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Title: Intravenous gene transfer throughout the brain of infant Old World primates using AAV

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Abstract: Adeno-associated viruses (AAVs) have emerged as dependable and ubiquitous tools for researchers and clinicians alike since their discovery as dependoparvoviruses. In the decades since the earliest descriptions of recombinant AAVs, hundreds of clinical trials serve as a testament to their potential for safe and long-term expression of genetic payloads. In recent years, the field has focused on engineering novel capsids to expand the therapeutic opportunity landscape for gene therapy into disorders not previously approachable with natural AAV serotypes, while in parallel, the neuroscience community has focused on several engineered AAV variants, e.g. AAV-PHP.B, that traverse the restrictive blood-brain-barrier (BBB) to systemically deliver genetically-encoded tools to the rodent brain. However, the majority of AAV capsid engineering efforts targeting the brain have thus far focused on increasing gene-transfer to the rodent central nervous system (CNS), and direct efforts in non-human primates (NHPs) are sparse. Some recently engineered capsids, e.g. AAV.CAP-B10, now enable systemic gene transfer to the brain of the common marmoset (Callithrix jacchus), a New World primate species. But few comparable options exist for Old World primates, which are more evolutionarily related to humans compared to marmosets and are well-established animal models of human cognition, neurodevelopment, neuroanatomy, and physiology. To enable research and for greater therapeutic translatability, it is imperative that we advance AAV development for systemic gene transfer to the brains of Old World primates. Here, we describe the identification and characterization of AAV.CAP-Mac, an engineered AAV9 variant that efficiently transduces throughout cortical and subcortical brain regions of infant Old World primates after IV administration. We identified CAP-Mac after 2 rounds of initial selection of an engineered AAV9 capsid library in the adult common marmoset (Callithrix jacchus) followed by a final round of selection in infant macaques, where CAP-Mac-delivered transgenes were enriched 10- and 6- times more than those delivered by the AAV9 parent in viral DNA and whole RNA brain extracts, respectively. Looking across Old World primate species, CAP-Mac efficiently transduces the brain of both the rhesus macaque (Macaca mulatta) and the green monkey (Chlorocebus sabaeus), achieving broader CNS distribution via an IV route compared to intrathecal administration. Furthermore, CAP-Mac targets neurons in the brain much more effectively than AAV9, highlighting the opportunity for broader and more diverse study of those cells in primates, as well as the potential for increased therapeutic benefit. To exemplify CAP-Mac's immediate research utility, we delivered a cocktail of three fluorescent proteins for multicolor labeling of neurons throughout the Old World primate brain and subsequent morphological tracing. By characterizing CAP-Mac across NHP species, we aim to both expand the AAV toolbox available to researchers interested in studying the Old World primate CNS and highlight the utility of engineering AAVs for increased translatability and potential therapeutic benefit.