Development of optimized ATP7B gene therapy vectors with increased potency for the treatment of Wilson's Disease

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Abstract

Wilson's Disease (WD) is caused by autosomal recessive, loss-of-function mutations in the ATP7B gene which lead to pathological accumulation of copper in the liver, brain, and other tissues. Symptoms of WD include Parkinson's Disease-like neurological defects and hepatic defects associated with cirrhosis. ATP7B is a transmembrane copper pump that is too large to effectively package into adeno-associated virus (AAV). As such, efforts have been made to engineer ATP7B minigenes that can be used as gene therapies for WD. By applying our vector optimization platform to ATP7B, we created a construct that far exceeds the expression of published gene therapy approaches. Here we show that by altering various components within the construct, we can achieve higher expression in various in vitro models and over 100-fold higher expression in the liver of ATP7B-null animals. This may allow us to use a lower viral dose which could improve safety outcomes in patients and lower manufacturing hurdles including cost. Moreover, AAV8-mediated overexpression of our miniATP7B in ATP7B-null mice reduced ALT activity and spleen size, suggesting improved liver function, and decreased immune response. Together, these results indicate a potent and effective new gene therapy for the treatment of Wilson's Disease.

ATP7B in Wilson's Disease

- The prevalence of Wilson's disease is 1 in ~30,000 people[1]
- WD symptoms include elevated copper levels, liver cirrhosis, and dystonia/bradykinesia[2]
- WD is caused by loss-of-function homozygous mutations in the ATP7B gene[3]
- ATP7B encodes a transmembrane copper pump that is 1465 amino acids and is too large to package into AAV[4]
- When copper increases, ATP7B translocates to the lysosome and pumps copper into vesicles so that it can be excreted via the bile duct in the liver[5]

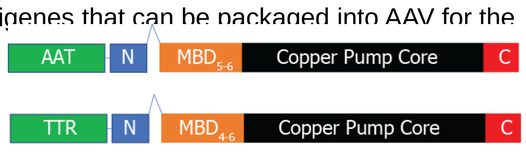
ATP7B Structure

Chang and Hahn. Handb. Clin. Neurol.; 142:19-34. 2017

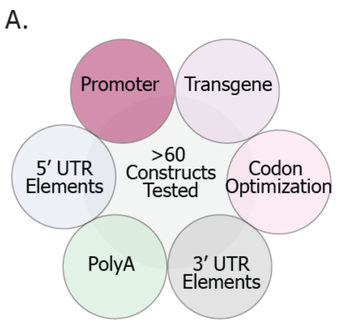
ATP7B Localization

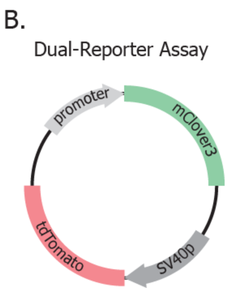
Polishchuk et al. Dev. Cell; 29(6):686-700. 2014

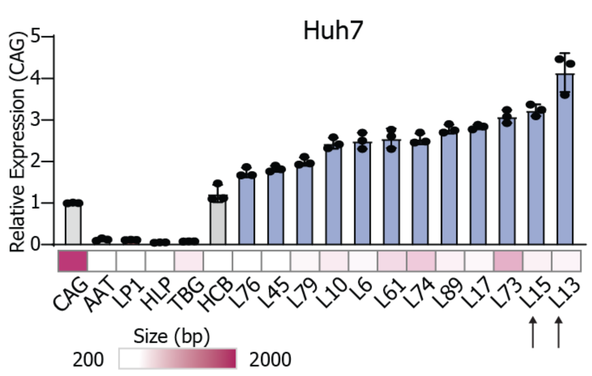
- Efforts have been made to design ATP7B minigenes that can be packaged into AAV for the purpose of human gene therapy
  - Anc80-AAT-miniATP7B-sPolyA[6]
  - AAV9-TTR-miniATP7B$_{co}$-sPolyA[7]

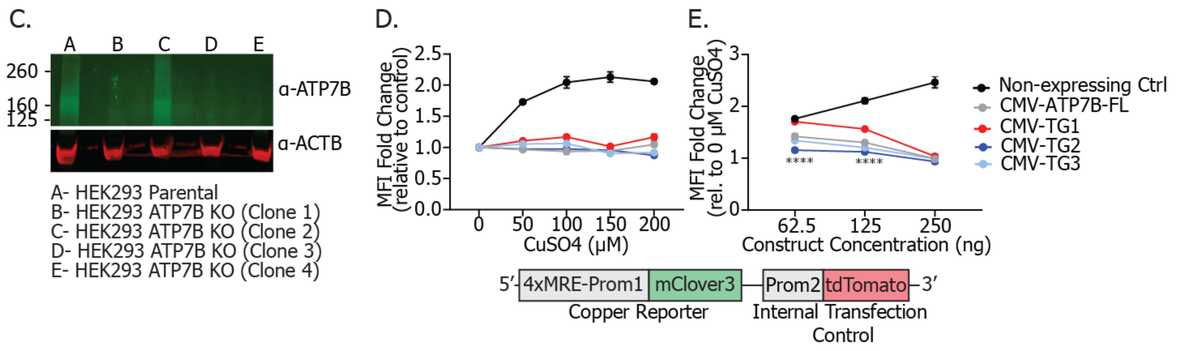


Identification of potent promoters and miniATP7B transgenes for application to Wilson's disease



