Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance

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PICALM is a highly validated genetic risk factor for Alzheimer’s disease (AD). We found that reduced expression of PICALM in AD and murine brain endothelium correlated with amyloid-β (Aβ) pathology and cognitive impairment. Moreover, Picalm deficiency diminished Aβ clearance across the murine blood-brain barrier (BBB) and accelerated Aβ pathology in a manner that was reversible by endothelial PICALM re-expression. Using human brain endothelial monolayers, we found that PICALM regulated PICALM/clathrin-dependent internalization of Aβ bound to the low density lipoprotein receptor related protein-1, a key Aβ clearance receptor, and guided Aβ trafficking to Rab5 and Rab11, leading to Aβ endothelial transcytosis and clearance. PICALM levels and Aβ clearance were reduced in AD-derived endothelial monolayers, which was reversible by adenoviral-mediated PICALM transfer. Inducible pluripotent stem cell–derived human endothelial cells carrying the rs3851179 protective allele exhibited higher PICALM levels and enhanced Aβ clearance. Thus, PICALM regulates Aβ BBB transcytosis and clearance, which has implications for Aβ brain homeostasis and clearance therapy.

PICALM, the gene encoding the phosphatidylinositol-binding clathrin assembly (PICALM) protein1,2, is important for endocytosis and internalization of cell receptors3–6. PICALM also mediates intracellular trafficking of endocytic proteins7,8. Several genomewide association studies have shown the association of PICALM with AD9–13, a neurological disorder characterized by neurovascular dysfunction, elevated Aβ, tau pathology and neuronal loss14–16. However, the role of PICALM in disease pathogenesis remains elusive.

PICALM was originally postulated to affect disease by modifying trafficking of Aβ precursor protein (APP)9. Recent studies suggested that PICALM protects neurons from Aβ toxicity by reversing Aβ effects on clathrin-mediated endocytosis and/or by directing APP transport to the terminal degradation pathway by autophagosomes, which reduces Aβ production17. In contrast, viral-mediated silencing of PICALM in the hippocampal neurons in APP-overexpressing mice has been shown to diminish Aβ production, resulting in a moderate reduction in Aβ load18. Furthermore, PICALM influences the ratio of Aβ42 to total Aβ in neurons through clathrin-mediated endocytosis of γ-secretase19.

In addition to its neuronal functionality, PICALM is abundantly expressed in brain capillary endothelium20,21, a site of the BBB in vivo14, which provides a major pathway for Aβ clearance from the brain into circulation14,22,23. Thus, PICALM is ideally situated to regulate the function of brain capillary endothelial receptors, including receptors that mediate Aβ clearance such as the low-density lipoprotein receptor related protein 1 (LRP1), which binds Aβ and is a key Aβ clearance receptor at the BBB and vascular cells22–27. Thus, we hypothesized that PICALM influences Aβ clearance across the BBB and, at the molecular level, regulates the function of LRP1 in brain endothelial cells22–24. Our data suggest that endothelial PICALM has a central role in Aβ clearance and transcytosis across the BBB, which is critical for regulation of Aβ levels and homeostasis in the brain.

RESULTS

PICALM reductions in brain endothelium in AD

We found robust expression of PICALM in microvessels in aged control human brains without dementia by immunocytochemistry (Fig. 1a), immunoblotting (Fig. 1b,c) and double fluorescence immunostaining for PICALM and endothelial–specific Lycopersicon esculentum lectin (Fig. 1d and Supplementary Fig. 1a), indicating that ~65% of the endothelial cell surface area labeled with lectin was positive for PICALM in the hippocampus and cortex (Fig. 1e). PICALM levels in isolated cortical microvessels from control human brains were more than 1.7-fold

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Figure 1 PICALM reductions in brain capillary endothelium in AD. (a) PICALM and Aβ immunostaining in the prefrontal cortex of an age-matched control (Braak I, left) and AD case (Braak V–VI, right). Scale bars represent 20 μm. (b) Immunoblotting for PICALM, von Willebrand Factor (vWF), β3-tubulin, glial fibrillary acidic protein (GFAP) and GAPDH (loading control) in isolated microvessels and microvessel-depleted brains from controls (Braak 0–I) and AD cases (Braak V–VI). (c) Quantification of PICALM-positive area (percentage) occupying lectin-positive endothelial capillary profiles in the prefrontal cortex (Ctx) and the CA1 hippocampal subfield (Hpc). Data are presented as mean ± s.e.m., n = 6 cases per group; P < 0.05 by ANOVA followed by Tukey’s post hoc tests. (d) PICALM (green), lectin-positive endothelial capillary profiles (magenta) and MAP2-positive neurons (red) in the hippocampus (CA1) of an age-matched control (Braak I) and AD cases (Braak III and V–VI). Scale bar represents 20 μm. (e) Correlations between PICALM-positive area (percentage) occupying lectin-positive endothelial capillary profiles in the prefrontal cortex (Ctx) and the CA1 hippocampal subfield (Hpc). Data are presented as mean ± s.d., n = 9 controls (Braak 0–I) and 7 AD cases (Braak V–VI); P < 0.01 by Student’s t test. (f–i) Correlations between PICALM-positive area (percentage) occupying lectin-positive endothelial capillary profiles (percentage) in the prefrontal cortex and AD dementia (CDR), Braak stages, Clinical Dementia Rating (CDR, h) and Mini-Mental State Examination (MMSE, i). Each point in f–i is an individual value from 50 (f,g), 28 (h) and 37 (i) controls and AD cases, CDR and MMSE were not available for all cases. Significance was determined by Pearson and Spearman rank correlation analysis.

Higher than in capillary-depleted brain homogenates containing neurons and glia (Fig. 1b,c). In advanced AD (Braak stage V–VI), compared with controls (Braak stage I), PICALM levels were reduced in cerebral microvessels (Fig. 1a,d and Supplementary Fig. 1a) by 55–65%, as shown by immunoblotting (Fig. 1c) and double staining for PICALM and lectin, respectively (Fig. 1e). In contrast, immunostaining for PICALM and the neuronal marker microtubule-associated protein 2 (MAP2) revealed a moderate increase in PICALM levels in neurons in advanced AD (Braak V–VI) compared with controls (Fig. 1d), consistent with a previous report.28 When comparing 30 AD cases (Braak stage III–IV and V–VI) with 20 controls (Braak stage 0–I to III) (Supplementary Table 1a), we found that PICALM endothelial levels inversely correlated with Aβ load (Fig. 1f), Braak stage (Fig. 1g) and clinical dementia rating (Fig. 1h), and positively correlated with mini-mental state exam scores (Fig. 1i). As in humans, PICALM levels in murine brain microvessels were more than twofold higher than in capillary-depleted brain homogenates (Supplementary Fig. 1b). Notably, elevated Aβ levels in APP-overexpressing APP<sup>Swt</sup>/PICALM<sup>−/−</sup> mice or in endothelial cultures did not affect PICALM levels (Supplementary Fig. 1c–e), ruling out Aβ as a PICALM suppressor. Thus, endothelial loss of PICALM in AD is associated with greater Aβ and AD pathology and worse cognitive impairment.

