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Naturally Occurring Variations at the 501 and 706 Residues on AAVHSC16 Contribute to Reduced Liver Tropism and Slower Serum Clearance

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AAVHSCs, derived from human hematopoietic stem cells, are naturally occurring adeno-associated virus (AAVs) with one to four unique variations in the capsid when compared to fellow Clade F member, AAV9. The unique variations of the panel of 15 AAVHSCs contribute to their distinct biodistributions both in mice and non-human primates (NHPs). One AAVHSC, AAVHSC16, displays lower liver tropism compared to other AAVHSCs in multiple species and does not induce any elevation of alanine transaminase (ALT) or aspartate transaminase (AST) levels at high doses, up to 1E14 vg/kg, in NHPs. AAVHSC16 has two unique amino acid variations compared to other AAVHSCs, F501I and Y706C. Both naturally occurring variations of AAVHSC16 are surface exposed on the capsid. We employed mutagenesis to determine the contribution of each naturally occurring variation to the unique biodistribution of AAVHSC16. The F501I and Y706C variation was introduced onto the AAVHSC15 capsid and biodistribution was determined through tissue specific vector genomes (vgs) for each capsid in albino C57 mice at doses of 1E13 and 1E14 vg/kg at six-weeks post-dose. Both AAVHSC15 F501I and Y706C modified capsids displayed lower liver tropism compared to wild-type AAVHSC15, but liver transduction was not as low as the parent AAVHSC16, indicating a synergistic effect of both variations on liver tropism. Mutagenesis and binding experiments indicated that the 501I variation drastically changed terminal galactose binding while the 706C variation had unaltered glycan binding. These data indicate that the lower liver tropism observed with 706C is due to an influence on non-glycan binding steps of transduction. To understand the relationship between clearance to overall biodistribution including liver tropism, we investigated the clearance from blood of AAVHSC15, AAVHSC16 and the two AAVHSC15 alternate capsids containing the 501I and 706C variations in albino C57 mice. Blood was collected at various time points over 48 hours and both whole blood and serum was assayed for vgs. AAVHSC15 displayed rapid clearance from blood while AAVHSC16 cleared at a slower rate, although both capsids reached a similar level of vgs in the blood at the latest time point, 48 hours post-dose. No differences were observed between the level of vgs between whole blood and serum, indicating that differences in immune cell transduction in the peripheral blood was not contributing to differences in the rate of clearance between capsids. The clearance rate of the AAVHSC15 modified capsids appeared to correlate with their liver tropism, as the AAVHSC15 Y706C modified capsid had slower clearance and lower liver tropism compared to the AAVHSC15 F501I modified capsid. These data support previously published hypotheses that liver tropism and blood clearance rates are linked. The 501I naturally occurring variation appears to be contributing to the differences in liver tropism and clearance rate through changes in glycan binding due to its proximity to residues defined as key for galactose binding on AAV9. The 706C naturally occurring variation is contributing in a non-glycan binding manner. Continued structure and function studies will help to understand the relationship of the 706C residue on AAVHSC16 with the reduced liver tropism, slower clearance rate and increased safety profile in NHPs. Additionally, further characterization of the contribution of each naturally occurring variation to the

tropism and transduction kinetics of each AAVHSC will allow for rational capsid selection or modulation for developing therapeutic applications.