant basic fibroblast growth factor (Tocris, Abingdon, UK), and 10 µM retinoic acid (Sigma-Aldrich)]. At day 8 of differentiation, cells were enzymatically dissociated (Accutase®, Corning) and seeded on tissue culture plastic surfaces (TCPS) coated with collagen (isolated from human placenta, Sigma-Aldrich)/fibronectin (bovine plasma, Sigma-Aldrich) at concentrations of 80 µg/cm² and 20 µg/cm² respectively. At day 9 of differentiation, iPSC-derived BMECs were maintained in EC-/- medium [EC medium supplemented with 1% PDS] for 24 hours. Experiments were conducted at day 10 of differentiation.

Immunofluorescence

Cells were quickly washed with ice-cold PBS and fixed in 4% paraformaldehyde (PFA, Electron Microscopy Sciences, Hatfield, PA, USA) and blocked for 30 minutes at room temperature (RT) in presence of PBS supplemented with 10% goat serum (Thermofisher) supplemented with 0.2% Triton-X100 (Sigma). Cells were incubated overnight at 4ºC in primary antibodies targeting BCRP (1:100, Milipore, RRID: AB_11213795), claudin-5 (1:100, Life Technologies, RRID: AB_2533200), GLUT1 (1:100, Thermofisher, AB_10979643), GLUT3 (1:100, Thermofisher, AB_2809974), GLUT4 (1:100, Thermofisher, AB_11153908), MRP1 (1:100, Millipore, RRID: AB_2143819), occludin (1:100, Life Technologies, AB_2533101) and P-gp (1:50, Thermofisher, AB_1233253) diluted in 10% goat serum (PBSG). Primary antibodies detection occurred by incubation with goat-anti mouse Alexa Fluor® 555-conjugated secondary goat anti-mouse (Life Technologies) for 1 hour at room temperature. Cells were observed at 200X magnification (20X long-distance dry objective) and acquired using a Leica DMI-8 inverted epifluorescence microscope (Leica Microsystems, Wetzlar, Germany). Images were processed using ImageJ (Image J,
NIH, Bethesda, MD). Relative fluorescence was quantified using the built-in function in ImageJ. Background fluorescence was subtracted from unlabeled cells incubated with the secondary antibody only.

**TEER and permeability experiments**

Barrier tightness was measured by assessing both transcellular electrical resistance (TEER) and fluorescein permability (paracellular tracer). TEER was measured using an EVOHM STX2 chopstick electrode (World Precision Instruments, Sarasota, FL, USA). For each experiment, three measurements were performed for each insert, and the average resistance obtained was used to determine barrier function. Fluorescein permeability was assessed by incubating 10 μM sodium fluorescein (Sigma-Aldrich) in the donor (apical) chamber, with sampling in the donor (basolateral) chamber every 15 minutes for up to 60 minutes. Fluorescein permeability ($P_e$) was calculated using the clearance slopes obtained by extrapolation using the following formula:

$$\frac{1}{(P_e \cdot S)} = \frac{1}{(P_t \cdot S)} - \frac{1}{(P_f \cdot S)} \text{ with } P_e = \frac{P_e \cdot S}{S}$$

$P_t$ and $P_f$ indicative of the clearance slopes of samples and blank (empty coated) filters, and $S$ indicative of the the insert surface area (cm$^2$).

**Drug uptake assay**

Cells were incubated in the presence of 10 μM Rhodamine 123 (P-gp substrate, Sigma), FL-BOPIDY (BCRP substrate, Sigma) or CM-DCFDA (MRP substrate, Sigma) for 1 hour at 37ºC followed by cell lysis using RIPA buffer (Thermofisher). For assessing the contribution of efflux pump in the drug uptake, cells were pre-incubated for 1 hour in presence of 5 μM cyclosporine A (CsA, P-gp inhibitor, Sigma), 1 μM Ko143 (BCRP inhibitor, Sigma) or 10 μM MK571 (MRPs inhibitor, Sigma) and maintained during the incubation with drug efflux
substrate. Fluorescence in cell lysates was assessed using a SynergyMX² ELISA plate reader (Bio-Tek, Winooski, VT, USA). Relative fluorescence units (RFU) were normalized against the total protein content and the protein levels were determined by bicinchoninic acid assay (BCA, Thermofisher). Fluorescence values (expressed as relative fluorescence unit or RFU) obtained from cell lysates in the absence of inhibitor (named as controls) were normalized to the protein content and expressed as RFU/µg protein.

