Directed Evolution of AAV2 Libraries Yields Capsids with Improved Performance in the Central Nervous System and Cross-Species Translatability (Abstract 992)

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Introduction

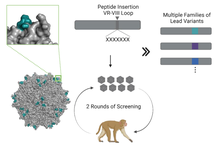
Broad delivery of genetic therapies throughout the brain and spinal cord has been a significant challenge. Intravenous delivery of engineered Adeno-Associated Virus (AAV) capsids capable of crossing the blood-brain barrier (BBB) is a promising strategy for achieving broad and uniform transduction of the CNS.

Current efforts to engineer AAV capsids for improved transduction of the CNS have largely focused on AAV9, primarily due to its ability to cross the BBB more efficiently than other serotypes. Alternative AAV serotypes may offer distinct advantages regarding tropism, manufacturability, and safety profile. Considering this, we investigated other AAV serotypes as the starting point for engineering with a focus on AAV2, which has been extensively studied and utilized in clinical trials, providing a wealth of information to aid engineering efforts.

AAV2 NHP Library Screening

To identify an AAV2 variant able to cross the BBB, we modified surface-exposed loops of AAV2 known to be important for brain and liver tropisms and employed our high-throughput, non-human primate (NHP)-based engineering platform to evolve capsids with CNS enrichment.

A Library Design & Screening Strategy



B Variant Characterization Strategy

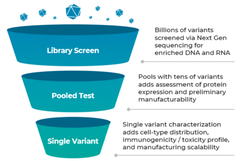
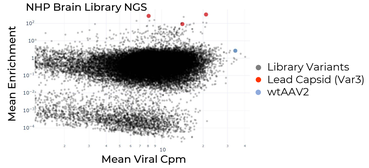


Figure 1. Directed evolution and characterization strategies for AAV2 variants.

A. Library design strategy where a highly diverse library was used in two rounds of NHP screening.
B. Variants are characterized through a large library, followed by a select number of variants in a pool, and finally progress to single variant characterization.

Following two rounds of screening in NHPs, we identified three capsid families with distinct consensus sequence motifs, that showed notable BBB-penetrance and liver de-targeting capabilities.

A



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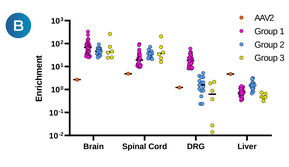


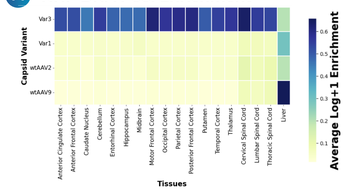
Figure 2. Directed evolution of AAV2 variants identified multiple high performing capsid families.

A. Second round of library screening in cynomolgus macaques showing mean CNS enrichment for each library variant.
B. Performance of three lead groups of variants in the brain, spinal cord, dorsal root ganglion (DRG), and liver compared to wtAAV2.

Cross-Species Pool Characterization

Pool studies in NHPs and rodents testing multiple AAV2 capsid families highlighted one family (Var3) with remarkably improved brain penetrance relative to wtAAV2 and wtAAV9.

A NHP DNA Pool Enrichment



B Murine DNA Pool Enrichment

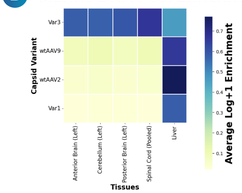


Figure 3. Robust CNS performance identified for multiple capsids in a small pool.

A. A small pool of capsids, including wtAAV2, was dosed in cynomolgus macaques. Nucleic acid was extracted from multiple brain and peripheral tissues and sequenced via NGS. Average enrichment log(Tissue Cpm / Viral Cpm)+1 across animals is plotted.
B. Parallel study in C57/BL6 mice showing relative DNA enrichment of pool capsids across tissues.

Murine Single Variant Characterization

Single variant characterization of Var3 in rodents showed extensive brain and spinal cord transduction.

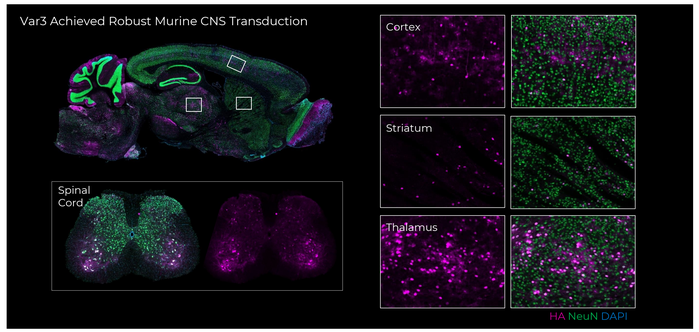


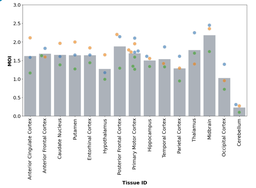
Figure 4. Var3 robustly transduces murine brain and spinal cord.

