Reengineering an brain endothelial-specific adeno-associated virus for transcytosis into the CNS in mice

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Abstract

Adeno-associated viruses (AAV) are small, non-pathogenic viruses that are used by researchers to deliver genetic material into the brain. Recent efforts have utilized small amino acid insertions to the capsid protein, essentially loops, to enhance crossing the blood brain barrier (BBB) for better treatment of the CNS. The first step in crossing the BBB for AAVs is to be taken in by brain endothelial cells, where they may be escorted into the brain parenchyma by a process termed "transcytosis". The engineered AAV "X1.1" efficiently targets brain endothelial cells but is not transcytosed; presumably because of its tight association with low-density lipoprotein receptor-related protein 6 (LRP6). In our project, we characterized different engineered strains of X1.1 with weaker LRP6 affinity to determine how well they were able to deliver genetic cargo by transcytosis into the brain rather than deliver payload to brain endothelial cells. To determine transduction, the AAVs encode the green fluorescent protein (GFP). Six new variants of X1.1 were characterized that had single amino acid substitutions in the engineered loop. Some of the variants we tested did not enter endothelial cells or the brain; however, two of the variants were able to enter the CNS, showing targeting for brain endothelial cells, neurons and glia. Two variants were analyzed further: we performed antibody staining for neuronal and glial markers to quantitate the transduction of these cell types. By learning the sequence determinants of how AAVs trancytose brain endothelial cells, we can deliver therapeutic contents more effectively.

Introduction

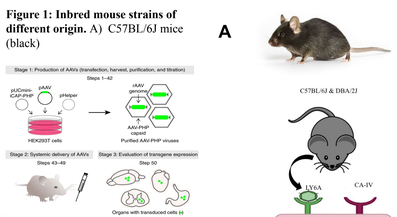


Figure 1: Inbred mouse strains of different origin. A) C57BL/6J mice (black)

Figure 2: Production of engineered AAV's, a systemic delivery and evaluation of expression in the brain (Challis, R.C., et al. 2019 Systemic AAV vectors for widespread and targeted gene delivery in rodents. *Nat Protoc* 14, 379–414)

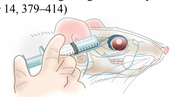


Figure 3: Retro-orbital Systemic Injection - noninvasive, simple procedure, systemic spread throughout the body/brain, potential liver toxicity at high doses

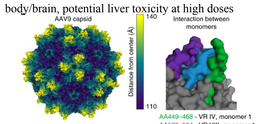


Figure 4: The AAV9 capsid proteins amino acid structure is engineered to contain random additional amino acids and then selected through directed evolution to obtain AAVs that have better delivery to the brain (Goertsen, D., et al., 2022 AAV capsid variants with brain-wide transgene expression and decreased liver targeting after intravenous delivery in mouse and marmoset. *Nat Neurosci* 25, 106–115).

- A biochemical approach was taken to select novel AAVs based on known receptor binding assays
- The pipeline includes characterizing these viruses and identifying their tropism by using mice as an animal model
  - Our objective is to engineer a virus that can be broadly useful for gene therapy, specifically one capable of effectively crossing the blood-brain barrier
- An engineered AAV virus encapsulated the gene for green fluorescent protein (GFP), functioning as a reporter gene to visualize the successful delivery of the virus.

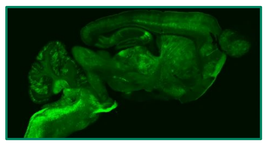


Figure 5: Sagittal mouse brain injected with PHP.B, an engineered AAV, showing successful gene delivery and widespread transduction of neurons in the brain (Challis et al).

- X1.1 efficiency targets endothelial cells without transcytosis, preferring to endocytose instead in mice
- X1.1 has enhanced blood brain barrier crossing via the binding of LRP6
- x1.1 has a 82-85% transduction of GLUT1+ cells throughout the CNS, it has a high efficiency for endothelial cells
- Our goal is characterize new variants that have weaker binding to LRP6, allowing us to test the hypothesis that weaker affinity for LRP6 would favor transcytosis into the brain rather than payload delivery to endothelial cells