Diminished Aβ clearance in Picalm<sup>−/−</sup> mice

To address whether PICALM is involved in Aβ clearance across the BBB in vivo and whether it contributes to worsening AD pathology, we next studied clearance of human Aβ40 and Aβ42 in Picalm<sup>−/−</sup> mice (generated as shown in Supplementary Fig. 2a). Complete knock-out of Picalm was embryonic lethal, but Picalm<sup>−/−</sup> mice developed normally and had normal blood glucose, hepatic and renal analyses (Supplementary Fig. 2b–m) and did not show behavioral changes in the first 9 months (Supplementary Fig. 2n–q). Compared with their
Figure 2 Diminished Aβ clearance in Picalm<sup>+/−</sup> mice. (a) Immunostaining for PICALM (red) and endothelial-specific lectin (blue) in brain microvessels from Picalm<sup>+/+</sup> and Picalm<sup>+/−</sup> mice. Scale bar represents 20 µm. (b) Relative abundance of PICALM protein compared with β-actin, as determined by immunoblotting and densitometry analysis in brain microvessels and microvessel-depleted brain homogenates in Picalm<sup>+/+</sup> and Picalm<sup>−/−</sup> mice. P < 0.05 by Student’s t-test; NS, non-significant. Data are presented as means ± s.e.m. from 3–4 mice per group. (c–f) Brain retention of Aβ40 (left), Aβ42 (middle) and 14C-inulin (right) (c), Aβ40 and Aβ42 clearance across the BBB (d), and by interstitial fluid (ISF) bulk flow (e) and plasma levels of Aβ40 or Aβ42 (f) after 30 min of intracerebral administration of human synthetic Aβ40, Aβ42 and 14C-inulin into the caudate nucleus of 3-month old Picalm<sup>+/+</sup> and Picalm<sup>−/−</sup> mice. Aβ in brain and plasma was determined using human-specific Aβ40 or Aβ42 ELISA. Means ± s.e.m., n = 6 mice per group. Statistical significance by Student’s t-test.

Picalm deficiency diminished Aβ40 and Aβ42 efflux across the BBB by 41% and 61%, respectively (Fig. 2d and Online Methods), but did not affect Aβ efflux via ISF compared with controls (Fig. 2e). We found 48% and 65% lower plasma levels of Aβ40 and Aβ42, respectively, in Picalm<sup>+/−</sup> mice compared with control Picalm<sup>+/+</sup> mice (Fig. 2f), confirming impaired Aβ clearance from brain to blood.

Figure 3 Diminished Aβ clearance and accelerated pathology in APP<sup>sw/0</sup>Picalm<sup>+/−</sup> mice. (a, b) ISF Aβ<sub>40</sub> and Aβ<sub>42</sub> levels monitored by in vivo hippocampal microdialysis of 3-month-old APP<sup>sw/0</sup>, Picalm<sup>−/−</sup> and APP<sup>sw/0</sup>, Picalm<sup>+/+</sup> mice. Baseline Aβ levels were monitored for 3 h. (c) The elimination half-life of ISF Aβ40 and Aβ42 determined after administration of compound E (γ-secretase inhibitor, 20 mg per kg, intraperitoneally). (d) Representative cortex and hippocampus sections stained with human Aβ-specific antibodies in 3-month-old APP<sup>sw/0</sup>, Picalm<sup>−/−</sup> and APP<sup>sw/0</sup>, Picalm<sup>+/+</sup> mice showing no Aβ deposition. Scale bar represents 100 µm. (e, f) Representative hippocampus and cortex sections stained with human Aβ-specific antibodies in 9-month-old APP<sup>sw/0</sup>, Picalm<sup>−/−</sup> and APP<sup>sw/0</sup>, Picalm<sup>+/+</sup> mice showing accelerated Aβ deposition (e) and increased Aβ load (f). In a–c and f, data are presented as means ± s.e.m., n = 5–6 mice per group. Scale bar represents 100 µm. (g–i) Behavioral changes in 9-month-old APP<sup>sw/0</sup>, Picalm<sup>−/−</sup> and APP<sup>sw/0</sup>, Picalm<sup>+/+</sup> mice studied by nest construction (g), burrowing (h), novel object recognition (NOR) (i). Data are presented as means ± s.e.m., n = 12–14 mice per group. In g–i, statistical significance was determined by Student’s t-test. In h, boxplots represent the median (dark horizontal line), with the box representing the 25th and 75th percentiles, and the whiskers the 5th and 95th percentile.
Figure 4  Endothelial specific rescue of PICALM deficiency in the hippocampus of APPsw/0; Picalm+/– mice. (a) The scheme of endothelial-specific rescue of PICALM in APP in APPsw/0; Picalm+/–; Tie2-Cre mice using an AAV carrying a Cre recombinase-dependent expression cassette of Flag-Picalm transgene (Online Methods). (b) Expression of Flag-PICALM in endothelium of 5-month-old APPsw/0; Picalm+/–; Tie2-Cre mouse after AAV-Flex-Picalm administration in the hippocampus. Lectin, endothelial-specific marker. Co-injection of AAV-Syn1-GFP showed insignificant expression of Flag-PICALM in neurons (<3%). Scale bar represents 20 µm. (c-e) Endothelial-specific expression of PICALM in the ipsilateral hippocampus of APPsw/0; Picalm+/–; Tie2-Cre mice injected with AAV-Flex-Picalm (b) reduced Aβ deposition (c,d) and Aβ40 and Aβ42 accumulation (e) at 6 months of age compared with the contralateral hippocampus injected with AAV-Flex control. Cortex that was not injected with AAV-Flex-Picalm showed no changes in Aβ load (c,d). Red lines in d indicate average values. P < 0.01 by paired Wilcoxon signed rank test. Scale bar represents 100 µm. (f,g) Bilateral hippocampal administration of AAV-Flex-Picalm compared with AAV-Flex (control) improved behavior in 6-month-old APPsw/0; Picalm+/–; Tie2-Cre mice. Data are presented as means ± s.e.m., n = 10 mice per group. P < 0.05 by Student’s t-test.

Effects of Picalm deficiency and endothelial-specific rescue in APPsw/0 mice

To address whether Picalm deficiency can influence Aβ pathology, we crossed transgenic APPsw/0 mice, which develop Aβ elevation and correlative memory deficits9, with Picalm+/– mice. PICALM expression in APPsw/0; Picalm+/– mice was reduced by ~70% in microvessels and ~50% in capillary-depleted brain compared with their littermate controls (Supplementary Fig. 3a,b). Using hippocampal in vivo microdialysis27 (Online Methods), we found a substantial 2.4- to 2.5-fold increase in the steady-state levels of soluble Aβ40 and Aβ42 in brain ISF of 3-month-old APPsw/0; Picalm+/– mice compared with age-matched littermate controls, respectively (Fig. 3a,b). After intraperitoneal injection of the γ-secretase inhibitor Compound E, the half-life of Aβ40 and Aβ42 in brain ISF27 was increased in APPsw/0; Picalm+/– mice compared with APPsw/0; Picalm++/+ controls from 1.2 to 1.9 h and 1.4 to 2.5 h, respectively (Fig. 3c), suggesting that the increase of ISF Aβ levels was a result of diminished Aβ clearance. The increase in ISF Aβ levels preceded Aβ and amyloid deposition. Deposits were absent in 3-month-old APPsw/0; Picalm+/– mice (Fig. 3d), but began to accumulate at 6 months of age, as shown by the increase in Aβ load in the hippocampus and cortex and accelerated development of cerebral amyloid angiopathy (Supplementary Fig. 3c,d). Notably, 9-month-old APPsw/0; Picalm+/– mice had a substantial 3.5–4-fold increase in Aβ load in the cortex and hippocampus (Fig. 3e,f), which was associated with worse performance in behavioral tests, including nest construction, burrowing, novel object location and novel object recognition (Fig. 3g–i), compared with age-matched littermate controls.

