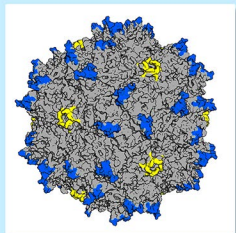


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The AAV8 capsid with the 3-fold symmetry axis (blue) and 5-fold pore (yellow)

Characterization of a Novel Mutant Virus from the AAV8 Capsid Library for Liver Gene Therapy

Different serotypes of Adeno-associated virus (AAV) have unique surface loops that confer distinct receptor recognition, which affects the overall tropism and transduction efficiency of the virus. Serotype AAV8 has been demonstrated to serve as an effective liver gene therapy vector in animal models of genetic liver diseases, but the results of human clinical trials have been inconsistent. We hypothesized that the effectiveness of AAV8 as a human gene therapy vector can be improved by modifying its surface loops to increase transduction efficiency and change tropism. However, AAV capsid structural biochemistry is not fully understood, making it difficult to predict structure with specific function. We used a mutagenesis approach to randomize a six amino acid stretch of one of the AAV8 surface loops, generating a library of mutant viruses where each of those six residues could be any amino acid. One mutant capsid isolated from the library, AAV880, transduced human liver cells with 10-fold higher efficiency than wild type AAV8. We then investigated the mechanisms involved in the increase in transduction of the mutant capsid, and found that AAV880 had increased binding on human liver cells compared to that of AAV8. Competitive inhibition assays were performed to potentially identify any novel glycan receptors on the mutant AAV8 capsids. AAV880 was not inhibited by soluble keratin sulfate and moderately inhibited by soluble heparan sulfate. The same virus was strongly inhibited by soluble chondroitin sulfate, which suggests the novel surface loop generated from the capsid library binds chondroitin sulfate to facilitate entry. AAV880 must be validated on more biologically relevant human liver models, such as humanized liver mice or *ex vivo* human liver organoids, prior to application for liver gene therapy.