

NEXT GENERATION TYROSINE-MUTANT AAV8 SHOWS HIGH MOUSE CORNEAL ENDOTHELIAL TRANSDUCTION IN VIVO

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Purpose: Recently, point mutations in surface-exposed tyrosine residues have been shown to increase AAV transduction significantly. We examined the efficacy of conventional AAV8 and next generation tyrosine-mutant AAV8 vectors to deliver genes into corneal endothelium *in vivo*.

Methods: The study, which used female C57BL/6 mice, was approved by the institutional animal care and use committee and experiments were performed in accordance with the tenets of the ARVO statement for the use of animals. Ketamine and Xylazine hydrochloride were administered for anesthesia. Two microliters of AAV8 titer ($\sim 10^{10}$ vg/ml) was injected into the anterior chamber of the eye from four different clock-positions with the Hamilton Microinjection Syringe System under an operating microscope. Eyes were then washed with water and sponge-dried. Slit lamp microscopy was utilized for clinical eye exam. Stereomicroscopy and immunocytochemistry techniques measured the levels and location of transgene. Delivered-transgene expression was quantified with real-time PCR and western blot analyses.

Results: Both, conventional and tyrosine-mutant AAV8 vectors successfully transduced mouse corneal endothelium *in vivo*. Mutant AAV8 showed significantly higher transgene delivery into corneal endothelium than the conventional AAV8. Injections performed from 12 or 3 o'clock position showed highest targeted transgene delivery into corneal endothelium. Low-to-mild transgene delivery was also detected in the stroma. The tested AAV8 showed no apparent side effects or toxicity.

Conclusions: Tested AAV8 vectors are efficient to treat corneal endothelial dystrophies via gene therapy. Future studies will determine optimal doses of conventional and tyrosine-mutant AAV8 vectors to deliver therapeutic genes into corneal endothelial *in vivo*.

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