To specifically address the role of endothelial PICALM in transvascular Aβ clearance, we performed a rescue experiment. We generated APPsw/0; Picalm+/–; Tie2-Cre mice and an adeno-associated viral (AAV-Flex-Picalm) construct for delivery of a Cre-dependent expression cassette (Flex-Picalm) specifically to brain endothelium in the hippocampus (Fig. 4a). After administration of AAV-Flex-Tdtomato into the hippocampus of 5-month-old APPsw/0; Picalm+/–; Tie2-Cre mice, more than 50% of lectin-positive endothelial vascular profiles expressed Tie2-Cre–dependent Tdtomato (Supplementary Fig. 4a). Co-injection of AAV-Flex-Tdtomato and AAV-Syn1-GFP revealed that less than 3% of hippocampal neurons expressed Tdtomato. Together, these data confirm Tie2-Cre–dependent endothelial-specific expression of transgene and minimal leakage in neurons. Similar results were obtained after administration of AAV-Flex-Picalm into the hippocampus of 5-month-old APPsw/0; Picalm+/–; Tie2-Cre mice with Tie2-Cre–dependent Flag-PICALM expression in more than 50% lectin-positive endothelial vascular profiles, and we observed negligible expression in neurons after co-injection of AAV-Flex-Picalm and AAV-Syn1-GFP (Fig. 4b). PICALM re-expression in endothelium of APPsw/0; Picalm+/–; Tie2-Cre mice after AAV-Flex-Picalm administration in the ipsilateral hippocampus diminished Aβ load, Aβ40 and Aβ42 levels by 63%, 46% and 37%, respectively, compared with the contralateral hippocampus injected with AAV-Flex (control) virus (Fig. 4c–e and Supplementary Fig. 4b). Moreover, bilateral administration of AAV-Flex-Picalm in the left and right hippocampus improved behavior in APPsw/0; Picalm+/–; Tie2-Cre mice compared with AAV-Flex (control), as shown in a separate group of mice (Fig. 4f,g). These data strongly support our hypothesis that brain endothelial PICALM has a central role in regulating Aβ clearance from the brain by controlling its efflux at the BBB.

Neither Picalm+/– nor APPsw/0; Picalm+/– mice showed changes in Aβ production and processing15,16, brain microvascular expression of
HA-LRP4 and Flag-PICALM were used as loading controls. HA-tagged C-terminal LRP1 mutants (LRP4T100) by antibody to Flag (IP: Flag) in HEK293T cells after transfection with Flag-PICALM and hLRP1 C-terminus fusion protein (GST-LRP1C). (followed by 1 min at 37 °C to initiate LRP1 internalization. Values at 4 °C were taken as 100%. Data are presented as means ± s.d. from three primary isolates in triplicate. P < 0.05 by Student’s t test. (e) Coimmunoprecipitation of PICALM, CHC and clathrin adaptor protein α-adaptin (AP-2) by LRP1-specific antibody (IP: LRP1) in BEC 30 s or 5 min after stimulation with Aβ40 (1 nM); IgG, non-immune IgG. (f) LRP1 internalization in control BEC (vehicle) and after transfection with scrambled siRNA (si. Scramble) and/or siRNAs targeting PICALM (si. PICALM) or CHC (si. CHC). Aβ40 (1 nM) was applied for 15 min at 4 °C followed by 1 min at 37 °C to initiate LRP1 internalization. Values at 4 °C were taken as 100%. Data are presented as means ± s.d. from three primary isolates in triplicate. P < 0.05 by ANOVA followed by Tukey’s post hoc tests. (g) In vitro binding of human recombinant PICALM to GST-tagged LRP1 C-terminus fusion protein (GST-LRP1C). (h) C-terminal mutants of the human LRP1 minigene (LRP4T100). (i) Co-immunoprecipitation of HA-tagged C-terminal LRP1 mutants (LRP4T100) by antibody to Flag (IP: Flag) in HEK293T cells after transfection with Flag-PICALM and HA-LRP4T100 mutants. HA-LRP4 and Flag-PICALM were used as loading controls.

Figure 5 PICALM/clathrin-dependent endocytosis of Aβ-LRP1 complex by brain endothelial cells. (a,b) Colocalization of LRP1-Aβ40 complex with PICALM (a) and CHC (b) in human BECs within 30 s of FAM-Aβ40 (250 nM) treatment. (c) Immunostaining for LRP1, PICALM and CHC without Aβ (–Aβ). DAPI, nuclear staining (blue). Insets, higher magnification of boxed region. Scale bars represent 10 μm. (d) Quantification of LRP1 puncta colocalized with PICALM (a,c) and with CHC (b,c), and FAM-Aβ40 puncta colocalized with LRP1 and PICALM (a,b). Data are presented as means ± s.d. from three primary isolates in triplicate. P < 0.05 by Student’s t test. (e) Coimmunoprecipitation of PICALM, CHC and clathrin adaptor protein α-adaptin (AP-2) by LRP1-specific antibody (IP: LRP1) in BEC 30 s or 5 min after stimulation with Aβ40 (1 nM); IgG, non-immune IgG. (f) LRP1 internalization in control BEC (vehicle) and after transfection with scrambled siRNA (si. Scramble) and/or siRNAs targeting PICALM (si. PICALM) or CHC (si. CHC). Aβ40 (1 nM) was applied for 15 min at 4 °C followed by 1 min at 37 °C to initiate LRP1 internalization. Values at 4 °C were taken as 100%. Data are presented as means ± s.d. from three primary isolates in triplicate. P < 0.05 by ANOVA followed by Tukey’s post hoc tests. (g) In vitro binding of human recombinant PICALM to GST-tagged LRP1 C-terminus fusion protein (GST-LRP1C). (h) C-terminal mutants of the human LRP1 minigene (LRP4T100). (i) Co-immunoprecipitation of HA-tagged C-terminal LRP1 mutants (LRP4T100) by antibody to Flag (IP: Flag) in HEK293T cells after transfection with Flag-PICALM and HA-LRP4T100 mutants. HA-LRP4 and Flag-PICALM were used as loading controls.

major Aβ transporters, including P-glycoprotein, LRP1 and RAGE, or Aβ-degrading enzymes, including neprilysin and insulin-degrading enzyme, compared with their respective controls (Supplementary Fig. 5), which make these mechanisms unlikely contributors to decreased Aβ clearance and/or increased Aβ accumulation.

**PICALM/clathrin-dependent endocytosis of Aβ-LRP1 complex by endothelial cells**

To elucidate the molecular mechanism(s) underlying PICALM regulation of Aβ clearance across the BBB, we studied Aβ internalization and trafficking in primary human brain endothelial cells (BECs) and in an **in vitro** model of the BBB. We found that Aβ40 bound to the cell surface LRP1 in BECs at 4 °C, as reported previously. (Supplementary Fig. 6a, Supplementary Table 1 and Online Methods), and that 6-carboxyfluorescein-labeled (FAM)-Aβ40-LRP1 complex colocalized rapidly (≤30 s) with PICALM (Fig. 5a) and proteins responsible for PICALM/clathrin-dependent internalization of ligands, at 37 °C, including the clathrin heavy chain (CHC, Fig. 5b–d) and clathrin adaptor protein α-adaptin (AP-2; Supplementary Fig. 6b). Similar to Aβ40, FAM-Aβ42, but not scrambled FAM-Aβ42 (Supplementary Fig. 6c,d), led to a rapid increase in colocalized Aβ-LRP1/PICALM puncta. Rapid recruitment...
of PICALM and the clathrin endocytic apparatus to LRP1 was confirmed by co-immunoprecipitation analysis 30 s after Aβ40 treatment at 1 nM, a concentration corresponding to Aβ40 levels in the CSF.6,16 (Fig. 5e). In contrast with PICALM, which remains associated with LRP1 over longer periods of time, CHC and AP-2 dissociated early from LRP1 (Fig. 5e), consistent with rapid uncoating of clathrin from internalized vesicles.6 siRNA inhibition of PICALM or CHC inhibited Aβ40-induced LRP1 internalization (Fig. 5f), suggesting that endocytosis of Aβ-LRP1 complex requires both PICALM and clathrin.

To further understand PICALM interaction with LRP1, we studied binding of human recombinant PICALM to a human recombinant glutathione S-transferase (GST)-tagged LRP1 C-terminus fusion protein, which indicated direct binding of PICALM to LRP1 C-terminus (Fig. 5g), but not to GST (Supplementary Fig. 6e). Although this experiment was carried out in the absence of cells, we cannot rule out the possibility that some intermediary proteins in the cellular milieu can facilitate and/or influence PICALM binding to LRP1. Using co-immunoprecipitation analysis after transfection of cells with PICALM and various mutants of the C-terminus LRP1 cytoplasmic tail,13 we found that PICALM binding to LRP1 required the YXXL motif (Fig. 5h,i).

