Formin protein Daam2 modifies neural circuit and neurovascular unit in the CNS

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BACKGROUND

Astrocytes are the most diverse and abundant glial cells in the CNS, and play dynamic roles in synaptogenesis, neurotransmission, neural circuit formation, and blood-brain-barrier formation. Consequently, dysregulation of astrocytes is closely associated with numerous neurological disorders and malignancies. Extraordinarily complex morphology of astrocytes is essential for their diverse functions during development as well as in pathological condition. Despite its' importance, yet the molecular and cellular mechanisms that link astrocyte morphology to neural circuit and neurovascular development remain largely undiscovered. Formin proteins are key regulators of the cytoskeleton and cellular morphology; however, their role in astrocytes morphogenesis and associated physiological functions is unknown. Formin protein Daam2 is highly expressed in astrocytes while its' function in astrocyte morphology and function remains enigmatic.

METHOD

For loss- and gain-of-functional studies during development and in disease model, whole-body and astrocyte-specific conditional knockout mice for Daam2, and viral manipulation are used. To decipher morphological alterations in astrocytes, sparse single cell labeling method is used and images are analyzed by IMARIS. Astrocytic calcium imaging and electrophysiological recording of neuronal activity were performed to investigate physiological alterations by Daam2 loss. To reveal function of Daam2 in pathological condition, photothrombotic stroke is induced and integrity of neurovascular unit is analyzed by examination of BBB leakage and cellular markers.

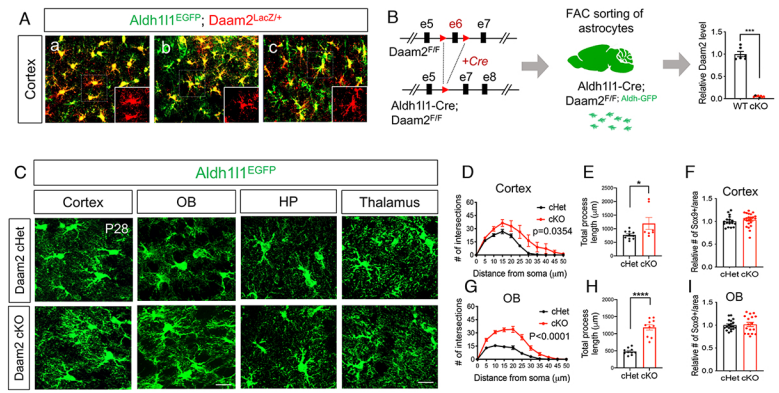

Fig 1: Loss of Daam2 increases morphological complexity of astrocytes in the brain. (A) Immunostaining from Daam2$^{LacZ/+}$ animals with Aldh1l1-EGFP reporter indicates Daam2 (Red) is expressed in astrocytes (Green). (B) Schematic of the generation of astrocyte specific deletion of Daam2 in Aldh1l1-Cre and Daam2-floxed mice. Quantitative RT-PCR in FAC-sorted astrocytes confirms significant reduction of Daam2 transcript from Daam2 cKO mice. (C) Representative images of astrocytes labeled with Aldh1l1-EGFP reporter in various brain regions of Daam2 cHet and cKO mice at postnatal day 28. (D-E, G-H) Overall complexity of astrocytes with Aldh1l1-EGFP reporter was measured by Sholl analysis and total process length. (F, I) Quantification of astrocyte marker, Sox9+ cells in cortex and olfactory bulb of Daam2 cHet and Daam2 cKO mice.

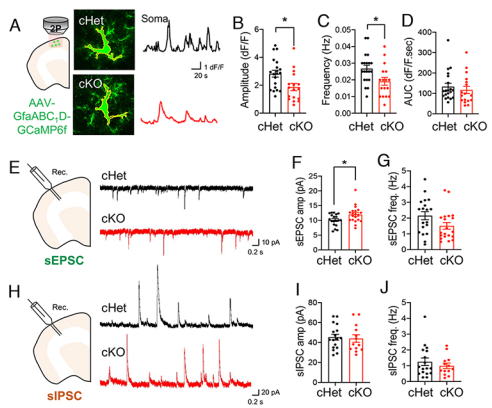

Fig 3: Deletion of Daam2 alters astrocytic calcium dynamics and synaptic activity. (A) Schematics of astrocytic calcium imaging from ex vivo slices and representative images of spontaneous calcium signaling (green) of Daam2 cHet and cKO mice injected with AAV-GFAP-GCaMP6f virus. Approximate territory of astrocytes including soma and multiple microdomains is outlined in yellow. Representative traces of GCaMP6f signal in Daam2 cHet and cKO astrocytes are shown in black and red respectively. (B-D) Quantification of amplitude, frequency, and area under curve (AUC) of △F/F GCaMP6f signal events. Daam2-deficient astrocyte have lower amplitude and frequency of signal. (E, H) Schematics of whole cell recording in ex vivo brain slices and representative traces of spontaneous EPSC and IPSC in neurons in cortex from Daam2 cHet and cKO mice. (F-G, I-J) Quantification of amplitude and frequency of sEPSC and sIPSC in each genotype.

