Persistent and stable therapeutic levels of transgenic FVIII expression following AAV delivery to adult and infant hemophilic dogs

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Introduction

- AAV5-FVIII gene therapy is approved in the U.S. and conditionally approved in Europe for the treatment of adults with severe hemophilia A
- The majority of FVIII is thought to be expressed by non-integrated episomal structures
- AAV-FVIII treatment during childhood could prevent the development of arthropathy and improve quality of life
- The factors influencing hepatocyte transduction and kinetics of AAV-cFVIII vector processing remain unknown

Aims

To evaluate the influence of:

1. Treatment age
2. Codon optimization
3. Vector dose

on FVIII expression and AAV-FVIII genome processing kinetics in a canine model of severe hemophilia A

Methods

- Neonatal, infant, and adult severe hemophilia A dogs (Table 1) received a single infusion of codon-optimized or wild-type AAV-canine FVIII
- Serial liver biopsies (Figure 2) underwent droplet digital PCR and histological analyses

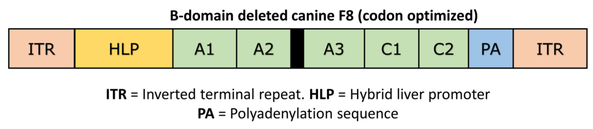

Figure 1. Structure of the AAV5-cFVIII vector

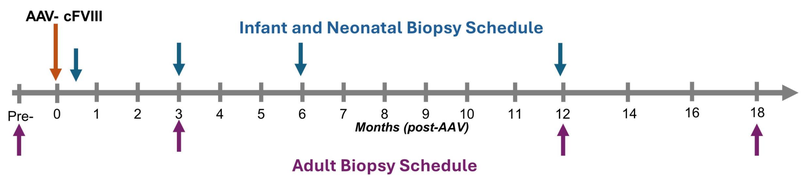

Figure 2. Study timeline

Cohort	N	Avg Age at Treatment	Vector (AAV-cFVIII)	Sex (% M)	Avg Treatment Weight (kg)	Target Dose (vg/kg)	Avg Total Vector Genomes
Neonatal	2	2 weeks	Codon-optimized	0	1.10	2.0e14	2.2e14
Infant	3	2 months	Codon-optimized	33.3	4.33	2.0e14	8.67e14
Adult WT	3	6.0 years	Wild-type	100	12.60	6.0e13 – 2.0e14	1.53e15
Adult CO (6e13)	4	4.3 years	Codon-optimized	100	16.3	6.0e13	1.07e15
Adult CO (2e14)	3	3.9 years	Codon-optimized	100	17.0	2.0e14	3.69e15

Table 1. Summary of AAV-cFVIII treatment cohorts. M, male; WT, wild-type; CO, codon-optimized; AAV, adeno-associated virus; BDD, B-domain deleted; vg, vector genomes; cFVIII, canine factor VIII; Avg, average.

Results

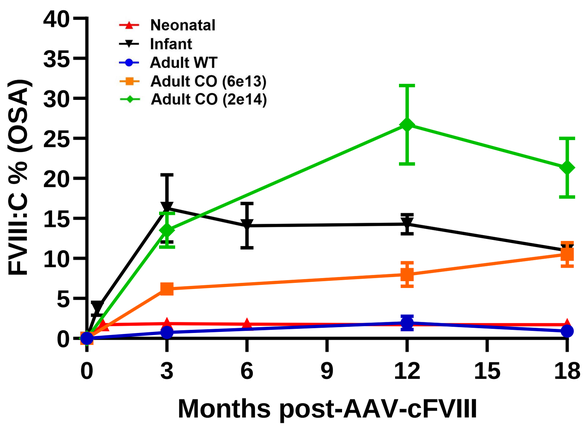

Figure 3. Stable therapeutic FVIII:C expression was seen in adult and infant hemophilia dogs treated with CO-AAV-cFVIII. OSA, one-stage assay, lower level of quantification = 2%. ±SEM.