**Glucose uptake assay**

Cells were incubated in presence of $[^{14}C]$-D-glucose (0.4 μCi/mL) for 1 hour at $37^\circ$C. Following incubation, cells were briefly washed with ice-cold PBS and lysed with RIPA buffer. In experiments involving GLUT1 inhibition, cells were pre-incubated in presence of 10 μM glucose transporter inhibitor II (Millipore-Sigma, Danvers, MA) for 1 hour prior incubation with glucose. Radioactivity in cell lysates was assessed using liquid scintillation cocktail (Scintisafe 30%, Thermofisher) and quantified with a Beckman-Coulter LS6500 (Beckman-Coulter, Brea, CA). Glucose uptake levels were normalized by the total amount of protein in samples.

**Glycolytic flux analysis**

Glycolytic flux analysis was assessed using a Seahorse Xf24 cell flux analyzer (Agilent Technologies, Santa Clara, CA). Cells were seeded on custom-designed 24-well plates (Agilent Technologies) at day 8 of differentiation and allowed to grow for 48 hours. At the day of experiments, cell medium was replaced by glucose-free medium provided with the glycolytic stress test kit (Agilent) for 2 hours prior experiment. Cell medium was replaced once with glucose-free medium and initiated measurement. At 20 minutes of incubation, 10 mM D-glucose was added in the incubation chamber, followed by the addition of 1 μM of oligomycin at 40 minutes and finally addition of 100 mM 2-deoxy-D-glucose (2-DG) at
60 minutes timepoint. Experiments were terminated at 90 minutes of experiment.

Flow cytometry

At day 10 of differentiation, cells were enzymatically dissociated with accutase, centrifuged and resuspended in medium containing 5 µM JC-1 dye (Thermofisher) for 30 minutes at 37ºC. In experiments involving FCCP treatment, cells were simultaneously treated with 50 nM FCCP. Following incubation with JC-1 dye, cells were washed by centrifugation and resuspension in 200 µL PBS for flow cytometry analysis.

In experiments involving acridine orange (AO), cells were maintained for 24 hours in EC-/medium or in serum-free EC medium to induce serum starvation. Following such treatment, cells were centrifuged and stained with 1 µg/mL acridine orange (AO, Sigma-Aldrich) dissolved in PBS and allowed to stain for 15 minutes, following the protocol of Thome and colleagues. Fluorescence detection in samples were performed using a FACSVerse flow cytometer (BD Biosciences, San Jose, CA). Fluorescence PMTs were calibrated on unstained cells and set for the remaining of the experiments.

Lysosensor

Live cells were incubated in presence of 1 µM Lysosensor™-Green DND 189 for 5 minutes, followed by a brief wash with ice-cold PBS and fixation with 4% paraformaldehyde. Cells were counterstained with 300 nM DAPI solution and immediately processed for imaging under the Leica DMi-8 inverted fluorescence microscope at 20X.

Statistics

Data are represented as mean ± S.D. from at least three independent experiments.

Statistical analysis was performed using one-way analysis of the variance (ANOVA) using parametric (Dunnett) tests. Statistical analysis was performed using GraphPad Prism 8.0 (Graphpad Software, La Jolla, CA). A p-value lesser than 0.05 (P < 0.05) was considered as
indicative of a statistic difference between one or more groups.

Results

PSEN1-BMECs but not PSEN2-BMECs showed impaired barrier function

Assessment of the barrier function in these iPSC-derived BMECs monolayers was the first outcome investigated in this study (Fig. 1). PSEN1-BMECs displayed a lower immunoreactivity to claudin-5 and occludin (Fig. 1A), such lower immunoreactivity was further confirmed by quantification of protein expression by fluorescence intensity (Fig. 1B). PSEN1-BMECs displayed overall lower expression of these two tight junction (TJ) proteins, whereas PSEN2-BMECs showed no differences to the control iPSC-line.