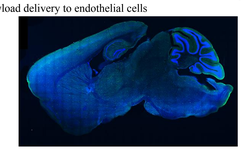


Figure 6: X1.1 Demonstrates strong vascular labeling

Methods

- We use HEK293 cells in order to package our capsid and cargo together so that we can produce enough virus to inject at a dose of 3.00E+11 vg (viral genomes) per mouse
- The virus was administered using retro-orbital injection and after 3 weeks of incubation the mice are perfused and the organs collected
- We took the brains and sectioned 100 micron sagittal slices and stained them using the cell markers NeuN (neurons) and s100B (astrocytes) and GLUT1 (endothelial).
- They were then imaged on a Nikon Confocal microscope and the images were quantified for co-staining of GFP and the cellular markers

Results

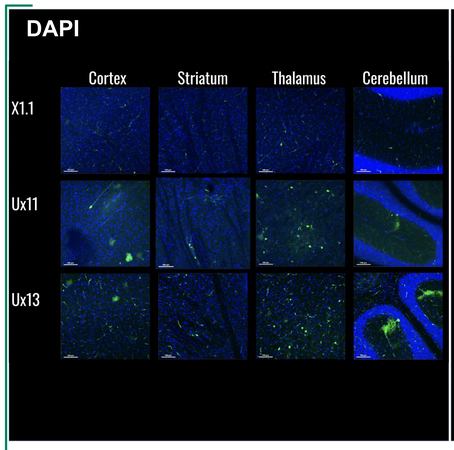


Figure 7: Staining for Dapi: Confocal images were taken after using IF to stain for cell nuclei (blue) to analyze the expression pattern of the AAV-CPP6 capsid reporter gene GFP (green).
Figure 8: Antibody Staining Graphs

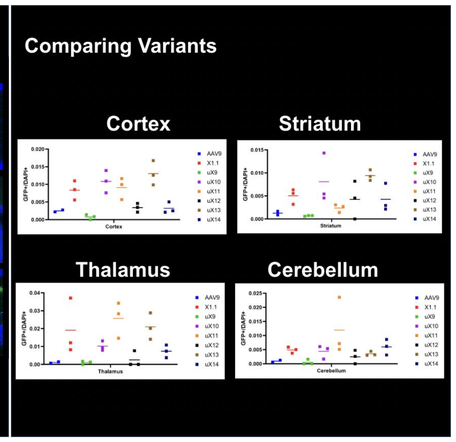


Figure 9: Comparing variants: Graphs of GFP+/DAPI+ cells for the variants across different areas of the brain
Figure 10: Staining for neuronal marker: Confocal images were taken after using IF to stain for neurons using NeuN (red) to analyze the expression pattern of the UX11 and UX13 capsid reporter gene GFP (green).

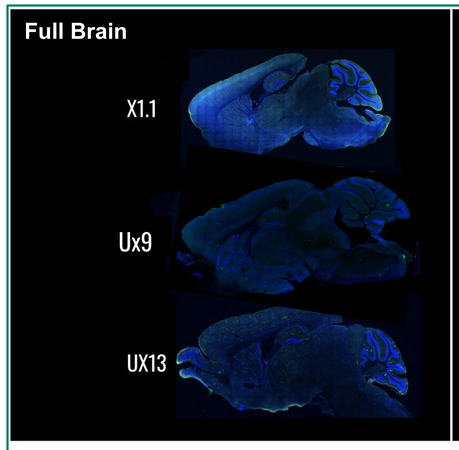


Figure 11: Full Brain: 20x full brain confocal images were taken after using IF to stain for DAPI and GFP
Figure 12: Staining for GLUT1: Confocal images were taken after using IF to stain for neurons using GLUT1 (red) to analyze the expression pattern of the UX11 and UX13 capsid reporter gene GFP (green).