To determine the specificity of Aβ as a ligand that enhances the binding of PICALM to the cytoplasmic tail of LRP1, we studied other LRPLigands, including apolipoprotein E (apoE) and activated α2-macroglobulin (α2M*). Incubation of BECs with astrocyte-derived lipided apoE3, apoE4 (ref. 32) and α2M* did not result in a rapid binding of PICALM to LRP1, whereas Aβ40 binding to apoE3, apoE4 and α2M* inhibited PICALM binding to LRP1 (Supplementary Fig. 7a). Consistent with these data, binding of Aβ40 to apoE4, apoE3 and α2M* inhibited Aβ40-induced internalization of LRP1 (Supplementary Fig. 7b,c). Collectively, these data suggest that binding of Aβ to the ectodomain of LRP1 has a unique conformational effect on its cytoplasmic C-terminus tail, enhancing the binding of PICALM, which initiates PICALM/clathrin-dependent endocytosis of Aβ-LRP1 complex.

PICALM associates with LRP1 during Aβ transcytosis across an endothelial monolayer

We next used an in vitro model of the BBB6,10 to study the role of PICALM in Aβ transport across fully confluent (~97%) human brain endothelial monolayer co-cultured with pericyte-conditioned medium, as pericytes critically influence the BBB properties.13–15 The endothelial monolayer had a typical cobblestone pattern of the zonula occludens 1 (ZO-1) tight junction protein and a cortical distribution of the F-actin cytoskeleton and expressed PICALM (Fig. 6a). A transmonolayer electrical resistance (TEER) of ~280 Ω cm² and a low paracellular permeability constant (P) of ~1.21 × 10⁻⁶ cm s⁻¹ was determined for dextran (molecular weight, 40 kDa), a metabolically inert polamer molecule, confirmed formation of the barrier.

To ascertain whether our endothelial monolayer model is suitable for Aβ transport studies, we determined cell polarity for two key Aβ transporters, LRP1 and the receptor for advanced glycation end products (RAGE), which are expressed in brain endothelium in vivo mainly at the abluminal (basolateral) and luminal (apical) side, respectively,14 as confirmed by signal intensity profile analysis across capillary lumens in human brain (Supplementary Fig. 8a,b). Consistent with in vivo findings, LRP1 and RAGE chiefly colocalized toward the basolateral and apical membrane of the endothelial monolayer, respectively (Supplementary Fig. 8c). PICALM associated with LRP1-Aβ40 complex in the endothelial monolayer rapidly after Aβ application to the basolateral membrane (Fig. 6b), as it did in BEC cultures (Fig. 5a,d,e).

The proximity ligation assay (PLA)32,36 confirmed that PICALM associates rapidly with LRP1 in the endothelial monolayer after Aβ40 (1 nM) addition to the basolateral membrane with a peak at 30 s and a plateau over 4 min (Fig. 6c,d). In contrast, LRP1-clathrin association peaked in 30 s and was followed by a sharp decline in 1 min (Fig. 6d), consistent with co-immunoprecipitation data showing rapid dissociation of clathrin from internalized LRP1 (Fig. 5e).

To further study the roles of LRP1, PICALM and CHC in Aβ internalization at the basolateral endothelial membrane and the basolateral-to-apical transendothelial transport (transcytosis), we used siRNA silencing. Aβ40 (1 nM) internalization was rapid, with a t1/2 of ~17 s (Fig. 6e), as reported for LRP1-mediated endocytosis.15 siRNA inhibition of LRP1, PICALM or CHC substantially diminished Aβ internalization by >90% in 1 min compared with control scrambled siRNA (Fig. 6e), suggesting that all three molecules are essential for Aβ internalization. Basolateral-to-apical transcytosis of Aβ40 (1 nM, determined by Aβ40 ELISA measurements in the basolateral and apical chambers corrected for paracellular diffusion by subtracting diffusion values of inulin, a metabolically inert polamer molecule; Online Methods) was inhibited by the receptor-associated protein.
Figure 7 PICALM interacts with Rab5 and Rab11 during Aβ transcytosis across endothelial monolayer. (a) Colocalization between PICALM (red) and Rab5 (green) in primary human BMECs cultured with FAM-Aβ40 (250 nM) for 2 min. (b) Lack of association between PICALM (red) and Rab7 (green) in BMECs cultured with FAM-Aβ40 for 5 min. (c) Colocalization between PICALM (red) and Rab11 (green) in BMECs cultured with FAM-Aβ40 for 5 min. DAPI, nuclear staining (blue). Insets, high-magnification depicting colocalization. In a–c, scale bars represent 10 μm. (d) Quantification of colocalization between PICALM and Rab5, Rab7 or Rab11 puncta in a–c. Data are presented as mean ± s.e.m. from three primary isolates in triplicates. P < 0.05 by Student's t test. (e,f) Colocalization of FAM-Aβ40 (green) with Rab5 (magenta, upper) or Rab11b (magenta, bottom) 2 and 4 min after Aβ internalization at the basolateral side of endothelial monolayer, respectively. Arrows denote colocalized white puncta. Scale bar represents 10 μm. (g) PLA of PICALM-Rab11 association (relative abundance) 0, 2 and 4 min after addition of Aβ40 (1 nM) to the basolateral membrane. (h) PLA of PICALM-Rab5 association (relative abundance) 0, 2 and 4 min after addition of Aβ40 (1 nM) to the basolateral membrane. In i, j and k, data are presented as mean ± s.e.m. from three primary isolates in triplicates. P < 0.05 by ANOVA followed by Tukey’s post hoc test. (l,m) Inhibition of Rab5 (l) and Rab11 (m) GTPase activity by si.PICALM compared with si.Scramble control.

(RAP) and LRPI-specific antibodies, but not by other low-density lipoprotein receptor–specific antibodies (Supplementary Fig. 8d). LRPI siRNA, but not scrambled siRNA, also inhibited Aβ40 basolateral-to-apical transendothelial transport (Supplementary Fig. 8d). These data confirm that Aβ binding to LRPI initiates Aβ clearance at the basolateral membrane of the monolayer, as previously suggested, and that LRPI is required for Aβ transcytosis across the monolayer. As expected, silencing PICALM and CHC inhibited Aβ40 transcytosis across the monolayer by approximately 85%, as shown within 30 min of the unidirectional basolateral-to-apical transendothelial Aβ transport (Fig. 6f,g). Within 5 min of Aβ application to the basolateral membrane of the endothelial monolayer, Aβ-LRP1 internalized complex was already found near the apical membrane of the monolayer (Supplementary Fig. 9a), confirming basolateral-to-apical trans-endothelial transport.

PICALM guides Aβ trafficking to Rab 5 and Rab 11 leading to Aβ endothelial transcytosis

Given that PICALM remains associated with LRPI over longer periods of time in contrast to CHC and AP-2 that dissociate rapidly from internalized Aβ-LRP1 vesicles (Figs. 5e and 6d), we next traced intracellular trafficking of PICALM and Aβ-LRP1 using molecular markers for different steps of the endosomal pathway. Because Rab5 and Rab7 regulate Aβ endosomal trafficking in neurons, we asked whether PICALM interacts with Rab GTPases following Aβ-LRP1 internalization. Using subconfluent endothelial cultures, we found
that PICALM and Aβ40-LRP1 complex colocalize with Rab5- and EE1A-positive early endosomes within 2 min of Aβ40 (1 nM) treatment (Fig. 7a and Supplementary Fig. 10a,b). However, Rab7, a GTPase that directs fusion of late endosomes with lysosomes leading to degradation of ligands (Fig. 7b) or the lysosomal-specific marker LAMP1 (Supplementary Fig. 10c)38. Rather, PICALM colocalized with Rab11 (Fig. 7c), a GTPase that regulates recycling of vesicles controlling transcytosis and exocytosis of ligands. Aβ42 also colocalized with Rab5 and Rab11 (Supplementary Fig. 11a,b). In the absence of Aβ, PICALM minimally colocalized with Rab5, Rab7 and/or Rab11 (Supplementary Fig. 11c–e). These data suggest that PICALM likely regulates trans-endothelial Aβ trafficking by shunting Aβ away from a degradation pathway toward a transcytotic pathway, consistent with previously demonstrated clathrin-independent functions of PICALM2,8,17. Notably, the peak co-localization between PICALM and Rab5 (Fig. 7a,d) was somewhat higher than the peak co-localization between PICALM and Rab11 (Fig. 7c,d), suggesting that transfer of PICALM-containing endocytic vesicles from Rab5 to Rab11 could potentially be a rate-limiting step in Aβ transcytosis.

