Developing an AAV-based Gene Therapy for MYBPC3 Mutation-Associated Hypertrophic Cardiomyopathy

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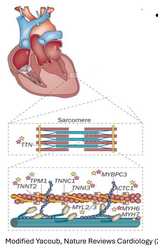
Abstract

Hypertrophic cardiomyopathy (HCM) is a highly prevalent cardiovascular genetic disorder affecting approximately 0.2% (1:500) individuals worldwide. This disease which potentially results in heart failure or sudden cardiac death, is characterized primarily by left ventricular hypertrophy, diastolic dysfunction, myocyte disarray, and interstitial fibrosis. Loss-of-function mutations in *MYBPC3,* the gene encoding cardiac myosin-binding protein C (cMyBP-C), is one of the primary causes of genetic HCM. Most pathogenic mutations of *MYBPC3* arise via frameshift, nonsense, or conserved RNA splice site mutations on a single allele that results in protein truncation, which is more likely to degrade resulting in lower total cMyBP-C protein levels (haploinsufficiency). Haploinsufficiency of *MYBPC3* contributes to sarcomeric dysfunction and deregulation of contraction and relaxation in cardiac myocytes. Restoration of cMyBP-C haploinsufficiency offers a viable therapeutic approach for the treatment of HCM.

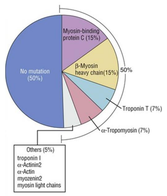
We are developing an AAV-based therapy to treat MYBPC3-associated HCM. We have engineered AAV vectors encoding human cMyBP-C protein. We showed that systemic delivery of AAV vectors effectively restores cMyBP-C to cardiomyocytes in a *Mybpc3* knockout (KO) mouse model. AAV vector treatment of neonatal *Mybpc3* KO mice (on P1) caused a significant reduction in heart weight to body weight ratio in comparison to the vehicle-treated group, indicating there was an improvement in cardiac hypertrophy. Our data also showed a correlation between the level of cMyBP-C protein restored in cardiomyocytes and the reduction in heart weight to body weight ratio. In addition, treatment of the *Mybpc3* KO mice with AAV vectors showed a significant reduction in HCM-associated biomarkers in the heart, including the genes encoding atrial and B-type natriuretic peptide and tissue inhibitor of matrix metalloproteinase-1, a marker of fibrosis which is a hallmark of this disease. Biodistribution studies demonstrated that our vector specifically targeted the heart as evidenced by the lack of protein and mRNA expression in liver and skeletal muscle. Furthermore, no clinical abnormalities or safety concerns were observed in our studies. These data demonstrate that our AAV vectors are capable of restoring cMyBP-C deficiency and improving cardiac hypertrophy and associated biomarkers in a murine model that resembles the human condition of HCM and support the continued development of this AAV-based gene therapy.

Biology and Disease Background



Modified Yacoub, Nature Reviews Cardiology (2014)

- Myosin-binding protein C (cMyBP-C) -
  - Encoded by the gene *MYBPC3*
  - A multi-modular structural protein component of the sarcomere.
  - Exclusively expressed in heart muscle cells and found in the cross-bride-bearing zone of the C region of the A band, forming doublet appearing transverse stripes.
  - A key regulator of cardiac contraction



Modified from Maron and Olivatto, Thoracic Key, Hypertrophic Cardiomyopathy

- Loss-of-function mutations in MYBPC3, is one of the primary causes of genetic HCM -
  - Mutations of MYBPC3, including frameshift, nonsense, or conserved RNA splice site mutations on a single allele, may result in haploinsufficiency (lower total cMyBP-C protein levels).
  - Haploinsufficiency of *MYBPC3* contributes to sarcomeric dysfunction and deregulation of contraction and relaxation in cardiac myocytes.

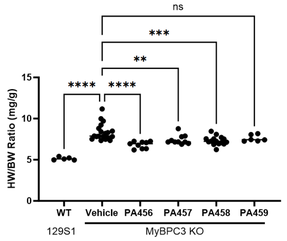
We aim to develop an AAV-based therapy to restore the normal expression and function of cMyBP-C for the treatment of HCM caused by *MYBPC3* haploinsufficiency.