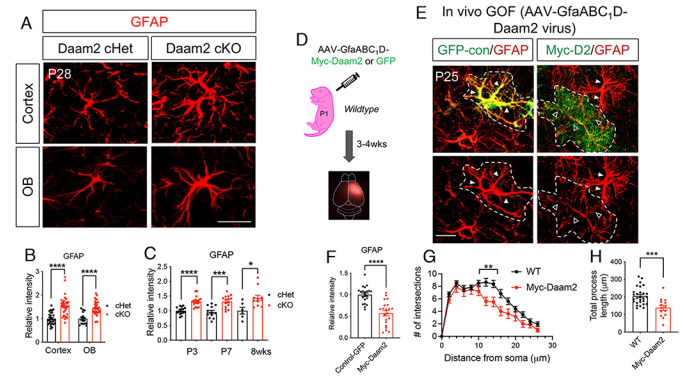

Fig 2: Deletion of Daam2 increases intermediate filament GFAP whereas overexpression of Daam2 reduces GFAP and morphological complexity. (A) Representative images of GFAP immunostaining in Daam2 cKO mice compared to controls in cortex and OB at P28. (B) Quantification of GFAP immunostaining in Daam2 cHet and cKO mice in fortex and PB at P28. (C) Quantification of GFAP immunostaining in Daam2 cHet and cKO mice from P3 to adult. (D) Schematic of virus injection to overexpress Daam2 in the cortex. (E) Overexpression of Daam2 in wildtype revealed reduced GFAP intensity compared to animals injected with control virus. (F-H) Quantification of GFAP immunostaining in control virus-positive cells and Daam2 overexpressing cells. Sholl analysis and quantification of total length of filament with overexpression of Daam2

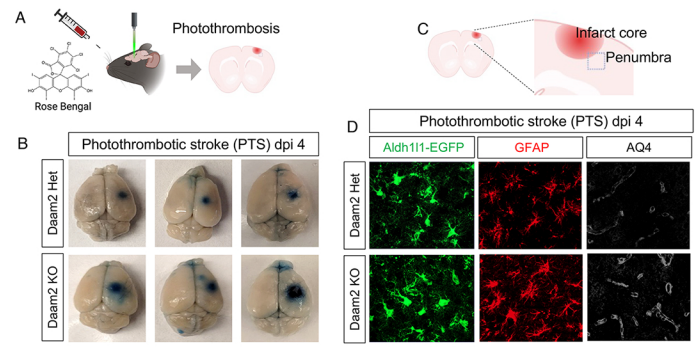

Fig 4: Loss of Daam2 results in increased microvascular bleeding after ischemic injury. (A) Schematics of Photothrombotic stroke (PTS) in mice induced by Rose Bengal dye. (B) Microvascular bleeding after 4 days post PTS is visialized by injection of Evans Blue dye. Daam2 KO animal shows increased leakage after injury compared to Het animal. (C) Schematics of stroke induced-injury in cortex. Penumbra is imaged in (D) for reactive astrocytes. (D) Increased reactive gliosis is observed in Daam2 KO with Aldh1l1-EGFP and immunostaining of GFAP. Astrocyte endfeet marker, Auaporin 4 (AQ4) is enhanced in Daam2 KO.

RESULTS

We found Daam2 is enriched in astrocytes in multiple brain regions. Loss of Daam2 in astrocytes results in increased morphological complexity of astrocytes and expression of GFAP in cortex. Morphological changes induced by loss of Dama2 elicits inhibition effects on astrocytic calcium dynamics in cortex. Moreover, loss of Daam2 in astrocytes modifies synaptogenesis and resulted in increased excitatory synaptic transmission but no changes in inhibitory synaptic activity in cortex. Since astrocyte is also important for blood-brain-barrier function, we investigated function of Daam2 in Photothrombotic stroke induced injury model. We found that loss of Daam2 also increased Aquaporin 4 expression, suggesting alterations in astrocyte endfeet and cerebrovascular function. More, loss of Daam2 results in cerebrovascular impairment and aggravates BBB leakage by injury, leading to delayed tissue repair.

CONCLUSION

This work focuses on critical functions of Daam2 in astrocytes during CNS development. Using a combination of complementary loss- and gain-of-functional studies, in vivo calcium imaging, electrophysiological recording, and injury model, we revealed new mechanisms regulating morphological maturation, physiological properties of astrocytes. Further, we found changes in astrocyte morphology can differentially influence excitatory and inhibitory circuit function during brain development. Importantly, we found function of Daam2 in neurovascular unit, providing further insight into that alterations in morphological complexity also modify astrocytic contacts with the cerebrovasculature through endfeet processes.