An Evolved AAV Variant with Enhanced Brain and Spinal Cord Tropism and Translation across Primate Species

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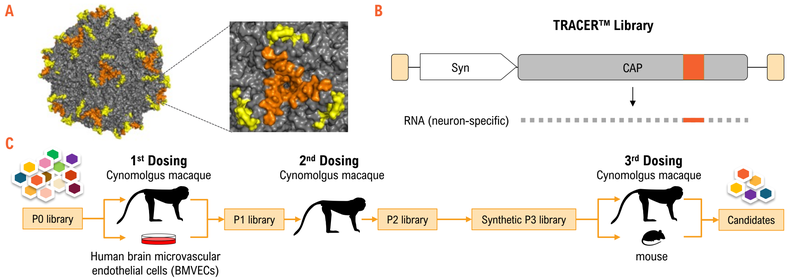
SUMMARY

- We applied the TRACER™ RNA-driven evolution platform to several peptide display libraries generated by random amino acid modifications of surface-exposed regions of AAV9.
- Neuron-specific evolution was performed in Cynomolgus macaques and human BMVEC cells.
- We identified a variant, VCAP-103, showing enhanced CNS transduction in Cynomolgus macaque, african green monkey (AGM) and marmoset, but not in C57Bl/6 or BALB/c mouse.
- VCAP-103 was validated as an individual vector in adult Cynomolgus macaque and showed a robust improvement over AAV9 in viral genome biodistribution and transgene RNA expression across the brain and the spinal cord.

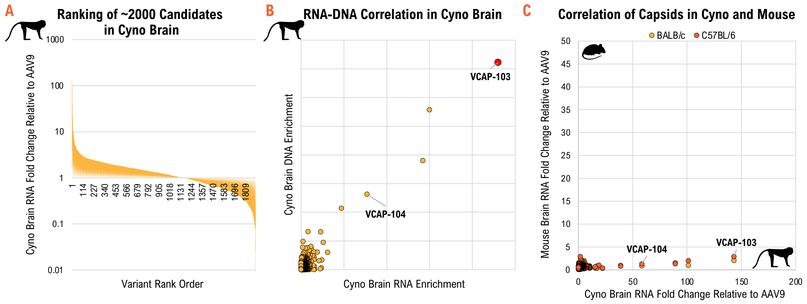
INTRODUCTION

Figure 1. AAV9-VR8 TRACER™ library design and screening



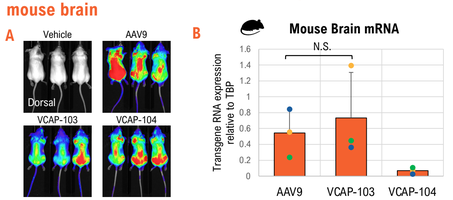
A) Viral structure of AAV9, the randomized region in VR8 is highlighted in orange. B) Neuron-specific construct design for RNA-driven AAV9VR8 library. C) Schematic diagram of the directed evolution process.

Figure 2. Synthetic P3 library NGS enrichment analysis in Cynomolgus macaque and mouse



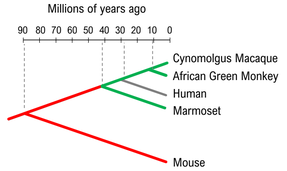
A) Ranking of all synthetic candidates in P3 Cynomolgus macaque biopanning according to average brain RNA fold change relative to AAV9. B) Correlation between Cynomolgus macaque average brain RNA fold change and viral DNA. C) Correlation between Cynomolgus macaque average brain RNA fold change relative to AAV9 and average rodent brain RNA fold change relative to AAV9 (BALB/c and C57Bl/6).

Figure 3. VCAP-103 and -104 do not outperform AAV9 in mouse brain



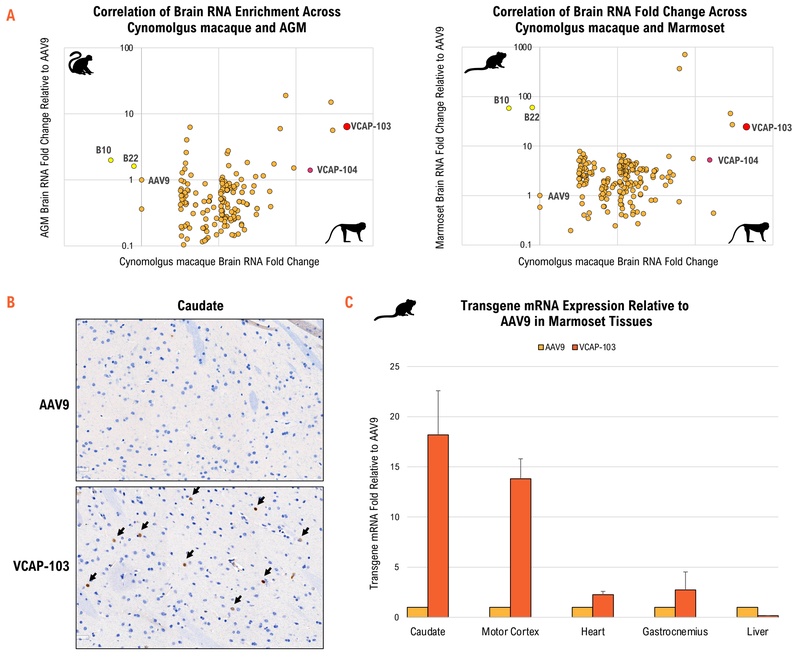
A) *In vivo* imaging of mouse dorsal side 27 days after intravenous (IV) injection of vehicle, AAV9, VCAP-103 and VCAP-104 with a firefly luciferase transgene. B) RT-qPCR quantification of transgene RNA from mouse brain. Numbers indicate average values normalized to TATA-box Binding Protein (TBP) as housekeeping gene. N.S.: non-significant.