Next, changes in the barrier function was assessed by measuring changes in TEER and fluorescein permeability (Fig. 1C&D). As expected, PSEN1-BMECs showed impaired barrier function compared to the two other iPSC lines, as a significantly lower TEER (\(- 150 \Omega \text{.cm}^2\)) and higher fluorescein permeability.

Such impaired phenotype appeared not limited to BMECs, as iPSC-derived neurons originated from PSEN1 iPSCs displayed an impaired formation of maturing neurons as represented by formation of neurites compared to control and PSEN2-neurons (Supplementary Fig. 1). Taken together, PSEN1 but not PSEN2, seems to impair the formation of tight BMECs monolayers.

PSEN1-BMECs have impaired drug efflux pumps activity

To further investigate the effect of mutations on PSEN1 and PSEN2 genes, changes in drug efflux pump transporters expression and activity were assessed in iPSC-derived BMECs (Fig. 2). No significant differences were observed in the expression of major drug efflux transporters were observed, although P-gp and MRP1 expression appeared lower in PSEN1-BMECs. Such decreased expression correlated with a decreased activity of both pumps.
PSEN1-BMECs showed higher drug uptake levels for rhodamine-123 (Fig. 2B, a P-gp substrate) and DCFDA (Fig. 2C, an MRPs substrate), and confirmed by the relative absence of change in cellular uptake following treatment with cyclosporine A (CsA, a P-gp inhibitor) or MK571 (a pan-MRP inhibitor). In contrast, no significant differences were observed in regards of BCRP activity. In conclusion, mutation in the PSEN1 gene may impair the activity of certain drug efflux transporters.

**PSEN1 and PSEN2-BMECs display impaired glucose uptake and metabolism**

Next, changes in glucose uptake and metabolism between iPSC lines was assessed (Fig. 3). No significant changes in glucose transporter isoforms (GLUT1, GLUT3, GLUT4) at the BBB were observed (Fig. 3A). However, PSEN1-derived BMECs showed a lower glucose uptake (Fig. 3B) compared to controls and PSEN2-BMECs. Although PSEN2-BMECs showed no significantly lower glucose uptake than controls, these cells still showed a slight decrease compared to control. In addition, both PSEN1-BMECs and PSEN2-BMECs failed to show inhibition of glucose uptake following treatment with glucose transporter inhibitor II (GTI). Notably, similar pattern was observed with iPSC-astrocytes (Supplementary Fig. 2), as PSEN1-astrocytes showed lower glucose uptake than control-astrocytes. However, all three groups showed significant decrease in glucose uptake following treatment with GTI.

To investigate the impact of such impaired glucose uptake on the cell metabolism, we investigated changes in glycolysis in iPSC-derived BMECs using a cell flux analyzer (Fig. 3D-F). Both PSEN1-BMECs and PSEN2-BMECs showed a basal extracellular acidification rate (ECAR) compared to control-BMECs. Both PSEN-BMECs showed a metabolic phenotype considered “quiescent”, compared to a “glycolytic” phenotype observed with control-BMECs. A detailed analysis of the glycolytic stress assay showed a decrease in glycolysis and non-glycolytic activity only in PSEN1-BMECs, whereas a decreased glycolytic capacity and glycolytic reserve were observed in both PSEN-BMECs.
In conclusion, mutations in PSEN genes maybe impairing glucose uptake and metabolism at the BBB.

**PSEN1 mutation impairs mitochondrial and lysosomal acidification**

Finally, we investigated changes in cell metabolic activity and investigated the effect of PSEN on mitochondrial and lysosomal function (Fig. 4). Firstly, changes in cell metabolic activity was assessed in iPSC-BMECs monolayers using an MTS assay (Fig. 4A). Interestingly, PSEN1-BMECs showed a higher cell metabolic activity than control and PSEN2-BMECs. Similar observation was done in iPSC-derived neurons (Supplementary Fig. 2B), whereas iPSC-derived astrocytes showed no differences in cell metabolic activity.

