Argininosuccinate lyase deficiency increases blood brain barrier permeability through altered endothelial junctional protein expression

Hsiang-chun Chang, MD, PhD,[1] Jordan Kho, PhD,[1] Zixue Jin, PhD,[1] Lindsay C Burrage, MD, PhD,[1] Sandesh CS Nagamani, MBBS, MD,[1] Rita Shack,[2] Robia G Pautler, PhD,[2] Hannah P Thompson,[3] Akihiko Urayama, PhD,[3] and Brendan Lee, MD, PhD[1]

1. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston TX, USA
2. Department of Integrative Physiology, Baylor College of Medicine, Houston TX, USA
3. Department of Neurology, McGovern Medical School, University of Texas Houston, Houston TX, USA

Pathogenic variants of argininosuccinate lyase (ASL) gene cause argininosuccinic aciduria (ASA), one of the urea cycle disorders. Independent of hyperammonemia episodes, patients with ASA also develop systemic complications, including liver fibrosis, refractory systemic hypertension, and progressive neurocognitive decline. We have previously demonstrated that ASL is critical for cell-autonomous nitric oxide (NO) production through its role in nitric oxide synthase complex assembly and channeling of the substrate arginine into the complex. However, it is unclear how nitric oxide deficiency contributes to the neurological symptoms in patients. In current study, we observed increased blood brain barrier permeability in hypomorphic $Asl^{neo/neo}$ mice and increased paracellular permeability in cultured human brain microvascular endothelial cells (hBMECs) with ASL modulation. RNA-sequencing experiment using hBMEC with ASL downregulation identifies significant changes in cell junctional proteins. Specifically, normal junctional proteins, including claudin 5 (CLDN5) and tight junctional protein 1 (TJP1) are downregulated while pathological junctional protein claudin 1 (CLDN1) is upregulated. Exogenous NO reduces the CLDN1 protein level in hBMECs with ASL dowregulation and restores the paracellular permeability. This effect is comparable to direct modulation of CLDN1 expression. Furthermore, NO supplementation *in vivo* restores the BBB integrity in $Asl^{neo/neo}$ mice. Together, our findings suggest that ASL regulates BBB integrity through alteration of cell junctional protein profiles, and may

have implications in novel therapies for patients with ASA.