Improving AAV-mediated FVIII transgene expression using enhancer sequences from the F8 locus

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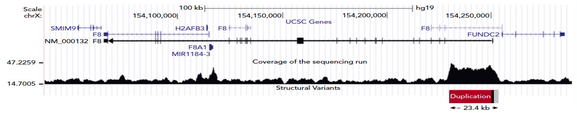
Introduction

- Remaining concerns with FVIII gene therapy include marked variability of factor levels and a decline in levels over time.
- Problems might be mitigated by the delivery of vectors possessing native regulatory elements to endothelial cells that normally express FVIII.
- Native elements might be more resistant to the putative epigenetic silencing that may be occurring in the years following vector delivery.
- Due to AAV5 packing limit of 4.9 kb, and large size of FVIII cDNA, regulatory elements that can be employed are ~ 400bps.
- A 23.4 kb duplication of F8 gene (F8 Padua) covering promoter, exon1 and large portion of intron 1 has been reported to significantly increase FVIII levels (3 to 4-fold).
- High levels of transgene expression might be achieved by incorporating hyper-active regulatory elements.

Aim

- To investigate transcriptional potential of FVIII-Padua enhancer elements *in vitro* and *in vivo*

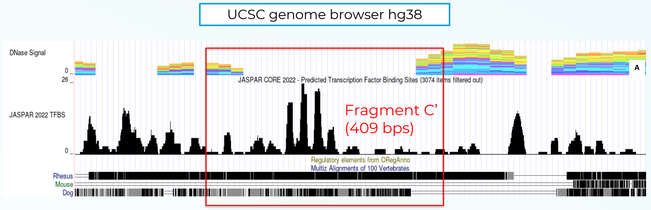


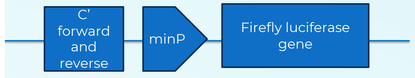
- **Figure 1:** Simioni et al (2021). Duplicated region of 23.4 kb at the 5' end of the Factor 8 transcript.

Methodology

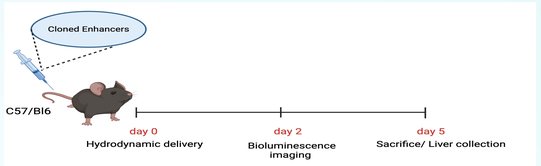
- A 400-bp sequence from intron 1 of F8 Padua or Fragment C' was cloned into a reporter plasmid in forward and inverted orientations. Fragments were cloned upstream of a minimal promoter already present in the vector deriving luciferase expression. Constructs were transfected into HEK293T, HepG2, HUVEC and HLEC (lymphatic endothelial) cells (n=4-6). Luciferase expression was measured.

UCSC genome browser hg38

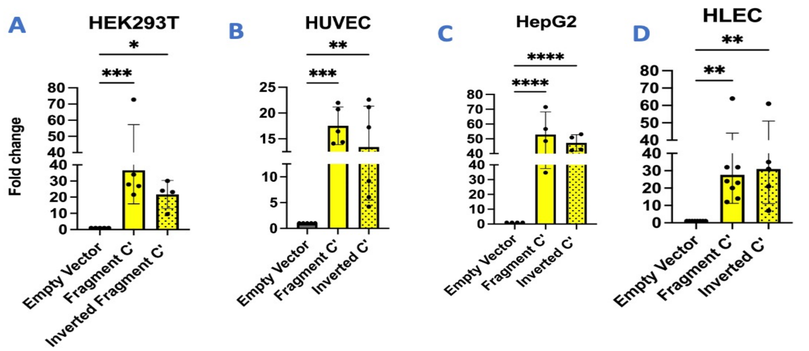




- Luciferase plasmids (100ug) were delivered to C57BL/6 mice (n=3-5) by hydrodynamic injection. In vivo imaging was performed 48 hours post-injection. Liver sections were snap frozen 4 days post-plasmid delivery to quantify luciferase activity in liver homogenates.



Results

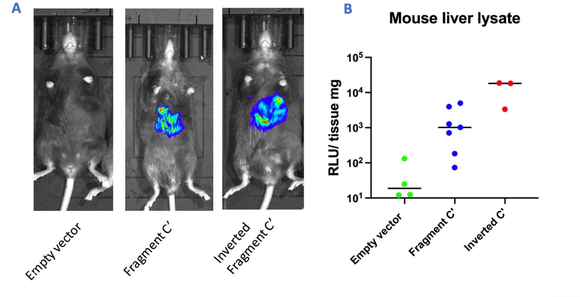


Luciferase activity from Normalized firefly enhancer element constructs in (A) HEK239T (B) HUVEC (C) HepG2 (D) HLEC cells

Data are normalized for empty vector (containing only the minimal promoter) and Renilla fluorescence. HUVEC: human umbilical endothelial cell, HLEC: human lymphatic endothelial cell.

Results

Luciferase expression measured both by whole-animal imaging of bioluminescence in vivo (A) and by direct luciferase assay of liver homogenates (B).



Conclusion

- Fragment C', significantly increased gene expression in both orientations in different cell types, and in mice, suggesting its role as ubiquitous transcriptional enhancer sequence.

Acknowledgments

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CBR THE CENTRE FOR BLOOD RESEARCH

References

1.Simioni P, et al. Partial F8 gene duplication (factor VIII Padua) associated with high factor VIII levels and familial thrombophilia. Blood. 2021;137(17):