CB 2679d-GT - A Novel Human Factor IX Variant Shows Enhanced Activity After Delivery Into Hemophilic Mice Using an AAV Capsid With High Liver Transduction

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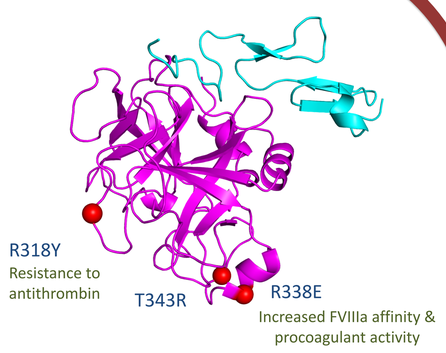
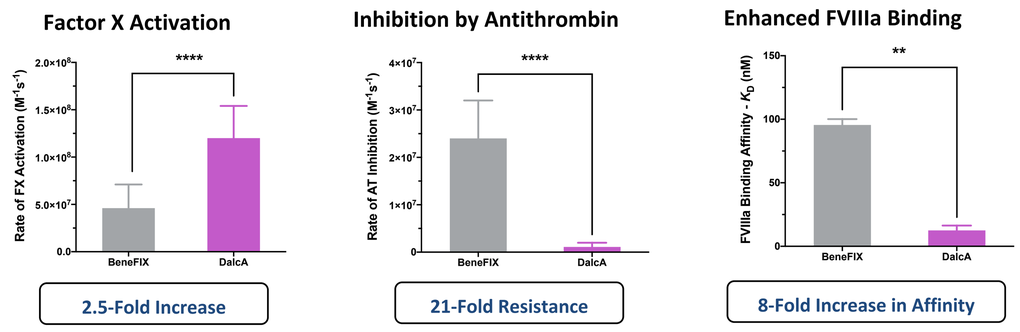
Background

Cb 2679-GT is an engineered next-generation coagulation Factor IX using rational protein design with enhanced functionality through triplet substitutions (R318Y, T343R and R338E) that increase catalytic activity, increase resistance to antithrombin inhibition and improve affinity for activated FVIII.

In a Phase 1/2 study these enhancements resulted in a 22-fold improved activity over BeneFIX® which has wild-type (wt) huFIX sequence.





(**** P<0.0001, *** P<0.001, ** P<0.01 and * P<0.05)

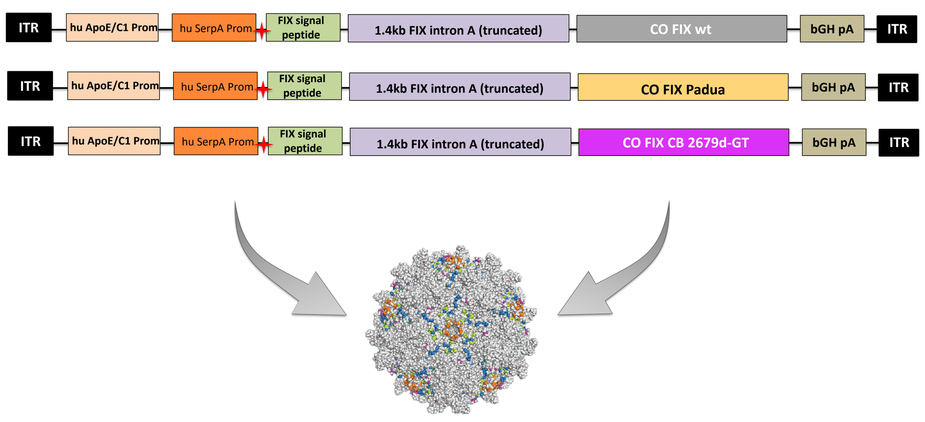
Previously it was demonstrated that a self-complementary AAV-DJ8 construct expressing CB 2679d-GT in hemophilia B mice significantly reduced tail clip bleeding time (4-5-fold) over that of Padua FIX, thus achieving a more rapid and robust hemostatic correction of bleeding and reduction in blood loss[1].

In this study we sought to achieve higher huFIX expression levels *in vivo* by using a different expression cassette as well as a novel AAV capsid for packaging.

Study Design

We constructed a single-stranded rAAV vector harboring a mouse codon-optimized huFIX sequence that contained a truncated version of the native FIX intron A. The transgene was cloned downstream of a strong liver specific ApoE/SerpA hybrid promoter. Mutations for Padua (R338L) and CB 2679d-GT (R318Y, T343R, R338E) were introduced by site-directed mutagenesis and rAAV vectors were packaged into the KP1 capsid. This capsid had been developed by directed evolution and was shown to exhibit strong transduction efficiency for human and murine hepatocytes, both *in vitro* as well as *in vivo*[2].

