

# Oligodendrocyte Dysfunction and Global CNS Dysmyelination Occur in Arginase Deficiency that is Prevented with AAV-based Hepatic Gene Therapy

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## AIMS

- To understand the effects and mechanism of Arginase 1 deficiency on the developing nervous system and how it relates to dysmyelination.
- To visualize and quantify the difference in expression of myelination and related proteins in the developing murine brains between wild type, Arginase 1 deficient, and AAV-treated Arginase 1 deficient mice.

## RESULTS

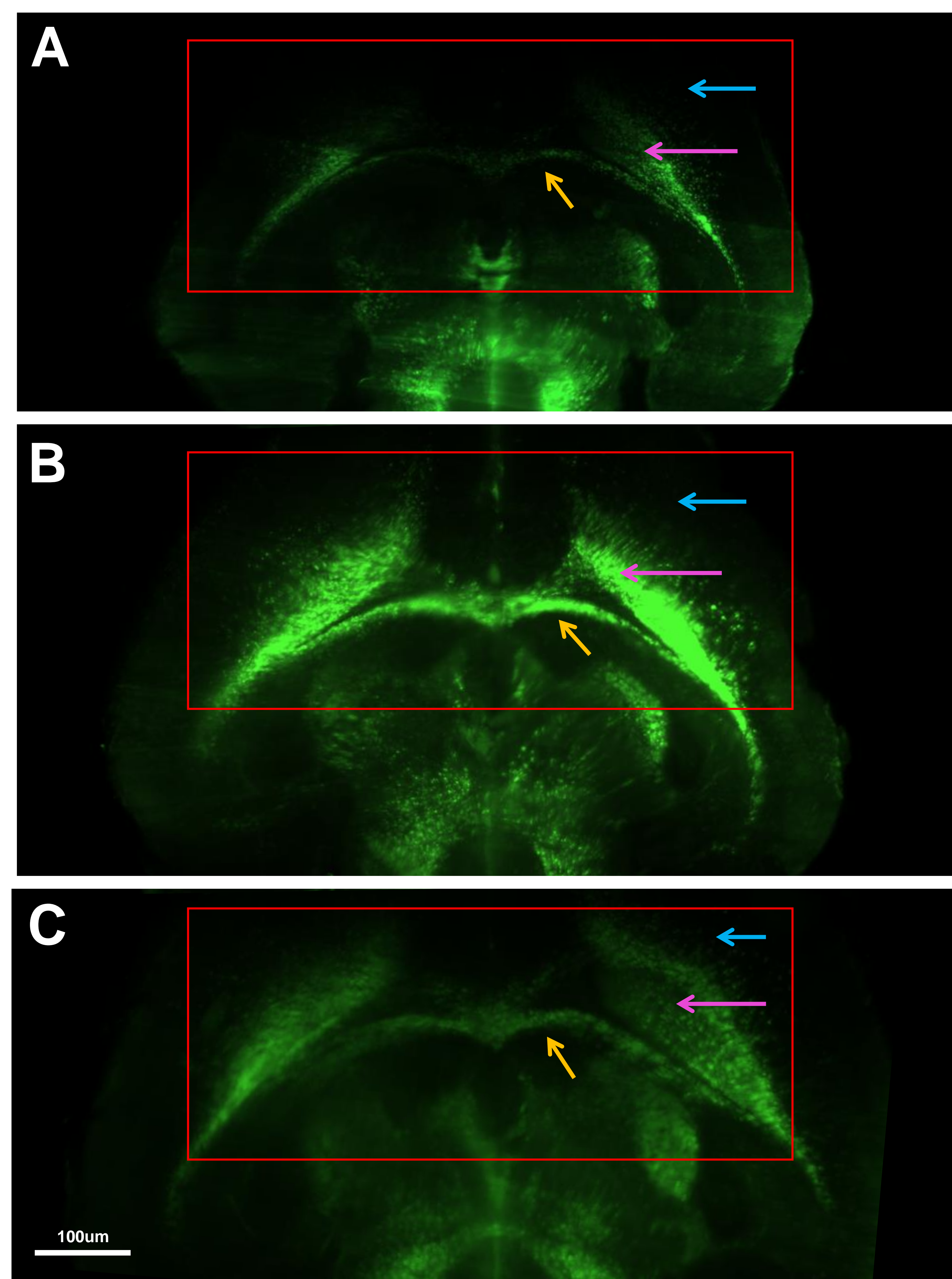


Figure 1: Imaris 3D rendered Arg1/PLP-eGFP CLARITY-cleared brains imaged with light sheet microscopy. Brains were imaged at 4x magnification in the transverse plane of (A) Arg1-KO (B) TKO and (C) WT P12 mice. Red box outlines the frontal lobe of the cortex. Arrows indicate regions of interest within CST. Blue arrow points to the corpus callosum (part of white matter motor cortex), pink arrow points to the caudoputamen (part of striatum); yellow arrow points to the fimbria (white matter leading to hippocampal commissure; part of striatum). Scale bar represents 100µm.