Figure 4. Rationale of capsid comparison across primate species



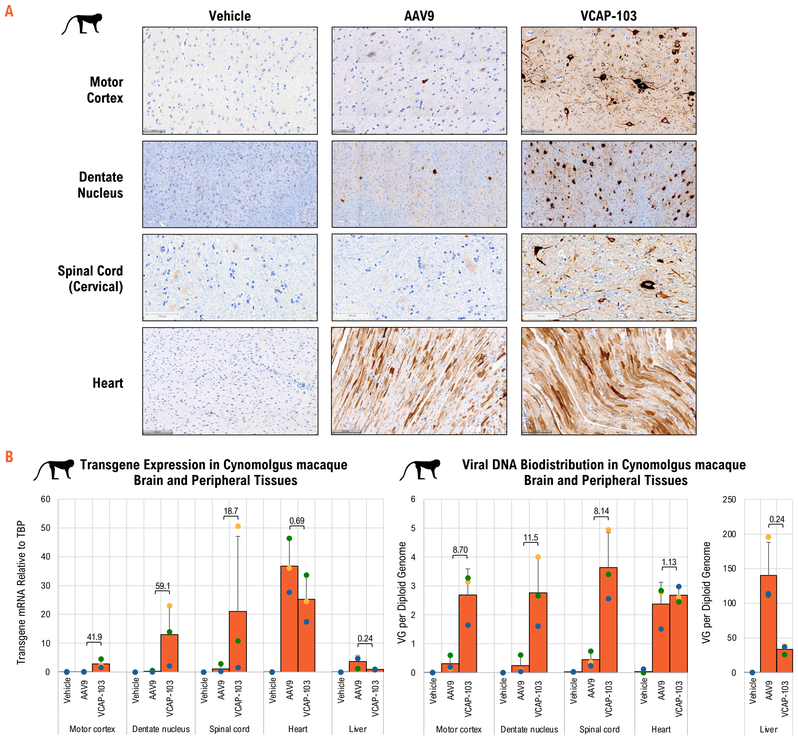
Phylogenetic tree to show the evolutionary descent of Cynomolgus macaque, african green monkey, marmoset, mouse and human.

Figure 5. Performance of VCAP-103 across primate species



A) Multiplexed NGS analysis of brain RNA enrichment in Cynomolgus macaque and AGM (left panel) or marmoset (right panel). Data indicate average brain RNA fold change relative to AAV9. Internal benchmark capsid AAV9, B10 and B22 are indicated. B) Immunohistochemistry staining of marmoset brain (caudate region) following low-dose injection with AAV9 and VCAP-103 carrying a nuclear reporter. C) RT-ddPCR quantification of transgene mRNA expression in marmoset brain and peripheral tissues. Values indicate fold change vs. AAV9.

Figure 6. Dose-response biodistribution and transduction of VCAP-103 in Cynomolgus macaque brain



A) Immunohistochemistry staining of Cynomolgus macaque brain, spinal cord and heart after dosing with AAV9 and VCAP-103. B) Quantification of transgene mRNA and viral genomes in Cynomolgus macaque tissues. RNA values (left panel) indicate RT-qPCR quantification relative to TBP, and viral DNA biodistribution (right panel) is indicated as viral genomes per diploid cell quantified by ddPCR. The values above the bars indicate the fold change vs. AAV9.

CONCLUSION

- VCAP-103 was selected for enhanced CNS transduction after 3 rounds screening in Cynomolgus macaque via TRACER™ platform.
- VCAP-103 brain tropism showed enhanced brain tropism in various primate species, including marmoset. **The cross-species tropism supports the possibility of translation in human patients.**
- In Cynomolgus macaque, VCAP-103 showed improved genome biodistribution and transgene RNA expression in brain (~40x vs. AAV9) as well as spinal cord (~20x vs. AAV9).
- VCAP-103 was well tolerated in Cynomolgus macaques with no detectable toxicity observed by clinical chemistry or histopathology.
- Engineering of VCAP-103 2nd generation capsid for improved CNS tropism is currently in process.