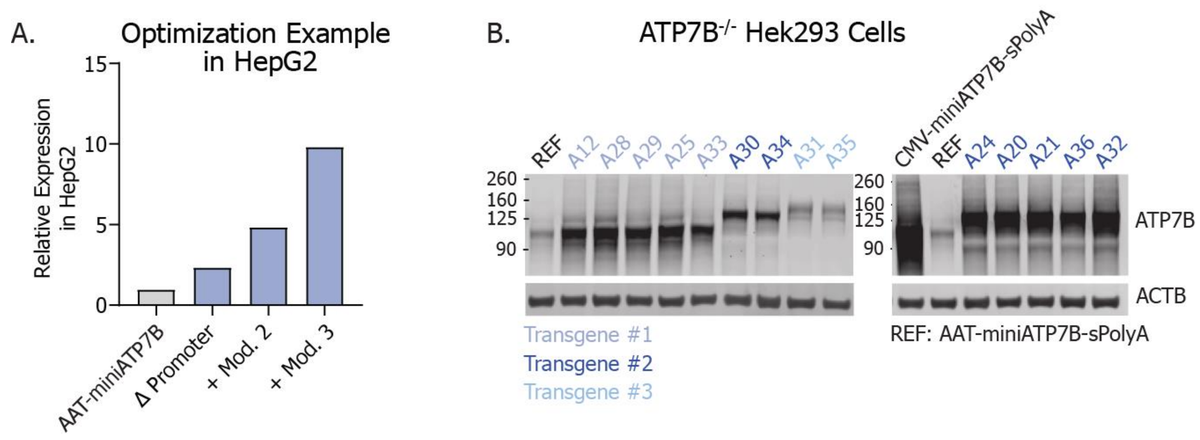


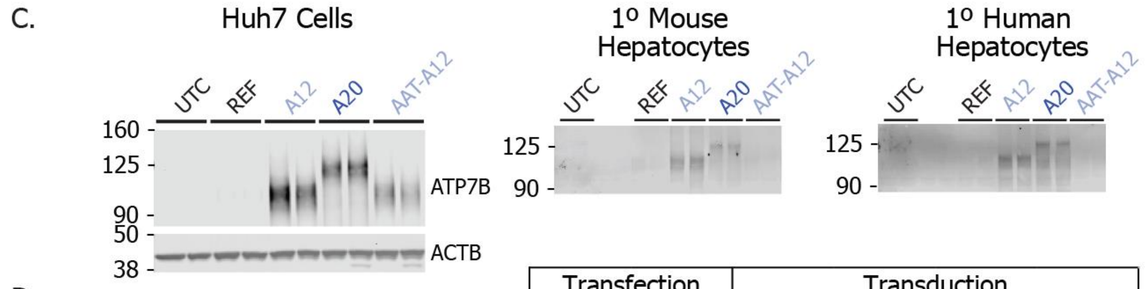




(A) MeiraGTx optimization platform considers multiple factors and combinations in the design of a gene therapy. (B) Using a dual-reporter flow-based assay in transfected human Huh7 cells, the activity of MeiraGTx liver-specific promoters were compared to commonly used promoters. (C) ATP7B KO HEK293 cells were generated using Crispr-Cas9 and validated by Western blotting. (D-E) MeiraGTx ATP7B minigenes were tested for copper pump activity in ATP7B KO cells using a copper reporter in combination with flow cytometry. mClover3 fluorescence increases upon treatment with copper sulfate. This increase is prevented by expression of either full-length ATP7B or mini-transgenes. Potency of transgenes was assessed by measuring copper reporter activity at decreasing concentrations of minigenes.