Immunofluorescent images from C57/Bl6 mice dosed with Var3 containing an HA tagged cargo. Tissue sections were imaged using anti-HA, anti-NeuN, and DAPI. Transduction across the spinal cord, cortex, striatum, and thalamus are highlighted.

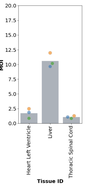
Cross-Species Single Variant Characterization

Further characterization of Var3 revealed a 50-fold increase in vector biodistribution in the brain and a 20-fold decrease in the liver compared to historical Capsida assessments of wild-type AAV9 in cynomolgus monkeys. These data suggest that the novel capsid is targeting a BBB cross mechanism that is conserved between rodents and primates.

A NHP DNA BioD



B NHP DNA BioD



C Murine DNA BioD

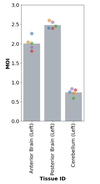


Figure 5. Multiplicity of Infection (MOI) values in the CNS of cynomolgus macaques and mice indicate robust brain penetrance of Var3.

A. MOI (vg/dg) values across 15 brain regions in cynomolgus macaques at a medium vector dose. Data points are color coded by animal and the gray bar shows the average across the three animals tested. For the motor cortex three tissue replicates were collected from each animal.
B. MOI values from heart, liver and spinal cord of NHPs.
C. MOI values across three brain regions in C57/Bl6 mice treated with a medium vector dose.

Variant Re-diversification Library

Continued engineering of the Var3 capsid has yielded next-generation variants with further enhancement in CNS transduction relative to the parent novel capsid and wtAAV2 in NHPs.

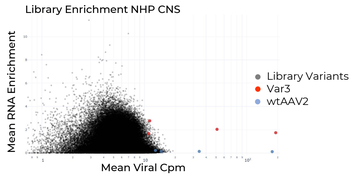


Figure 6. An NHP re-diversification library identified new Var3 variants with further improved CNS performance.

The lead AAV2 variant was re-diversified and tested in cynomolgus macaques. Mean CNS enrichment (brain sample CPMs / Viral Cpm) for each variant is plotted (gray circles). The parental variant Var3 (red circles) and wtAAV2 (blue circles) were titrated in at multiple viral CPMs.

Conclusions

Applying directed evolution with our high throughput automated screening, we identified AAV2-derived capsids with a combination of desirable characteristics, including robust CNS penetrance, cross-species translatability, and a reduced liver expression. The identified variants have the potential to be utilized in future gene therapy applications for CNS disorders.

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ASGCT Annual Meeting 2024

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PRESENTED ON TUESDAY

- Chen et al, AAV Gene Therapy Corrects Neurological Phenotypes with Clinically Relevant Doses in a Mouse Model of STXBP1-Related Developmental and Epileptic Encephalopathy (Oral Abs 38)

PRESENTED ON WEDNESDAY

- Knoll et al, CAP-002: Systemic AAV Gene Therapy with Next Generation Capsids for Treatment of STXBP1 Encephalopathy (Abs 504)
- Morales et al, Characterization of engineered AAV capsids from different HEK293 cell culture fractions, crude lysate versus cell pellet material (Abs 529)
- Volpe et al, Alternative Plasmid Designs Including Two Plasmid Transfection Systems for Improved Production and Packaging of Engineered AAV Capsids (Abs 530)

PRESENTED ON THURSDAY

- Shi et al, Directed Evolution of AAV2 Libraries Yields Capsids with Improved Performance in the Central Nervous System and Cross-species Translatability (Abs 992)
- Gejji et al, Separation of Empty and Full Engineered Adeno-Associated Virus Capsids Using a Weak Anion Exchange (Abs 1038)

PRESENTED ON FRIDAY

- McDowell et al, Systemic AAV Gene Therapy with CNS-Targeted Engineered Capsids Achieves Significant GCase Activity Increases in the Primate Brain to Support the Potential Treatment of GBA-PD (Oral Abs 274)