To confirm if such differences in cell metabolic activity were due to changes in mitochondrial potential, changes in JC-1 fluorescence (Fig. 4B). Under resting condition, control-BMECs showed a majority of cell events in high intensity red fluorescence. Following treatment with CCCP (a mitochondria uncoupler), most events shifted their fluorescence in the green fluorescence. In contrast, PSEN1-BMECs and PSEN2-BMECs showed an overall lower fluorescence intensity compared to control. In addition, the shift from the red fluorescence into green fluorescence was less distinct, with a significant number of events still occurring in the red fluorescence gate. Such results suggests an impaired mitochondrial function. Next, changes in lysosomal activity in regards of lysosomal acidification was assessed in iPSC-derived BMECs using Lysosensor® Green (Fig. 4C) and acridine orange (Fig. 4D). Control-BMECs displayed presence of peri-nuclear intense green fluorescent punctae following Lysosensor® Green staining, indicative of the presence of acidic lysosomes. In contrast, the presence of fluorescent punctae was almost inexsitant in PSEN1-BMECs, whereas presence of fluorescent punctae was present in PSEN2-BMECs. Such observation was confirmed by the use of acridine orange (AO, Fig. 4D). Control-BMECs were characterized by the presence of predominant high
PerCP/high FITC events indicative of acidic lysosomes (34). In contrast, PSEN1-BMECs showed a notable decrease in acidic lysosomes compared to control-BMECs. Although PSEN2-BMECs showed an important number of events in the high PerCP/high FITC quadrant, the presence of a tail in the low PerCP/low FITC quadrant is indicative of a possible partial impairment in lysosome acidification. Induction of autophagy by serum starvation triggered a shift from green to red fluorescence signal in all three group. In summary, PSEN mutations may impact mitochondrial function and lysosomal acidification at the BBB.

Discussion

Alzheimer’s disease is the most common neurodegenerative and irreversible disease with its steady increase amongst the population raises an important public health issue. Until now, no treatment for the disease have been identified and most of the literature has been mostly bringing a neuron-centric centric approach. Although there is a literature showing the contribution of the BBB in the pathophysiology of the disease, studies investigating the contribution of genetic mutations associated with AD (e.g. APP, PSEN1, PSEN2...) on the BBB dysfunction remains anecdotal. In this study, we investigated the effect of mutations in PSEN1 and PSEN2 on the BBB function, using iPSCs obtained from patients suffering from FAD. This study identified PSEN1, rather than PSEN2, as a possible gene important in the formation and maintenance of the BBB, as PSEN1-BMECs showed an overall worsened outcome than PSEN2-BMECs including formation of the BBB phenotype, glucose metabolism and mitochondrial function. Interestingly, such results correlated with previous findings reported by Searson and colleagues using an iPSC line sharing the same mutation in PSEN1 (35), as we both observed that PSEN1-BMECs BMECs showed poor barrier function (as measured by TEER and permeability with a paracellular marker), as well as a compromised activity. Furthermore, our group showed that MRP-mediated efflux
in PSEN1-BMECs was also affected, as well as glucose metabolism (glucose uptake, glycolysis), as well as glucose metabolism, mitochondrial function and lysosomal acidification. Our study also suggests that this effect maybe restricted to PSEN1, as the phenotype observed with PSEN2 mutant was milder, although signs of impaired glucose metabolism, mitochondrial function and lysosomal acidification were reported by our group. A limitation of our approach is the limited number of iPSC lines available from patients with FAD at the time of publication. Hence, future studies are aimed to investigate and confirm our observation by the inclusion of additional cell lines from patients with mutations in APP, PSEN1 and PSEN2 respectively.