Superior miniATP7B expression from MeiraGTx Constructs *in vitro*



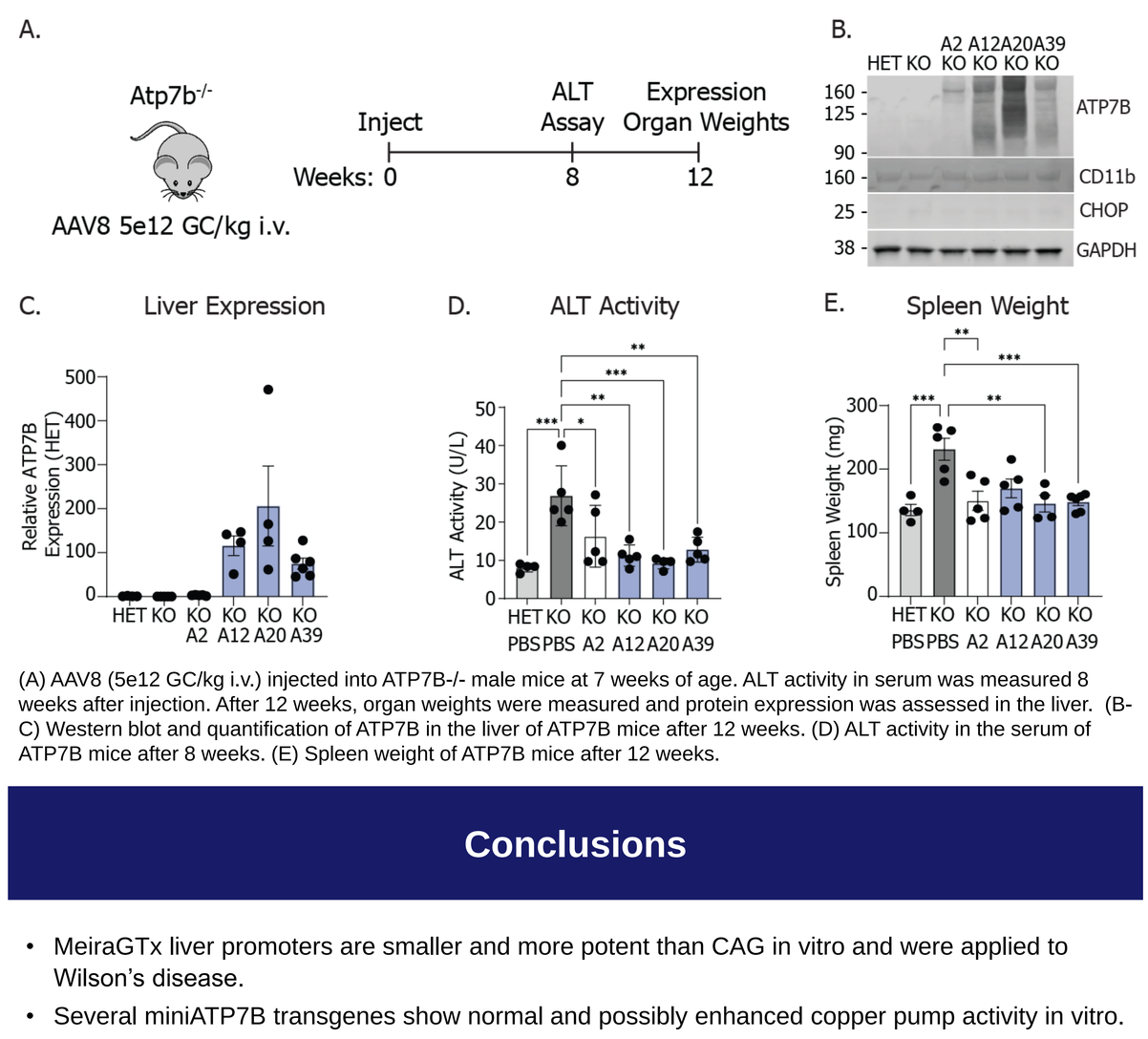


D.

			Transfection		Transduction			
ID	Promoter	ORF	AML12	HUH7	AML12	HUH7	Primary Human	Primary Mouse
REF	AAT	miniATP7B-sPolyA	0.33	0.03	0.03	0.06	0.05	0.10
A12	L15	ORF1	1.00	1.00	1.00	1.00	1.00	1.00
A39	AAT	ORF1	0.37	0.13	0.23	0.49	0.00	0.04
A20	L13	ORF2	1.87	0.52	0.87	0.93	0.70	0.68

(A) Example of the optimization of one MeiraGTx miniATP7B construct that leads to 10-fold higher expression in transfected human HepG2 cells. (B) Combinations of elements were tested to select the highest expressing miniATP7B constructs in transfected ATP7B KO HEK293 cells. (C) Highly-expressing top constructs were confirmed by transducing human Huh7 cells, primary mouse and human hepatocytes with AAV8. (D) Heatmap summarizes miniATP7B expression of the reference construct and MeiraGTx top hits across in vitro cellular models.

Over-expression of optimized miniATP7B rescues ALT and splenomegaly in ATP7B$^{-/-}$ mice



(A) AAV8 (5e12 GC/kg i.v.) injected into ATP7B-/- male mice at 7 weeks of age. ALT activity in serum was measured 8 weeks after injection. After 12 weeks, organ weights were measured and protein expression was assessed in the liver. (B-C) Western blot and quantification of ATP7B in the liver of ATP7B mice after 12 weeks. (D) ALT activity in the serum of ATP7B mice after 8 weeks. (E) Spleen weight of ATP7B mice after 12 weeks.

Conclusions

- MeiraGTx liver promoters are smaller and more potent than CAG in vitro and were applied to Wilson's disease.
- Several miniATP7B transgenes show normal and possibly enhanced copper pump activity in vitro.
- MeiraGTx optimization platform generated several promising ATP7B therapeutic constructs with high expression across diverse cellular models.
- Top MeiraGTx constructs express up to 200-fold more protein in the ATP7B$^{-/-}$ mouse model compared to endogenous levels without increasing inflammatory or ER-stress markers.
- Top MeiraGTx constructs also significantly rescue relevant phenotypes in a mouse model of Wilson's Disease.
- This increased vector potency may allow us to significantly decrease therapeutic dosage, potentially limiting manufacturing requirements and improving safety outcomes.

References

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