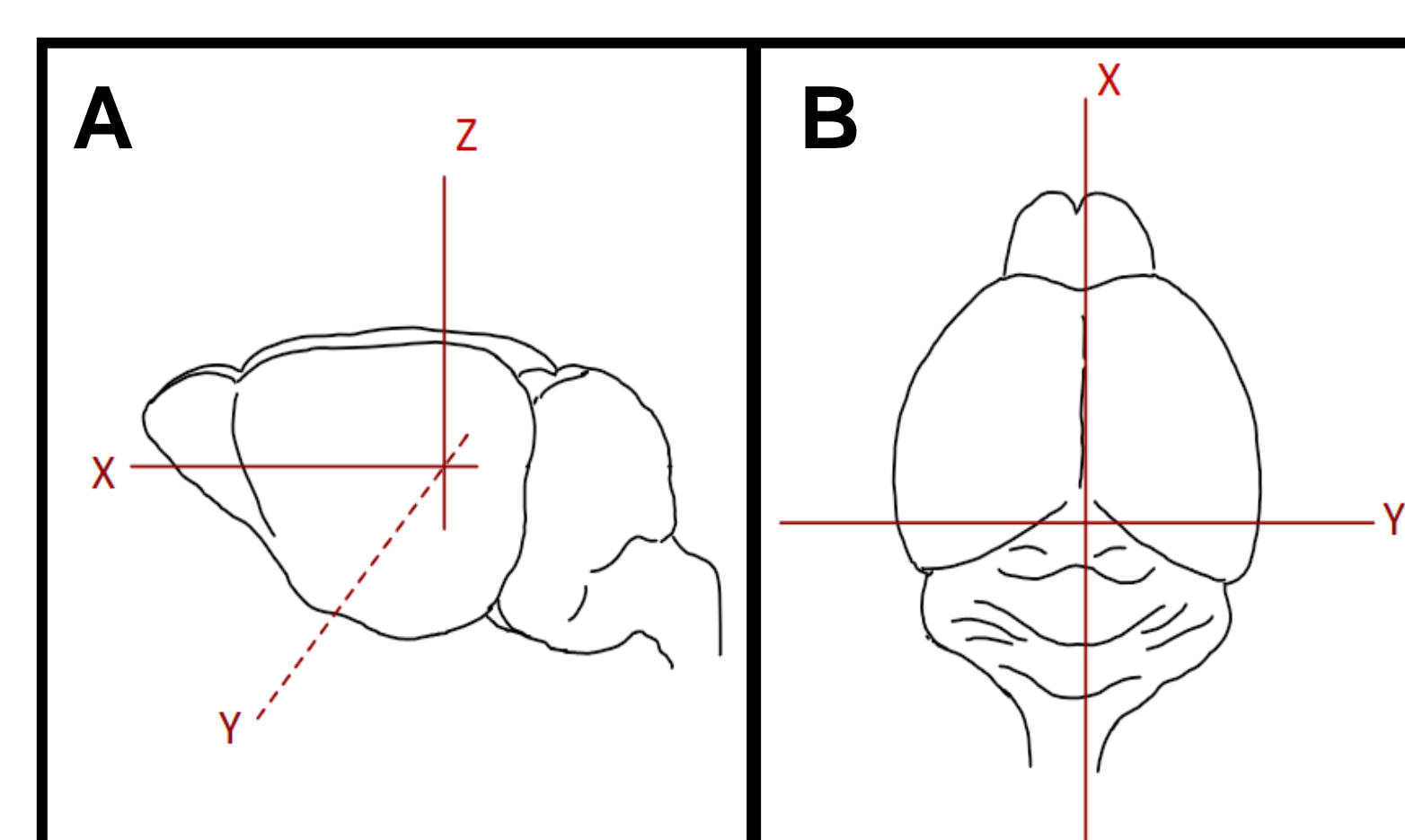


Figure 2: Orientation of brains for light sheet microscopy imaging. (A) Defines the orientation of the X-Y-Z axes used. (B) Shows position of brains in the transverse plane used to acquisition all images.

## BACKGROUND

### Arginase (Arg1):

- Arg1 functions predominantly in the liver as a catalyst in the urea cycle; Arginase deficiency (AD) results from a loss of hepatic Arg1.
- AD-associated neuromotor manifestations have recently been attributed to dysmyelination in the corticospinal tract (CST).

### Preliminary Data:

- Microarray data indicate a down-regulation of myelination and oligodendrocyte (OL) related genes in Arg1 knockout (Arg1-KO) mice (Liu et al., 2019).
- Our previous work in brain pathology for Arg1-KO mice shows decreased myelin density in subcortical white matter of the motor cortex and pyramidal tract (Liu et al., 2019).
- We seek to further understand the dysmyelination in the developing Arg1-KO CNS, explore potential mechanisms of its cause, and evaluate the efficacy of our gene therapy for its prevention.

## METHODS

### Visualization of CNS Myelination Levels:

- Post-natal day (P)12 Arg1/PLP-eGFP whole mouse brains were cleared by active CLARITY low current electrophoretic tissue clearing. Myelin proteolipid protein 1 (PLP) was used as a marker of myelination.
- Image acquisition performed by light sheet microscopy and 3D rendering done by IMaris.

### Electron Microscopy:

- Myelination was quantified in subcortical white matter and pyramidal tract of Arg1-KO, WT, and Arg1-KO AAV-treated (TKO) mice at time points P2, P6, P10, and P14 by electron microscopy. WT and TKO mice were also examined at 4 months.