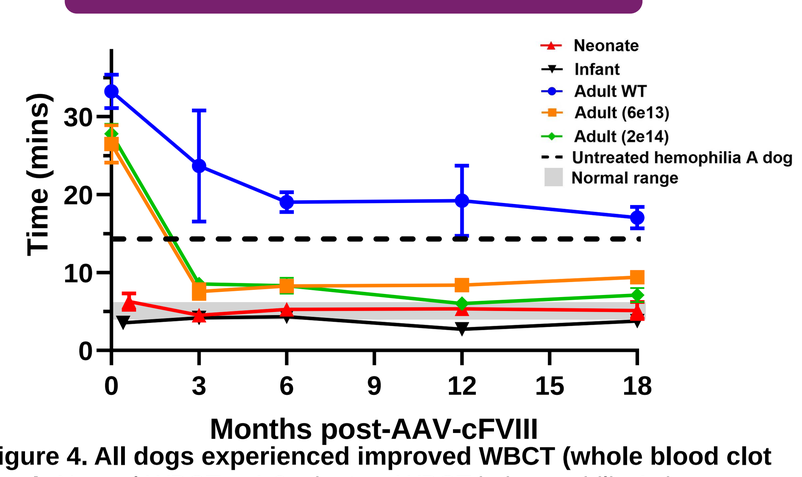

Figure 4. All dogs experienced improved WBCT (whole blood clot time). Normal WBCT = 4-6 minutes. WBCT in hemophilia A dogs = 13.9 minutes; ±SEM.

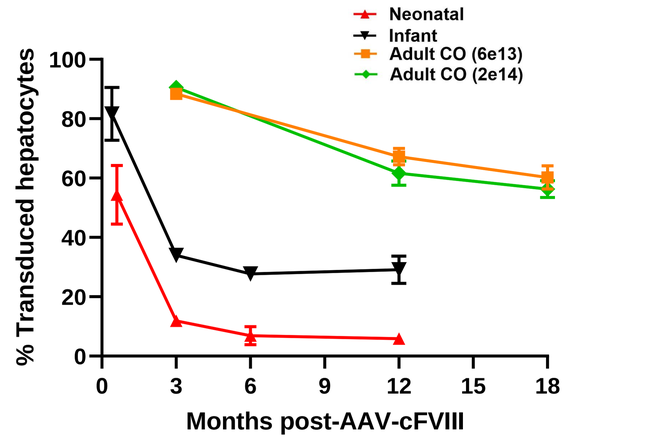

Figure 5. Vector hepatocyte transduction was high, declined over time, and then stabilized as measured by DNA *in situ* hybridization. ±SEM.

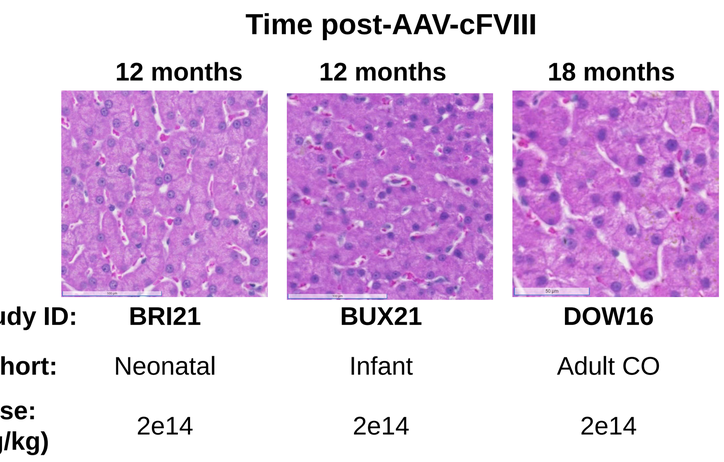

Figure 6. Histopathological analyses of liver biopsies at all time points were normal. Hematoxylin and eosin staining.

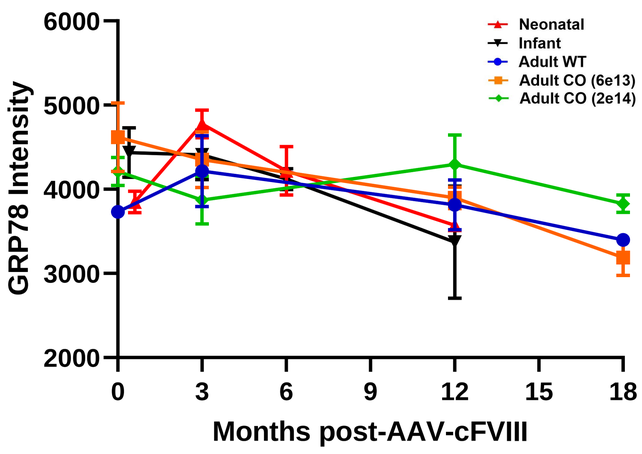

Figure 7. No elevation in ER stress or increased GRP78 staining in FVIII-expressing hepatocytes was observed. ±SEM.