Using an in vitro endothelial monolayer model of the BBB, we next found that FAM-Aβ40 associated with Rab5 and Rab11 in the endothelium within 2 and 4 min of its application to the basolateral membrane, respectively (Fig. 7c,f). PICALM-Rab11 association peaked at 4 min of incubation with Aβ40 at the basolateral membrane and remained at a plateau at 5 min (Fig. 7g,h), whereas PICALM-Rab5 association peaked at 2 min and declined in 3–4 min (Fig. 7h), as shown by PLA analysis. PICALM-Rab7 association was undetectable (Fig. 7h). PICALM interaction with LRP1, Rab5 and Rab11 at different time points after Aβ40 treatment has been corroborated by co-immunoprecipitation analysis (Fig. 7i).

Consistent with findings showing that Rab5 is necessary for the biogenesis of early and late endosomes44, dominant-negative Rab5-S34N mutant (Online Methods) inhibited Aβ transcytosis by more than 85% as compared with EGFP control (Fig. 7j,k). Dominant-negative Rab11-S25N mutant, but not Rab7-T22N mutant, also inhibited unidirectional Aβ40 basolateral-to-apical transport by 75% (Fig. 7j,k). These data suggest that Rab11 likely controls later stages of Aβ transcytosis across the monolayer, consistent with findings showing that Rab11 regulates trafficking of vesicles containing transcytosis and exocytosis of ligands. siRNA inhibition of Rab11b, but not Rab11a, blocked Aβ transcytosis (Supplementary Fig. 9b), consistent with findings that Rab11b is the major Rab11 isoform in brain endothelium, whereas Rab11a is a major isoform in epithelial cells40–42, as we confirmed (Supplementary Fig. 9c).

Notably, siRNA knockdown of PICALM inhibited Rab5 and Rab11 GTPase activity in the endothelial monolayers treated with Aβ40 by 80% and 95%, respectively (Fig. 7i,m), indicating that PICALM binding to Rab5 and Rab11 is critical for maintaining Rab5 and Rab11 GTPase activity during endothelial trafficking of Aβ. Consistent with findings showing that PICALM does not directly Aβ to lysosomes for degradation, Aβ was more than 95% intact within 30 min of unidirectional transcytosis and clearance across the monolayer (Supplementary Fig. 9d), as reported previously23. Collectively, our data indicate that PICALM controls the transcytotic pathway mediating Aβ clearance across the BBB.

**PICALM levels and Aβ clearance by endothelial monolayers from AD patients**

To determine the role of PICALM in Aβ clearance in AD brain endothelium (Supplementary Table 1b), we studied Aβ transcytosis across AD-derived endothelial monolayers co-cultured

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**Figure 8** Aβ transcytosis across AD-derived endothelial monolayer and iPSC-derived endothelium carrying the rs3851179 PICALM variants. (a) QRT-PCR and western blot analysis of PICALM in control and AD endothelial monolayers. (b) Diminished Aβ40 (1 nM) transcytosis across AD-derived endothelium from basolateral to apical side and reversal by adenosinergic-mediated Ad.PICALM re-expression. Ad.mLRP1, LRP1 minigene. In a and b, data are presented as mean ± s.e.m., from eight isolates in triplicate for control and AD monolayers. (c) Diagram of CRISPR/Cas9-based generation of isogenic iPSC lines homozygous for the protective (A) or non-protective (G) allele of rs3851179. gRNA, guide RNA. (d) SspI restriction digest of PCR products from iPSC genomic DNA at rs3851179 region. Asterisk denotes the CRISPR-Cas9–modified iPSC line. (e) Sanger sequencing of iPSCs at rs3851179 confirming independent isogenic lines homozygous for either the G or A variant. (f) FACS dot plot showing 15.7% of iPSC-derived endothelial cells via embryoid body (EB) formation were positive for endothelial markers CD31 and VE-Cadherin. (g) iPSC-derived endothelial cells co-cultured with pericyte-conditioned media formed monolayer in vitro with ZO–1–positive tight junctions (green). Scale bar represents 100 μm. (h,i) qRT-PCR and western blot analysis of PICALM (h) and Aβ40 (1 nM) transcytosis (i) in human iPSC-derived endothelial monolayers carrying the protective rs3851179 (AA) variant and the non-protective rs3851179 (GG) variant. In h.i, data are presented as means ± s.e.m. from six cultures for each rs3851179 variant in triplicate.
with pericyte-conditioned media. The AD endothelial monolayers had a normal cobblestone pattern, TEER values and paracellular permeability (Supplementary Fig. 12a–c). Consistent with diminished PICALM levels in AD brain endothelium in situ and in brain capillaries (Fig. 1), PICALM mRNA and protein levels were reduced by 34–35% in cultured AD BEC (Fig. 8a), resulting in ~50% diminished basolateral-to-apical transcytosis of Aβ across AD-derived endothelial monolayers compared with age-matched controls (Fig. 8b). LRPI levels were also reduced in AD brain endothelium22,23, whereas AP-2, CHC, Rab5 and Rab11b expression was comparable to controls (data not shown). Adenoviral-mediated transfer of PICALM compared with GFP control (Supplementary Fig. 12d) substantially improved Aβ transcytosis by 63%, whereas co-transfer of PICALM and LRPI mini-gene32 improved Aβ transport by 89% (Fig. 8b), suggesting that PICALM can be therapeutically targeted.

Aβ clearance by iPSC-derived endothelium carrying the rs3851179 PICALM variants

All AD-associated single-nucleotide polymorphisms (SNPs) in PICALM are located upstream of the coding region of the gene, and no mutation in the PICALM protein has been identified to influence AD risk10–13. Some AD-associated PICALM SNPs have been suggested, however, to influence PICALM expression, for example, rs659023 variants in peripheral blood mononuclear cells15. To address whether certain PICALM variants can influence PICALM expression and Aβ clearance in endothelial cells, we focused on highly validated and replicated rs3851179 PICALM variants whose rs3851179A allele is associated with a lower risk of AD than the rs3851179G allele9,10,12. To test the phenotypic differences between the minor protective rs3851179A allele and the major non-protective rs3851179G allele in human endothelial cells, we used inducible pluripotent stem cells (iPSCs). The iPSCs were generated from lymphoblasts from Coriell using the episomal plasmids and encoding factors as we described previously46. We next used CRISPR/Cas9 genome editing47 to generate the isogenic homozygous iPSC lines for the two homozygous allelic variants (Fig. 8c–e) followed by direct differentiation to generate bona fide endothelial cells48 and endothelial monolayers (Fig. 8f,g and Online Methods). iPSC-derived endothelial cells carrying the protective rs3851179A allele had 72–78% higher expression levels of PICALM mRNA and protein (Fig. 8h) and 120% higher Aβ clearance (Fig. 8i) than the non-protective rs3851179G allele.