Vector preparations were purified by double CsCl ultracentrifugation and injected into hemophilic mice via tail vein. Three different doses of rAAV were used for each group.



huFIX constructs were packaged into the novel AAV-KP1 capsid that had been derived by DNA shuffling and shown to exhibit strong transduction in vitro and in vivo.

Non-heparinized, citrated blood was collected at various timepoints after rAAV injection and FIX protein levels in the plasma was determined by ELISA using the respective recombinant proteins as standards. FIX activity was assessed by an activated partial thromboplastin time (aPTT) Factor IX single-stage clotting assay on an ACL-TOP instrument (Instrumentation Laboratories) using the recommended HemosIL® or SynthasIL® reagents and calibrators. Mice were sacrificed at 18 or 16 weeks post injection, and rAAV vector genome copy numbers and huFIX transcript levels were quantified in the livers using qPCR.

Results

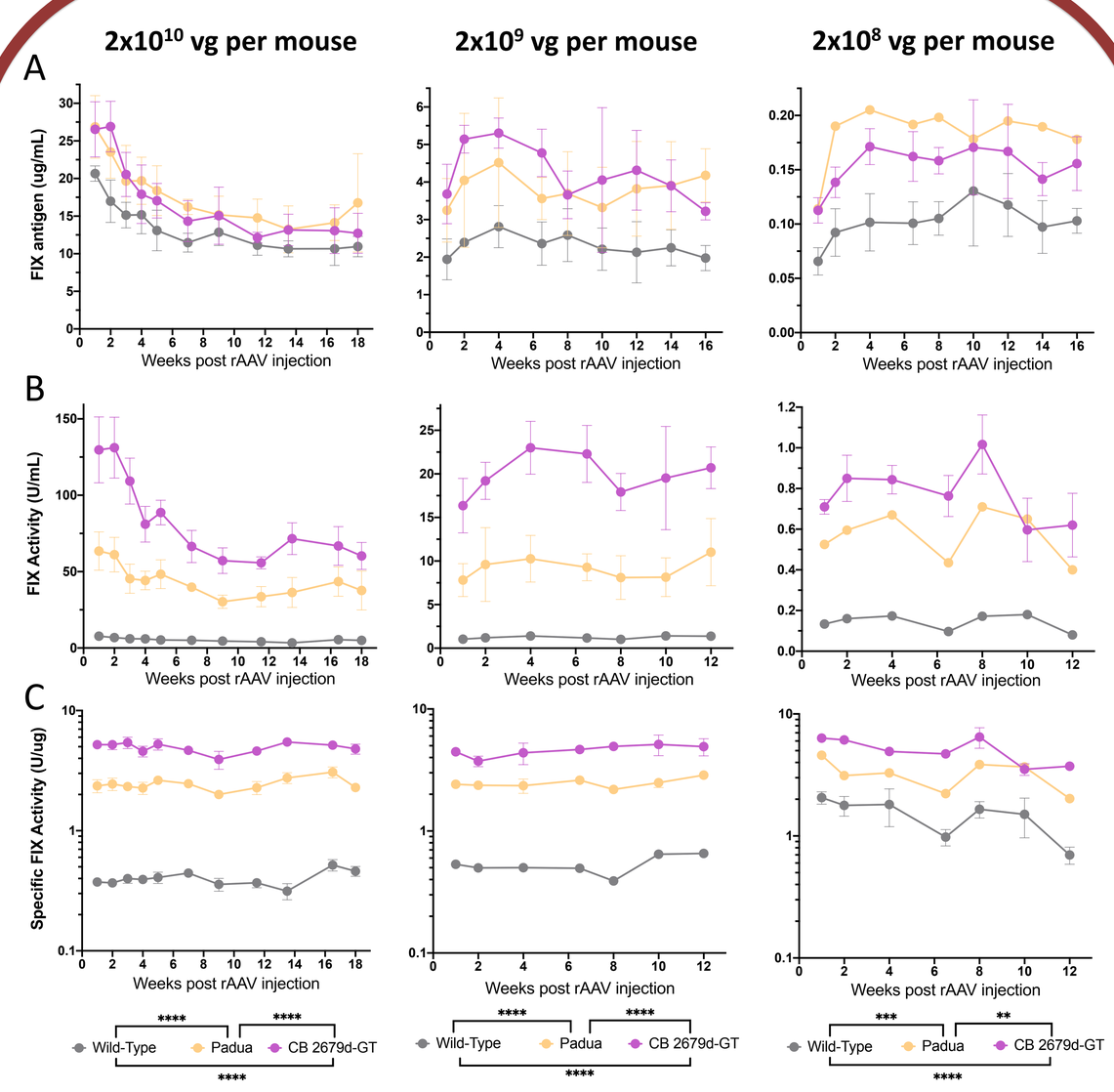


Fig 1: huFIX antigen levels (**A**), activity (**B**), and specific activity (**C**) in mouse plasma after rAAV injection using three different doses. FIX specific activity is calculated as the ratio of FIX activity/antigen. Data are presented as mean ± S.D. One mouse from the Padua 2x10[8] vg injection group died one day post injection, mean values are shown for this group. For the low dose injection experiments FIX activity data were not available for weeks 14 and 16. Statistical significance is indicated for the specific activity data. ** p=0.0011, *** p=0.0009, **** p<0.0001