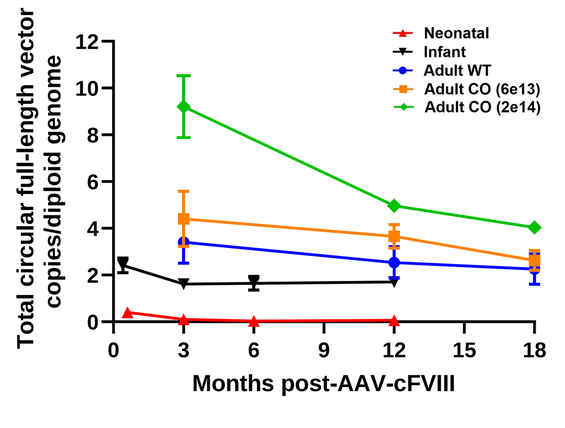

Figure 8. Full-length circular vector genomes were highest at early time points, then declined and remained stable for 12-18 months. ±SEM.

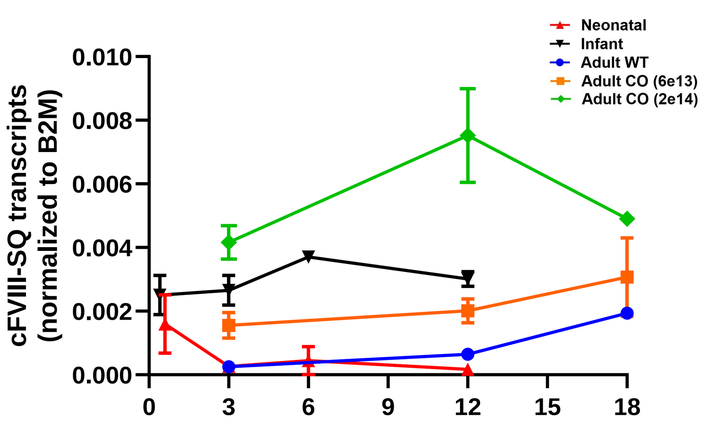

Figure 9. cFVIII mRNA levels were influenced by treatment age and dose. ±SEM.

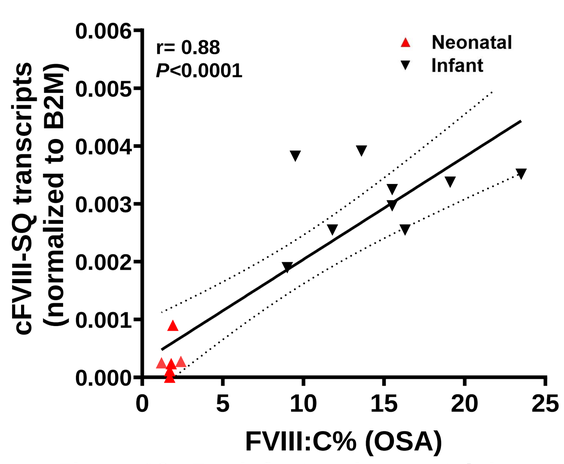

Figure 10. For infant and neonatal dogs, cFVIII mRNA levels positively correlated with FVIII activity. OSA, one-stage assay.

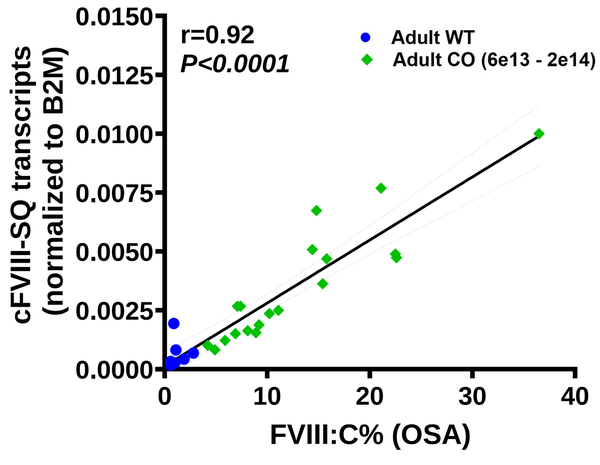

Figure 11. For adult dogs, cFVIII mRNA levels positively correlated with FVIII activity. OSA, one-stage assay.

Conclusions

- Adult and infant dogs treated with CO AAV-cFVIII displayed efficient dose-dependent hepatocyte transduction
- Hepatocyte transduction and circular full-length AAV-cFVIII copies were highest early post-treatment and maintained for 12-18 months with therapeutic transgenic FVIII:C expression (10-25%)
- Circular full-length vector genome levels positively correlated with plasma FVIII:C for infant/neonatal (r=0.88, p<0.001) and adult dogs (r=0.66, p=0.003) at later time points (data not shown)
- Neonatal-treated and WT-AAV-cFVIII-treated adult dogs had reduced FVIII transcript levels compared with other study cohorts

Acknowledgements

This study was supported in part by a Canadian Institutes for Health Research (CIHR) Foundation Grant FDN 154285, and by a grant from BioMarin Pharmaceutical Inc.