DISCUSSION

We found that PICALM reductions in brain endothelium in AD correlated with Aβ and AD neuropathology and cognitive impairment, whereas reduction and re-expression of PICALM in endothelial cells influenced Aβ clearance at the BBB, Aβ deposition in the mouse brain and phenotypic manifestations of behavior in mice. At a molecular level, using an in vitro endothelial monolayer that approximates BBB, we found that Aβ binding to the ectodomain of LRPI enhanced the binding of PICALM, which initiated PICALM/clathrin-dependent endocytosis of Aβ-LRPI complex, and PICALM remained associated with LRPI after Aβ internalization and directed Aβ trafficking to Rab5 and Rab11, leading to Aβ transcytosis (Supplementary Fig. 13). PICALM levels and Aβ clearance were greatly reduced in AD-derived endothelial monolayers, which was reversible by adenoaviral-mediated PICALM transfer. Using iPSC-derived human endothelial cells carrying rs3851179 PICALM variants, we found that the protective rs3851179 allele led to a higher PICALM expression and enhanced Aβ clearance by endothelial cells. AAV8 viral-mediated silencing of neuronal PICALM in the hippocampus modestly reduced Aβ production in APP/PS1 mice18, but whether a moderate reduction in Aβ load, as we observed, is beneficial for neurons after inactivation of PICALM is unclear, as PICALM also protects neurons against Aβ toxicity4. Mice with PICALM loss-of-function allele developed accelerated Aβ accumulation and had lower behavioral test performance than controls, raising the possibility that, in models of global Picalm deficiency, where PICALM is deleted in multiple cell populations (for example, endothelial cells, neurons), the effect of Picalm loss from endothelium causing faulty Aβ vascular clearance may override the discrepant reduction in Aβ secretion from neurons18. Future studies in transgenic mice with inducible PICALM deletion in neurons should be carried out to address its Aβ-independent effects, particularly as PICALM regulates axonal growth49 and turnover of synaptic vesicles and receptors50.

Our data revealed that PICALM guides intracellular trafficking of Aβ-LRPI to Rab5 and Rab11, leading to Aβ transcytosis across endothelial monolayer, which does not require clathrin beyond the early internalization steps. These findings are consistent with previously demonstrated clathrin-independent functions of PICALM in trafficking of endocytic proteins28,17,17.

At present, little is known about upstream regulators of PICALM expression. Using APP-overexpressing mice and brain endothelial cultures, we found that elevated Aβ does not suppress PICALM in endothelium. Multiple factors in AD including turbulent capillary flow, oxidant stress, hypoxia or inflammation can affect gene expression in endothelial cells44, potentially leading to PICALM reductions in endothelium. Identifying inhibitors of PICALM expression in AD endothelium will require future studies interrogating multiple genetic, environmental and local brain factors. Consistent with the idea that some AD-associated PICALM SNPs may influence PICALM expression22,42, using iPSCs, we found that the protective rs3851179 allele had a major effect on PICALM expression and Aβ clearance by endothelial cells. Future studies correlating PICALM levels in endothelium in human brain tissue with the genotype of patients in large cohorts of control and AD patients could provide additional information as to how different PICALM SNPs relate to each other and/or to other genes that influence Aβ clearance, as for example apoE or clusterin16, and interrogate the roles of vascular risk factors, environment and lifestyle. Collectively, our findings suggest that PICALM controls Aβ transport across the BBB and clearance from brain, and is therefore an important therapeutic target for Aβ clearance therapy.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHORS CONTRIBUTIONS

Z.Z., A.RS. and Q.M. designed and performed the experiments and analyzed the data. M.R.H., P.K., K.K., N.C.O., S.V.R., G.S., A.A. and T.S. performed the experiments. E.A.W. performed the pilot experiments. A.R. performed in vivo...
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ONLINE METHODS

Reagents. We used the following: 6-carboxyfluorescein-labeled (FAM)-Aβ40 (catalog #23514), FAM-Aβ42 (catalog #23526) and scrambled FAM-Aβ42 (catalog #60892) (Anaspec); unlabeled Aβ40 and Aβ42 (Biopure); anti-HA monoclonal antibody (catalog #H96/68, 1:5,000, western blot) and anti-Flag monoclonal antibody (catalog #F3165, 1:5,000 for western blot, 1:200 for co-immunoprecipitation) (Sigma); anti-Clathrin heavy chain monoclonal antibody (Thermo Scientific, catalog #MA1-065, 1:2,000 for western blot, 1:500 for immunostaining); rabbit monoclonal anti-LRP1 antibody (EPR3724) (Abcam, catalog #ab92544, 1:5,000 for western blot; 1:200 for immunostaining); rabbit polyclonal anti-PICALM antibody (Sigma, catalog #H96/0961, 1:5,000 for western blot, 1:200 for immunostaining); goat polyclonal anti-PICALM antibody from Santa Cruz (catalog #SC-6433, 1:2,000 for western blot, 1:100 for immunostaining); rabbit polyclonal anti-EEA1 antibody (catalog #2411), Rab5 β (catalog #3547), Rab7 (catalog #3967), Rab11 (catalog #5589), Rab11a (catalog #2413), Rab1b (catalog #2414) (Cell Signaling, 1:2,000 for western blot; 1:100 for immunostaining); mouse monoclonal anti-LAMP1 antibody (HA43) (Abcam, catalog #ab25630, 1:200 for immunostaining); rabbit polyclonal anti-AP2 alpha antibody (Abcam, catalog #ab116289, 1:2,000 for western blot); Rab5-534N (catalog #28045), Rab7-T22N (catalog #12660) and Rab11-S25N (catalog #12680) (Addgene). Human Rab11B gene was cloned from human adult brain cDNA library (Invitrogen, catalog #D8030) by polymerase chain reaction (PCR) and sub-cloned into pEGLP-C1 vector (Clontech) using BamHI site. Rab11B-S25N mutant was generated by site-directed mutagenesis using GeneArt mutagenesis system (Invitrogen, catalog #A13282). The constructs carrying the membrane-containing minireceptor of LRP1 and its mutants were provided by G. Bu (Mayo Clinic)26. Human PICALM cDNA clone (Openbiosystem, clone ID: BC22969) was subcloned into pCDNA5-Flag vector or pEASY-1 vector (Agilent, catalog #24005) using pShuttle-CMV vector (Agilent, catalog #24007) for viral production. Adenoviral vector carrying LRPI minigene (Ad.mLRPI) was prepared as we previously reported22. We also used a complete EDTA free cocktail of protease inhibitors and phosphatase inhibitors (Roche Applied Sciences); human recombinant RAP (EMD Biosciences); monoclonal mouse antibody P2-1 specific for human APP (1:1,000, 1 mg ml−1) and 22C11 that recognizes mouse and human APP (1:1,000, 0.5 mg ml−1) (Chemicon International); RAGE-specific IgG (1:500, 1 mg ml−1)24; rabbit anti-NICD antibody (Millipore, catalog #07-1232) and rabbit polyclonal anti-human Aβ (Cell Signaling Technology, catalog #8243; 1:200). Secondary antibodies were: Alexa 488-conjugated donkey anti-rabbit (Invitrogen; A11008; 1:200), Alexa 568-conjugated donkey anti-goat (Invitrogen; A11057; 1:200), Alexa 647-conjugated donkey anti-goat (Invitrogen; A21447; 1:200), Alexa 488-conjugated donkey anti-mouse (Invitrogen; A21202; 1:200), Alexa 647-conjugated donkey anti-mouse (Invitrogen; A31571; 1:200); HRP-conjugated donkey anti-mouse (Invitrogen; A16005; 1:5,000), and HRP-conjugated donkey anti-rabbit (Invitrogen; A16029; 1:5,000). Nuclei were stained with Hoechst 33342 (Invitrogen; 1:10,000).

