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Title: Transduction Profile of Engineered Adeno-Associated Viral Capsids in Mouse and Marmoset

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Abstract: Adeno-associated virus (AAV) is a promising viral vector for gene therapy due to stable expression in vivo and a strong clinical safety record. Unfortunately, naturally occurring AAV serotypes are inefficient transducers of many relevant tissues for gene therapy. As a result, high viral doses can be required for many disease indications to achieve therapeutic efficacy. Fortunately, AAVs are amenable to engineering efforts which can improve tissue tropism and specificity. Past engineering efforts by our lab have produced AAV variants with improved transduction capabilities of therapeutically relevant cell populations like the CNS in mice (AAV-PHP.eB), but the efficacy of those variants has not translated to other species. Many gene therapy relevant cell populations reside within tissue where direct administration is difficult. Systemic administration of AAV libraries through intravenous injection permits non-invasive transduction of these tissues, enabling subsequent variant selection within relevant cell populations. With the aim of enhancing viral tropism for refractory targets, libraries of AAV9 were selected for novel characteristics using multiplexed Cre-recombination based AAV targeted evolution (M-CREATE). Viral genomes from capsids that transduced tissues of interest across rodent Cre-lines are selectively amplified and recovered through the M-CREATE method, allowing simultaneous positive and negative selection of AAV variants. From our selections in mice, we are presenting three capsids: variant AAV.CAP-A4, which was identified for improved transduction of lung tissue, and variants AAV.CAP-B10 and AAV.CAP-B22 that, when administered systemically, can cross the blood-brain barrier and efficiently transduce neurons in adult mice and marmosets. The enhancement in AAV.CAP-A4 transduction is most specific to the lung, while the liver and other tissues targeted by AAV9 have similar transduction profiles. Both variants AAV.CAP-B10 and AAV.CAP-B22 display improved transduction across the marmoset brain relative to AAV9. In the cortex, AAV.CAP-B10 transduces neurons more readily than AAV9 or PHP.eB, while AAV.CAP-B22 displays a broader tropism. These novel variants enable robust, non-invasive gene delivery to the adult marmoset brain following IV administration. This work demonstrates that, through the M-CREATE method, novel AAV variants can be developed with sought-after transduction profiles in therapeutically relevant cell populations. We have also shown that relative improvements to the transduction profile obtained through in vivo selections in mice can be translated to non-human primates.