Study Design

- 4 constructs designed containing:
  - Human specific promoter
  - Optimized human Myosin Binding Protein C3 (MYBPC3) gene
- MyBPC3 KO mice treated at P0
- Tissue collected and analyzed 6 weeks post administration for biochemical and histological end points
- Statistical analysis: One way ANOVA with vehicle treated MyBPC3 group as control
- * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

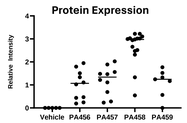
Results

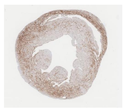
Decreased heart-to-body weight ratio in AAV treated MyBPC3 KO mice



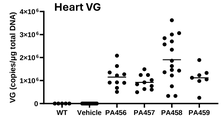
- A hallmark of Hypertrophic Cardiomyopathy is hypertrophy of the heart in which the heart becomes enlarged. Heart-to-body weight ratio (HW/BW) is a method of assessment for this parameter.
- Vehicle-treated MyBPC3 KO mice showed a significant increase ($p < 0.0001$) in HW/BW ratio compared to 129S1/SvlmJ WT mice
- PA456, PA457, and PA458 treated mice showed a significant reduction in HW/BW ratio compared to vehicle-treated MyBPC3 KO mice

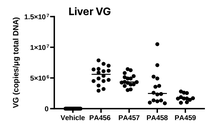
Elevated MYBPC3 protein expression in AAV-treated groups

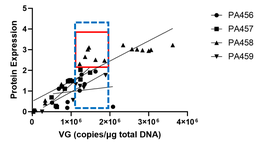




IHC for hMYBPC3 in PA458 treated mouse



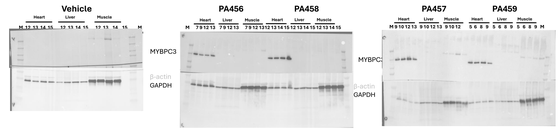




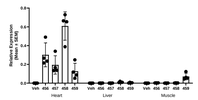
- Protein expression was measured via western blotting and vector copy number (VG) was measured via PCR in heart samples 6-weeks post treatment
  - Protein quantification was completed using ImageJ software by normalizing first to the β-actin loading control and then to an internal standard
- Analysis indicates that protein expression is significantly correlated to VG
- AAV-PA458 treated samples demonstrated the highest overall level of protein expression compared to other treatment groups
- At similar VG levels (blue box), PA458-treated mice (red box) showed highest MYBPC3 protein expression compared to other constructs
- IHC on heart samples from PA458 treated mice demonstrated robust hMYBPC3 signal

Heart-specific protein and mRNA expression of MYBPC3 in AAV treated groups

Protein Expression



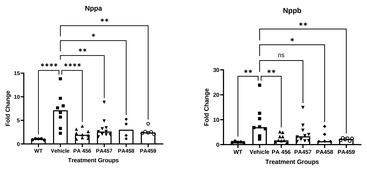
mRNA Expression



- Protein expression of MYBPC3 was detected only in heart tissue samples from treated animals; liver and muscle samples have no detectable level of MYBPC3 protein expression
- mRNA gene expression of MYBPC3 is detected in heart tissue samples, with highest level of expression in PA458 treated mice
- Livers showed highest level of VG copy # among the three tissues being analyzed (data not shown)
- PA459 shows some level of gene expression in muscle samples based on its design

Decreased mRNA expression of disease biomarkers in AAV treated groups

- *Nppa* encodes ANP (atrial natriuretic peptide)
- *Nppb* encodes BNP (B-type natriuretic peptide)
- Both hormones are secreted by the heart and involved in cardiac development, cardiorenal homeostasis, and implicated in response to cardiac injury and stress
- Both *Nppa* and *Nppb* are elevated in vehicle treated MyBPC3 KO mice compared to WT and significantly decreased with AAV treatment, with the exception of PA457 in *Nppb*



Conclusion

- Delivery of our AAV constructs resulted in a cardiac specific expression of MYBPC3 protein in a mouse model of MYBPC3 mutation-associated Hypertrophic Cardiomyopathy
- Treatment with these AAV constructs improved heart-to-body weight ratio, a parameter used to assess cardiac hypertrophy, a hallmark of Hypertrophic Cardiomyopathy
- PA458 demonstrates strong potential for development as an AAV-based gene therapy for MYBPC3 mutation-associated Hypertrophic Cardiomyopathy

Acknowledgements

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