Human postmortem studies. Tissue samples. Post-mortem paraffin embedded human frontal cortex and hippocampus samples were obtained from the Rush University Medical Center and the University of Southern California. Informed consent was obtained and the study approved by the Institutional Review Board of Rush University Medical Center and the University of Southern California. All autopsy cases underwent neuropathological evaluation of AD including assignment of Braak stages. Aged subjects that did not carry diagnosis of AD or another neurogenerative disease and showed neuropathological findings within the normal range for age were used as age-matched controls. MMSE and CDR information were available for most but not all individuals. Frozen brain tissue specimens were from the prefrontal cortical gray matter (Brodmann area 9, 10) and were snap-frozen and stored at −80 °C. A total of 20 controls and 30 AD individuals were used for histopathological analyses. The demographic information of all cases is provided in Supplementary Table 1a. This study used sample ND10689 from the NINDS Cell Line Repository (https://catalog.coriell.org/1/NINDS), as well as clinical data.

Histopathological analyses. Heat-induced antigen retrieval was performed following Dako’s protocol. For immunohistochemistry analysis of PICALM or Aβ, ImmPRESS Polymer-Bound Immunohistochemistry Reagents (Vector Laboratories) were used for visualization. For immunofluorescence analysis, species-specific fluorochrome-conjugated secondary antibodies were incubated for 1 h at 25 °C, and blood vessels were stained by Dylight 488–conjugated L. esculentum lectin for 1 h at 25 °C. All slices were scanned using Zeiss 510 confocal microscope with Zeiss Apochromat water immersion objectives (Carl Zeiss MicroImaging).

Isolation of cerebral microvessels and microvessel-depleted brain. These were isolated from frozen cerebral tissue samples using dextran gradient centrifugation followed by sequential cell-strainer filtration as we have previously described31,52.

Animals. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California and were consistent with US National Institutes of Health guidelines. All animals were included in the study. Animals of both sexes 3, 6 and 9 months old were used in the experiments. All animals were randomized for their phenotype information. All experiments were blinded; the operators responsible for the experimental procedures and data analysis were blinded and unaware of group allocation throughout the experiments.

Generation of Picalm+/− mice. Gene-targeting strategy for generating Picalm knockout mice is shown in Supplementary Figure 2. For details regarding generation of different Picalm mutant strains and conditional knockout mice, please see ref. 53. Genotyping of the mutant mice was performed by PCR of tail DNA with the following primers: PicalmFW CATAAAGTATGTTTCCGCTTACA and PicalmRV CCAATCTCAGGGGTCTAAAG.

Generation of APPsw/w0, Picalm+/−/w mice. APPsw/w0 mice expressing human APP transgene with the K670M/N671L (Swedish) double-mutation under control of the hamster prion promoter52 were crossed with Picalm+/− mice to generate Picalm-deficient APPsw/w0, Picalm+/−/w mice and their corresponding littermate controls. All experiments were performed using age-matched littermates.

In vivo BBB efflux. CNS clearance of unlabeled synthetic human Aβ40 and Aβ42 peptides was determined simultaneously with 14C-inulin (reference marker) in male Picalm+/− mice and littermate controls at 3 months of age using a procedure we described previously22,23. Brain and blood were sampled 30 min after Aβ injection and prepared for Aβ40 or Aβ42 ELISA27 and 14C-radioactivity analysis, as described previously22,23,25,54. The percentage of Aβ40 or 14C-radioactivity remaining in the brain after microinjection was determined as percentage recovery in brain = 100 × (Nb/N0), where Nb is the amount of Aβ40 or 14C-inulin remaining in the brain at the end of the experiment and N0 is the amount of Aβ40 or 14C-inulin simultaneously injected into the brain ISF, that is, the amount of intact Aβ40 based on ELISA and the d.p.m. for 14C-inulin, respectively. The percentage of Aβ cleaved through the BBB was calculated as \[ \frac{1}{2} \times \left( \frac{N_b(N_0 - N_b)}{N_0} \right) \times 100 \], using a standard time of 30 min.

In vivo microdialysis and ISF Aβ half-life determination. In vivo microdialysis was used to measure soluble Aβ40 and Aβ42 steady state levels in the hippocampus of awake, freely moving 3-month-old APPsw/w0, Picalm+/−/w and APPsw/w0, Picalm+/−/w mice, as we recently described in detail27. Microdialysates were collected every 60 min into polypropylene tubes in a refrigerated fraction collector (Havard Apparatus). A stable baseline ISF Aβ40 and Aβ42 concentrations were obtained within 4 h followed by an intraperitoneal injection of compound E (20 mg per kg, Millipore)27. The t1/2 of Aβ was calculated in GraphPad Prism 5.0 software using the slope (k) of the linear regression that included all fractions between drug delivery and when Aβ concentrations plateau (t1/2 = 0.693/k, where k = 2.303kt1/2).
Western ECL detection buffers (Millipore), exposed to CL-XPosure film (Thermo Scientific) and developed in a X-OMAT 3000 RA film processor (Kodak), or ChemiDoc XRS system from Bio-Rad. Please see Supplementary Figure 14 for original western blots.

**Tissue staining.** Mice anesthetized as described above were transcardially perfused with PBS containing 5 U ml⁻¹ heparin followed by 4% paraformaldehyde. OCT-embedded frozen brain tissue was cryosectioned at a thickness of 14–18 μm. All images were taken with a Zeiss 510 confocal microscopy or using the BZ 9000 all-in-one Fluorescence Microscope from Keyence, and analyzed using ImageJ software (US National Institutes of Health).

**Behavioral analyses.** Novel object location and recognition tests, nest construction test and burrowing test were performed as we reported²³,³².

**Endothelial specific rescue of PICALM deficiency.** Endothelial specific rescue of PICALM was achieved by adeno-associated viral delivery of a Cre-dependent expression cassette (Flex-Picalm) to 5-month-old APP/PS1; Picalm⁻/⁻; Tie2-Cre mice. The strategy was first validated with AAV-Flex-tatTomato virus (100 nl, 5 × 10¹² GC ml⁻¹) and AAV-Syn1-GFP virus (20 nl, 5 × 10¹² GC ml⁻¹) that were injected simultaneously into the hippocampus. AAV-Flex-Picalm construct was generated by cloning the coding region of mouse Picalm gene into the pAAV-FLEX-tatTomato (Addgene, Plasmid #28306) to replace the tatTomato. AAV-Flex-Picalm viruses were packaged by Penn Vector Core (6 × 10¹² GC ml⁻¹) and 100 nl was used for each injection. The HBD mutant AAV-DJ/8 serotype (Cell Biologics) was chosen for its high in vivo transduction efficiency and vascular tropism.⁵⁵

**Viral injections.** Surgical procedures were performed under general anesthesia with isoflurane (1–2%) using the SomnoSuite Small Animal Anesthesia System (Kent Scientific) and developed in a X-OMAT 3000 RA film processor (Kodak), or Western ECL detection buffers (Millipore) and developed in a X-OMAT 3000 RA film processor (Kodak), or ChemiDoc XRS system from Bio-Rad. Please see Supplementary Figure 14 for original western blots.

**Primary human brain endothelial cell cultures.** Isolation and characterization. We isolated and characterized BECs from rapid brain autopsies from the frontal cortex (area 9/10) from neurologically intact age-matched controls and AD patients as we previously described²⁴,²⁵,⁵⁶. For clinical and neuropathological analysis at least 20 cells from five different randomly selected fields in each culture (replicate) were selected.

**In vitro PICALM binding to LRP1.** Recombinant human PICALM protein (Transcript variant 1, 70.6 kDa) was purchased from Origene (catalog #TP313791). The LRP1 C-terminal sequence (312 nucleotides) was cloned from the Human Brain cDNA library by PCR and sub-cloned into pGEX-4T1 vector using BamH1 and NotI sites. GST and GST-tagged LRP1 C terminus fusion protein (330 amino acids) were produced in BL21 E. coli cells and purified with glutathione magnetic beads (Pierce, catalog #88822) following manufacturer’s procedure. In vitro binding assay was performed using 5 μg of PICALM and 2 μg of GST fusion proteins (on the beads) in Tris-buffered saline at 25 °C for 1 h, and eluted with 25 mM glutathione. HEK 293T cells. HEK 293T cells (ATCC) were cultured in high glucose DMEM medium supplemented with 10% FBS and penicillin (100 U ml⁻¹) and streptomycin (100 μg ml⁻¹) (Invitrogen) at 37 °C with 5% CO₂. For transfection, 60–70% confluent cells in 10 cm plates were transfected with 20 μg of maxi-prepped plasmid DNA using Lipofectamine LTX.