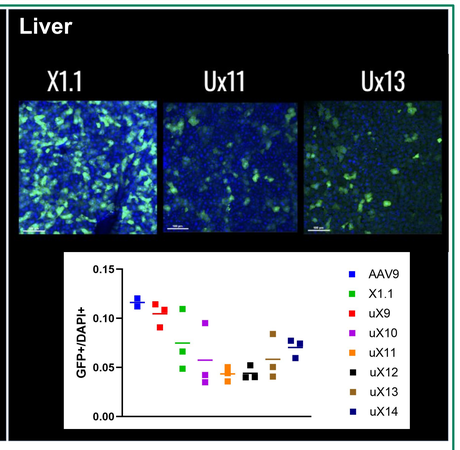
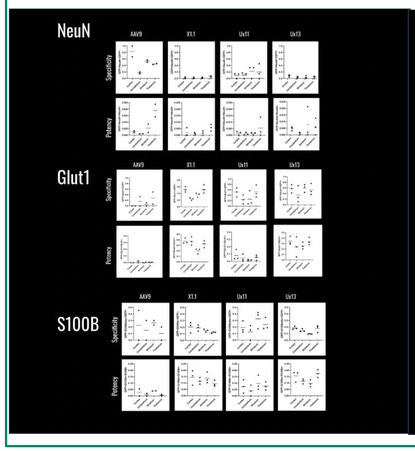
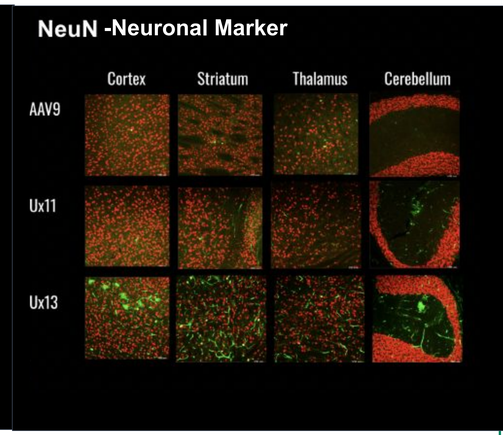
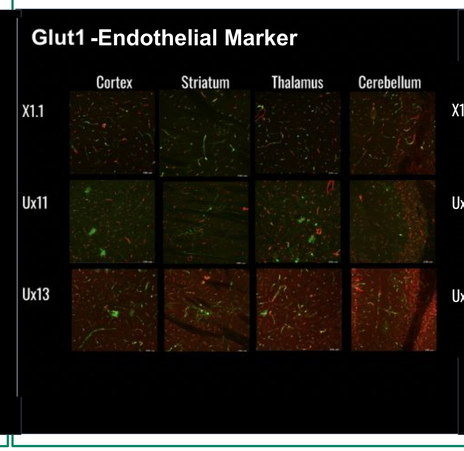
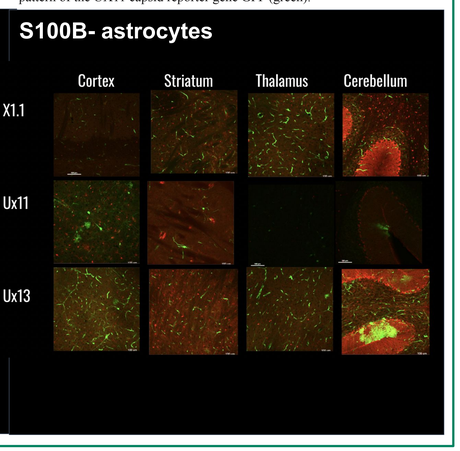


Figure 13: Liver: 20x liver confocal images were taken after using IF to stain for DAPI and GFP
Figure 14: Liver Transduction: Graph of GFP+/DAPI+ cells for livers
Figure 15: Staining for S100B: Confocal images were taken after using IF to stain for astrocytic cells using S100b (red) to analyze the expression pattern of the UX11 capsid reporter gene GFP (green).



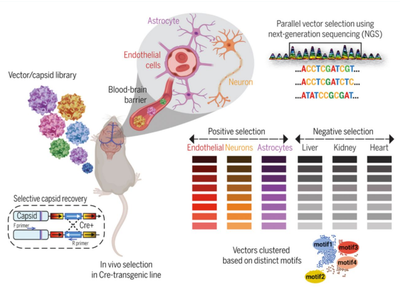






Conclusion & Future Directions

- AAV-UX11 and AAV-UX13 were most successful in efficiently crossing the BBB and infecting neuronal or glial cells.
- Due to the absence of viral expression in the brain/liver, further investigation into other organs might be necessary to fully characterize the tropism of these viruses.
- We will continue to characterize novel AAV capsids in order to continue building out an assortment of AAVs for a variety of gene delivery applications



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Key Sources

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Goertsen, D., Flytzanis, N.C., Goeden, N. et al. AAV capsid variants with brain-wide transgene expression and decreased liver targeting after intravenous delivery in mouse and marmoset. Nat Neurosci 25, 106–115 (2022)
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