### Protein Expression:

- Expression of myelin-basic protein (MBP), oligodendrocyte transcription factor 2 (Olig2), and neurofilament heavy chain protein (NeuH) were examined by western blot analysis on frontal cortex of Arg1-KO, WT, and TKO mice at P12.

## DISCUSSION

### CLARITY:

- Preliminary imaging of cleared P12 Arg1/PLP-eGFP brains shows decrease signal in Arg1-KO mice, most notably in regions of the CST. Signal is restored in P12 TKO brains.
- Observed decrease in PLP-eGFP signal appears more reduced in the Arg1-KO in regions originating in the cerebral cortex than in the structures of the mid brain. This could be in part due to the directionality of myelination development from brain stem to cortex.
- Greater signal intensity observed in TKO than WT speculated to be from repair or overcompensation of myelination due to delay between P2 AAV treatment and onset of oligodendrogenesis (prenatal).

### Electron Microscopy:

- Arg1-KO mice show reduced myelination in the CST with almost no myelination in the subcortical white matter and little in the pyramidal tract by P14; when found, OLs appear to be inactive
- Quantitatively, the subcortical white matter and pyramidal tract are severely dysmyelinated during development.
- In TKO mice, WT-level myelination density is maintained in CST in the long-term (4 months).

### Protein Expression:

- Western blot analysis of P12 mice suggests decrease in MBP, NeuH, and Olig2 expression in the frontal cortex of A1-KO mice. TKO mice show NeuH and Olig2 expression at levels comparable to WT.
- Olig2 and MBP reduction in Arg1-KO mice suggests a potential dysmyelination mechanism by impairment of OL development. Reduction of NeuH suggests axonal damage due to lack of myelination. Early postnatal gene therapy appears to restore OL function and prevent axonal damage.

## ACKNOWLEDGEMENTS

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