**In vitro model of the BBB.** A single monolayer of fully confluent (>97%) human BEC was produced by plating 5 × 10⁵ cells cm⁻² in the upper chamber of a 6-well or 24-well tissue culture inserts (BD BioCoat, catalog #356408) pre-coated with rat tail collagen I and fibronectin, as we reported²⁶. Cells were cultured in Endogrow media for 2 d and then switched to pericyte-conditioned medium in RPMI 1640 supplemented with 0.1% FBS to enhance the BBB properties including barrier function and polarity.⁵⁰,³²

**Immunocytochemistry.** The monolayers were washed in PBS, fixed in 4% paraformaldehyde for 10 min, blocked with 10% normal swine serum (Vector Laboratories) for 1 h at 25 °C and incubated with different primary antibodies specific for PICALM and LR1P1, CHC and LR1P1, and PICALM and Rab5, Rab7 or Rab11 overnight at 4 °C (see Reagents).

**In situ proximity ligation assay (PLA).** The PLA was performed as we previously reported²⁷.

**siRNA and Rab constructs.** siPICALM (catalog #s15799 and s1800), sLRP1 (catalog #s8279 and s8280), sCHC (catalog #s475 and s477), sRAB11a (catalog #s16702 and s16703), sRAB11b (catalog #s17647 and s17648) were purchased from Invitrogen. Two siRNAs against distinct sites were used to mitigate against off-target effects. Rab constructs were purchased from Addgene or generated as described in the Reagents section. BEC were transfected using Neon transfection system (Invitrogen) following the manufacturer’s instruction.

**Rab GTPase activity.** Rab5 and Rab11 GTPase activity was determined using Rab5 activation assay kit (NewEast Biosciences, catalog #83701) and Rab11 activation assay kit (NewEast Biosciences, catalog #83201), respectively.

**Adenoviral-mediated transfer.** BEC were transduced with adenoviral vectors carrying PICALM and LR1P1 minigene³² 24 h after plating. Cultures were then switched to pericyte conditioned medium within 24 h.

**Aβ internalization and transendothelial transport.** These experiments were carried out at 37 °C. Human unlabeled Aβ40 was added to the basolateral (lower) chamber at a final concentration of 1 nM. The amount of Aβ40 internalized by the monolayers was determined by human Aβ40 ELISA.²⁷ Aβ uptake by BEC was expressed in pmol mg⁻¹ protein as we previously described²¹. For transendothelial transport (transcytosis) studies, Aβ40 was added to the basolateral (lower) chamber at a final concentration of 1 nM simultaneously with ¹⁴C-inulin (molecular weight, ~5,000 kDa), a metabolically inert polar tracer that is not taken up by the vascular cells, as we reported²¹,²², ¹⁴C-inulin was used to correct for the non-specific paracellular leakage of transport across the monolayers, as reported²¹,²⁶. The intactness of Aβ40 (>95%) following transport across the BEC monolayer was confirmed in several assay buffer samples from the apical and basolateral chambers using high pressure liquid chromatography, as previously reported²¹,²⁷.

**Permeability of the endothelial barrier.** We used fluorescein isothiocyanate (FITC) labeled dextrans (40 kDa and 2,000 kDa; Invitrogen), as described²⁰. The BBB permeability to dextran was expressed as a permeability coefficient in cm/s as we described²⁰. Briefly, the volume cleared (AVC) of each time point was calculated using \( \Delta V = C_{\text{lower}} \times V_{\text{lower}} / C_{\text{upper}} \), where \( C_{\text{upper}} \) and \( C_{\text{lower}} \) are FITC-labeled dextran concentrations in upper and lower chambers, respectively, and \( V_{\text{lower}} \) is the volume in lower chamber. The volume cleared (AVC) was plotted against time, and the permeability surface area (PS) product was obtained from the slope by linear regression. The permeability coefficient (P) was then calculated by \( P = PS/s \), where s is the surface area of the filter (1.12 cm²). Finally, the permeability coefficient of cells (Pcell) was obtained by correcting the overall permeability coefficient (Pcell-filtered) for that of the cell-free filter (Pfilter) using \( 1/P_{\text{cell}} = 1/P_{\text{cell-filtered}} - 1/P_{\text{filter}} \). Pcell-filtered was determined on a separate series of experiments using the cell-free filter inserts only.

**iPSC culture.** The iPSCs were generated from lymphoblasts from Coriell (catalog ID: ND10689) using the episomal plasmids, encoding 6 factors using the cell-free filter inserts only.
ATATCTTGTGGAAAGGACGAAACACCGTGAGGTTTACTACTGCAAG-3' and R: 5'-GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCTT GCAGTAGTAAACCTCAC-3'.

**Electroporation.** Plasmids were electroporated using Nucleofector Kits for Human Dermal Fibroblast (Lonza) according to the manufacturer’s protocol. Briefly, after washing with PBS, iPSCs were treated with Accutase (Life Technologies) to dissociate single cells. These cells were suspended with Nucleofector solution and supplement (Lonza) and mixed with gRNA expression vector, Cas9 coding pSpCas9(BB)-2A-Puro (Addgene) and double-strand plasmid DNA for donor. The donor plasmid was constructed by cloning the flanking regions of rs3851179 amplified by primers as follows; F: 5'-ACCCATCACCCTTCTGTTTG-3' and R: 5'-TTTTCCAGCAAGTTGGGTTC-3'. Puromycin selections were started at 24 h after electroporation and continued 3 to 4 days at the concentration of 0.75 µg ml⁻¹. After withdrawal of puromycin, cells were cultured in mTeSR until suitably sized colonies appeared. Colonies were picked up and genomic DNAs were extracted using QuickExtract DNA Extraction Solution 1.0 (epicentre) according to manufacturer’s protocol. Target site was amplified by PCR using primers as follows; F: 5'-CCCGCTTCATAGGGTTATTG-3' and R: 5'-AACTCACCCCAGTCTCTTGC-3'. We confirmed suspected mutant clones by direct sequencing of the PCR products. We used Sanger sequencing of iPSCs at rs3851179 to verify independent isogenic lines homozygous for either the G or A variant.

**Endothelial differentiation.** To generate endothelial cells, we differentiated mutated and original iPSC clones as embryoid bodies (EBs) as described. Two weeks after differentiation, the cells were FACS sorted for endothelial markers CD31 and VE-Cadherin; the isolated double positive cells were characterized with monolayer and tight junction formation, did not express pericyte (PDGFRβ), neuronal (β3-tubulin) or astrocyte (GFAP) markers, and had TEER of ~230 Ω cm² cultured with addition of pericyte-derived conditioned media.

**Statistical analysis.** Sample sizes were calculated using nQUERY assuming a two-sided alpha-level of 0.05, 80% power, and homogeneous variances for the two samples to be compared, with the means and common s.d. for different parameters predicted from published data and our previous studies. Shapiro-Wilk test was used to test normality of the data. F test was conducted to ensure that the data meets the assumptions of the tests and the variance was similar between the groups that are statistically compared. Data were analyzed by Student’s t test for comparison between two groups; or by multifactorial analysis of variance (ANOVA) followed by Tukey’s post hoc tests for multiple comparisons; or paired Wilcoxon’s signed rank test for paired non-parametric comparison. Statistical significance for correlation analyses was performed by Pearson and Spearman rank correlation analysis tests. A P value less than 0.05 was considered statistically significant.

A **Supplementary Methods Checklist** is available.

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