A particular feature observed in our study was the lower glucose uptake in both PSEN1 and PSEN2 iPSC lines compared to control. Such lower uptake was accompanied by a lack of response to GLUT inhibition by GTI, and by a much lower ECAR values and glycolytic capacity compared to controls. Although the overall expression of GLUT1 appeared unchanged, we cannot exclude a possible impaired GLUT1 activity due to instrisic factor. GLUT1 has been documented to have a particular interactions with Aβ, as a recent study by Zlokovic and colleagues reported a worsened outcome in AD transgenic mice crossed with Slc2a1+/− deficient mice (22). Hence, our future direction will be to further investigate the relationships and interactions between Aβ peptides and GLUT1.

The effect of PSEN1 and PSEN2 on the BBB maturation and maintenance is intruiging. Both proteins are known to be part of the γ-secretase complex, which ultimately drives the formation of Aβ peptides. In addition, a survey of the literature also identified γ-secretase as an important modulator of the WNT signaling pathway(36, 37). WNT signaling is an important pathway involved in the development and maintenance of the BBB (38, 39). At this point, we cannot restrict and determine if the impairment of the BBB by PSEN1 is driven by an increase in Aβ production, or by an impairment of the endogenous WNT
signaling. A limitation of our study is the absence of documentation of Aβ1-40 and Aβ1-42 production by our BMECs monolayers. Assessing differential secretion of these Aβ peptides between PSEN mutants and control iPSC lines could help us better understand the contribution of each of these pathways on the BBB.

Finally, we have reported an impaired mitochondrial function (as seen by JC-1 staining) and lysosomal acidification. These are two components playing essential roles in the maintenance of energy homeostasis as well as vesicular trafficking. These two features remain largely undocumented at the BBB despite their important contribution in neurological diseases. Thus, a better understanding on how PSENs impact these pathways may increase interests in understanding the contribution of these pathways on the BBB dysfunction during neurological diseases.

Conclusion

In conclusion, this study suggests the importance of PSEN1 on the BBB development and maturation, as mutation in PSEN1 appears to have detrimental effect on the BBB function. Such study raises the importance to investigate the contribution of genetic disorders at the BBB, and the possible inclusion of a dysfunctional BBB in the pathophysiology of the disease.

Declarations

Availability of data and materials: Data are available upon request to the corresponding author.

Competing interests: The authors have no conflicts to disclose.

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Authors’ contributions: RP and SR have performed the experiments and analyzed the data. AA designed the experiments and redacted the manuscript.
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Supplementary Figures

**Supplementary Figure 1:** Phenotype of iPSC-derived neurons differentiated from the iPSC lines. Cells were differentiated into neurons following existing protocol (33, 40). Neurons were stained against nestin (red), bIII-tubulin. DAPI was used as nuclear counterstaining.

**Supplementary Figure 2:** Effect of PSEN1 and PSEN2 mutations on astrocytes glucose uptake and cell metabolic activity. (A) Glucose uptake assay in iPSC-derived astrocytes. Note the similar decrease in glucose uptake as observed in BMECs. Note the efficacy of GTI as a pharmacological inhibitor for GLUTs, as all three cell lines showed a significant decrease in glucose uptake. (B) Cell metabolic activity in astrocytes and neurons using an MTS-assay. Note the absence of differences in astrocytes, whereas a significant increased cell metabolic activity was observed in PSEN1-neurons.
PSEN2-BMECs show impaired tight junctions and barrier function. (A) Representative micrograph picture of claudin-5 and occludin immunofluorescence in iPSC-derived BMECs differentiated from control, PSEN1 and PSEN2 patients. Scale bar = 50 µm. (B) Semi-quantitative analysis of claudin-5 and occludin protein expression. Protein expression was quantified using fluorescence intensity obtained in random fields. Note the relative lower expression of claudin-5 and occludin in PSEN1 group compared to PSEN2 group. (C) TEER and (D) fluorescein permeability values in iPSC-derived BMECs at day 10 of differentiation.
Note the poor TEER value and higher permeability in PSEN1 group compared to other groups. N=3/group, ** denotes P<0.01 compared to controls.
Figure 2

A

Control    PSEN1    PSEN2

P-gp

BCRP

MRP1

B

![Bar graph showing cell uptake (RFU/μg protein) with and without inhibitors.]