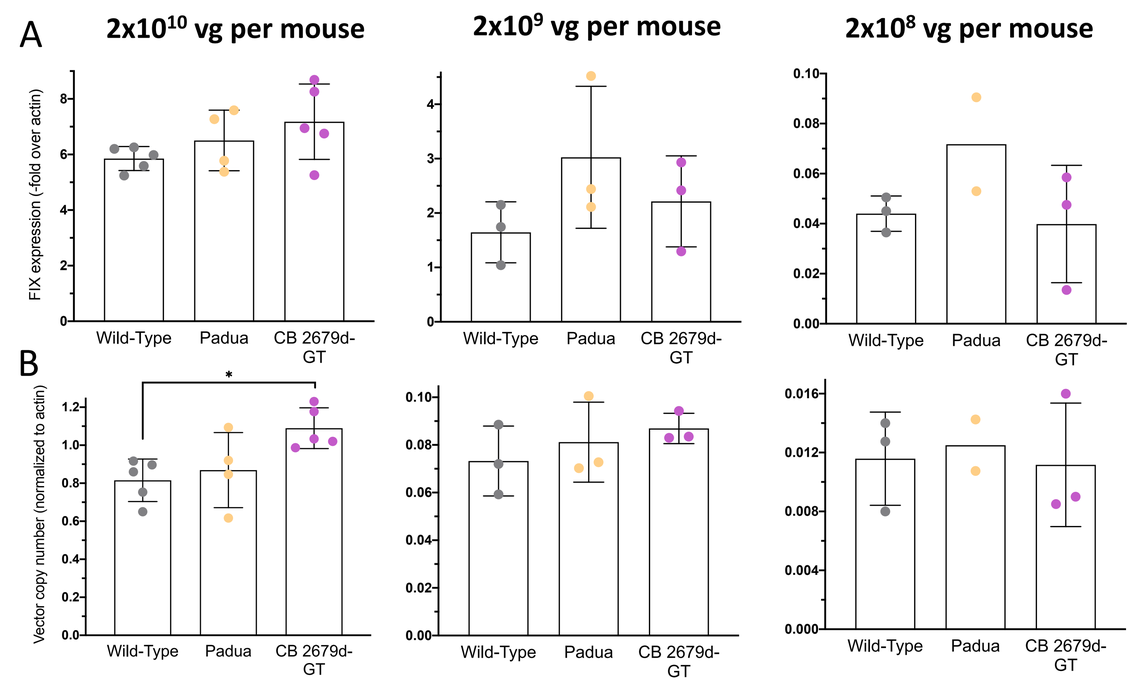


Fig 2: huFIX transcript levels (**A**) and vector copy numbers (**B**) in mouse livers after sacrificing the mice at week 18 (2x10[10] vg group) or week 16 (2x10[9] and 2x10[8] vg groups) post rAAV injection. Data are presented as mean ± S.D. Three technical replicates of each sample were analyzed by qPCR. Only statistically significant differences between groups are indicated (* p=0.025). One mouse from the Padua 2x10[8] vg injection group died one day post injection.

Conclusions

The new rAAV expression cassette in conjunction with the KP1 capsid was shown to exhibit robust and very strong huFIX expression after injection into hemophilic mice. When compared to the previous study dose-adjusted antigen expression was around 8- to 10-fold increased. At the highest dose used in this experiment (2x10[10] vg/mouse) huFIX levels were extremely high during the first two weeks, then dropped almost 2-fold, and remained stable throughout the experiment.

While rAAV vector copy numbers and FIX transcript levels were similar between wt, Padua, and CB 2679d-GT rAAV injected mice within the same dose groups, the animals injected with CB 2679d-GT rAAV had the highest huFIX activity levels for all three dose levels.

Specific huFIX activity in animals injected with CB 2679d-GT expressing rAAV was around 10-fold or 2-fold enhanced as compared to those injected with wt or Padua respectively (2x10[10] and 1x10[9] groups).

Bibliography

[1] Blouse GE, Nair N, Vandendriessche T, Chuah MK, Landau, J. (2019) *Haemophilia*, Vol 25, Supplement S1 P124

[2] Pekrun K, DeAlencastro G, Luo, Q.-J., Liu, J., Kim, Y., Nygaard, S., Galivo, F., Zhang, F., Song, R., Tiffany, M., Xu, J., Hebrok, M., Grompe, M., and Kay, M.A. (2019). Using a barcoded AAV capsid library to select for clinically relevant gene therapy vectors. JCI Insight 4.