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Title: Identifying Receptors for Enhanced Blood-Brain Barrier Crossing by Engineered Adeno-Associated Viruses

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Abstract: Efficient transcytosis of the blood brain barrier (BBB) is a key bottleneck in the development of molecules to study and treat the brain, particularly biologics requiring active transport. Our work on adeno-associated virus (AAV) engineering has yielded diverse capsid variants with enhanced central nervous system (CNS) infectivity upon systemic delivery in mice, marmosets, and macaques. However, the directed evolution methods by which we identified these capsids do not provide much insight into the mechanisms by which enhanced tropisms are conferred, complicating the application of engineered AAV both across model organisms and in potential human gene therapies. This dilemma is perhaps best illustrated by AAV-PHP.eB, whose enhanced CNS tropism was found to be restricted to mouse strains expressing membrane-localized Ly6a after unsuccessful application in non-human primates and select mouse strains. Here, we used surface plasmon resonance to assemble a panel of previously identified engineered AAVs with enhanced CNS tropism that do not interact with Ly6a. Administration of these capsids to the genetically diverse 129S1/SvImJ, CBA/J, DBA/2J, and NOD/ShiLtJ mouse strains showed enhanced CNS infectivity patterns consistent with non-Ly6a mechanisms for BBB transcytosis. We developed a cell culture screen of candidate membrane proteins selected based on their single-cell RNA sequencing profiles in C57BL/6J mouse cortex and tested a diverse panel of engineered AAVs. Using this screen, we identified a potential novel role for Ly6c1 as a molecular receptor in the BBB that enables enhanced CNS infectivity by multiple engineered AAVs. This receptor complements previously identified Ly6a-interacting AAVs as research tools in diverse mouse strains.