In a pooled, next generation sequencing (NGS) based screen, these Gen2 capsid variants showed up to 7-fold improved BBB-penetrance across broad CNS regions relative to the parental VCAP-101 or VCAP-102 capsids in NHPs. Upon intravenous dosing of cynomolgus macaques (*Macaca fascicularis*) with these Gen2 capsids expressing a self-complementary histone transgene at 3E13 vector genomes per kilogram (VG/kg), we observed upwards of 9 vector genomes per cell across broad CNS regions and upwards of 60% of cells being targeted, including both neurons and astrocytes. Gen2 capsids demonstrated further liver de-targeting relative to both the parental VCAP-101 and VCAP-102 capsids. Additional studies in mice reinforce the dual cell-type targeting of these Gen2 capsids. Furthermore, we identify the receptor for this family of capsid variants and demonstrate that the capsid-receptor binding is both specific and direct. Given the high conservation of this receptor across mammals, our data collectively suggest a novel, highly CNS-specific capsid for use in the clinic.

120. Second Generation AAV Capsids Reprogrammed to Bind Human Transferrin Receptor are Targeted to the Brain and De-Targeted from the Liver in Human TFRC Knock-In Mice

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Developing vehicles that efficiently deliver genes throughout the human central nervous system (CNS) will broaden the range of treatable genetic diseases. We developed a high-throughput *in vitro* screening approach to reprogram AAV9 to bind the human Transferrin Receptor (TfR1), a protein expressed on the blood-brain barrier (BBB). We showed that one TfR1-targeted capsid, BI-hTFR1, was actively transported across a human brain endothelial cell layer and achieved 40-50 times greater reporter expression in the CNS of human *TFRC* knock-in mice compared to AAV9 in wild type mice after intravenous delivery. The enhanced tropism of BI-hTFR1 was CNS-specific and absent in wild type mice (Fig. 1A). Through sequence diversification, we identified a second generation capsid, BI-hTFR1.2, that was capable of transducing more than 60 percent of NeuN-positive neurons in the cortex of human *TFRC* knock-in mice when dosed at 5E10 vg/mouse, while also being de-targeted from the liver (Fig. 1B). Similar to BI-hTFR1, BI-hTFR1.2 exclusively binds to human but not macaque or mouse TfR1. Using amino acid swaps between species, we pinpointed human-specific residues of TfR1 that are critical for the species-specific binding by BI-hTFR1 and BI-hTFR1.2. Importantly, there are no common human polymorphisms that would be predicted to directly impact the ability of these capsids to bind TfR1 in patients. Our work establishes these TfR1-targeting capsids as promising vectors for human CNS gene therapy.

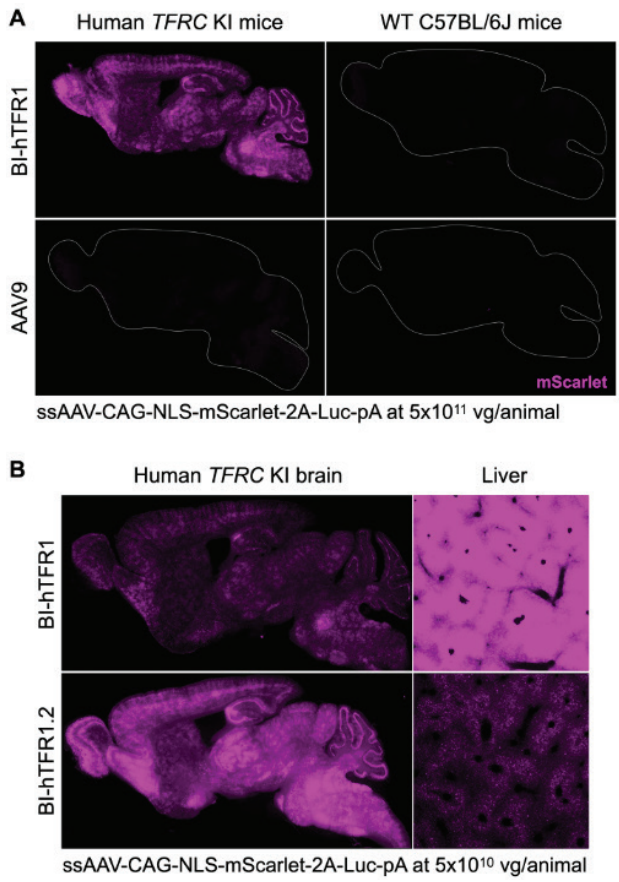

Figure 1. TfR1-targeted AAV capsids achieve efficient CNS-wide transgene expression in human *TFRC* knock-in mice. (A) BI-hTFR1 or AAV9 were intravenously injected into adult wild type C57BL/6J or *TFRC* knock-in mice at 5E11 vg/animal. Representative sagittal brain section images from three weeks post-injection are shown. **(B)** BI-hTFR1 or BI-hTFR1.2 were intravenously injected into adult *TFRC* knock-in mice at 5E10 vg/animal (~2.5E12 vg/kg). Representative sagittal brain and liver section images from three weeks post-injection are shown.

121. Engineering Viral Vectors for Acoustically Targeted Gene Delivery to the Brain across Species

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Spatially targeted gene delivery to the brain has the potential to treat prevalent neurological and psychiatric diseases. However, the site-specific delivery of vectors such as adeno-associated viruses (AAVs) is typically performed via invasive injections, limiting scope of clinical applications. Over recent decades, focused ultrasound blood-brain-barrier opening (FUS-BBBO) has emerged as a noninvasive procedure enabling the site-specific entry of small viral vectors into the brain from systemic circulation. However, when used in conjunction with natural AAV serotypes, this approach has limited transduction efficiency, and results in undesirable transduction of peripheral