- No inhibitor
- +CsA

Control    PSEN1    PSEN2

C

![Bar graph showing cell uptake (RFU/μg protein) with and without MK571.]

- No inhibitor
- +MK571

Control    PSEN1    PSEN2

D

![Bar graph showing cell uptake (RFU/μg protein) with and without Ko143.]

- No inhibitor
- +Ko143

Control    PSEN1    PSEN2
PSEN1-BMECs shows lower P-gp and MRP1 activity than control-BMECs and PSEN2-BMECs. (A) Representative micrograph pictures of P-gp, BCRP and MRP1 immunostaining in BMECs derived from the control, PSEN1 and PSEN2 iPSC lines. Scale bar = 50µm. Cell uptake assay of drug efflux substrate for assessing P-gp (B), BCRP (C) and MRPs (D) activity. Note the higher P-gp and MRPs efflux substrate uptake in PSEN1-BMECs compared to the two other iPSC lines, as well as an absence of increased of drug efflux substrate in PSEN1 following P-gp and MRPs inhibition by cyclosporine A and MK571 respectively. N=3/group, * and ** denote P<0.05 and P<0.01 respectively.
Figure 3

A

Control  PSEN1  PSEN2

GLUT1

GLUT3

GLUT4

B

-GLUT Inhibitor  +GLUT Inhibitor

Glucose uptake (µg/mg protein)

Control  PSEN1  PSEN2

C

Glutamine  25mM 2-glucose  Oligomycin  2-DG

ECAR (µP/min)

time (mins)

0 10 20 30 40 50 60 70 80 90

D

Control  PSEN1  PSEN2

Oxidative Phosphorylation

Mixed metabolism (oxidative/glycolysis)

OCR (pMoles/min)

ECAR (µP/min)

Quiescent  Glycolytic

0 15 30 45 60

E

Control  PSEN1  PSEN2

Glycolysis  Glycolytic Capacity  Glycolytic Reserve  Non-glycolytic Acidification

ECAR (µP/min)
Figure 3

Effect of PSEN mutations on glucose uptake and glycolysis. (A) Representative micrograph pictures of GLUT1, GLUT3 and GLUT4 immunostaining in BMECs derived from the control, PSEN1 and PSEN2 iPSC lines. Scale bar = 50µm. (B) Glucose uptake assay in iPSC-BMECs in absence or presence of 10µM Glucose Transporter Inhibitor II. Note the absence of glucose uptake inhibition in both the PSEN1 and PSEN2 iPSC lines. (C) Glycolytic flux analysis. Representative ECAR diagram following treatment with various inhibitor. Cells were incubated for 2 hours in glucose-free medium prior onset of experiment. Cells were maintained in medium with L-glutamine, and subsequently given 10mM D-glucose, followed by incubation with 1µM oligomycin (mitochondria respiratory chain inhibitor) and 2-deoxyglucose (100mM). (D) Energy consumption profile of iPSC-BMECs. OCR denotes oxygen consumption rate, ECAR denotes extracellular acidification rate. Note the shift of metabolic activity from “glycolytic” to “quiescent” phenotype. (E) Glycolytic parameters extrapolated. Noted the lower glycolytic capacity and reserve in PSEN1 and PSEN2 iPSC-BMECs compared to control iPSC-BMECs, whereas PSEN1 showed a lower glycolysis and non-glycolytic acidification rate. N=3/group, * denotes P<0.05 versus control group, # denotes P<0.05 versus non-inhibited group.

Figure 4
Figure 4

PSEN1 abnormally high cell metabolic activity is coupled with impaired mitochondrial function. (A) MTS assay in iPSC-derived BMECs. Cells were incubated in presence of MTS for 2 hours. MTS-conditioned medium was recovered and measured by spectrophotometry. Not the higher cell metabolic activity observed in PSEN1-BMECs compared to control-BMECs and PSEN2-BMECs. (B) JC-1 flow cytometry analysis.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.
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