A novel AAV gene therapy for treatment of MYBPC3 hypertrophic cardiomyopathy

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Introduction

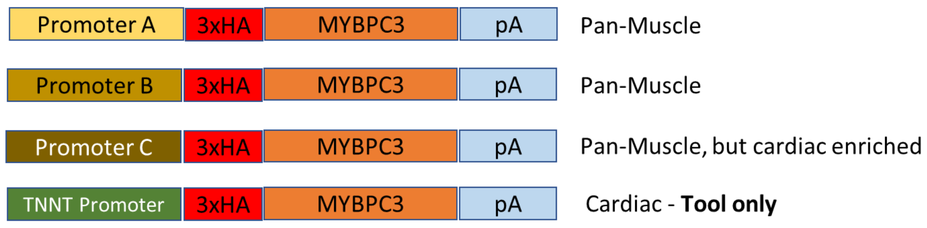
Mutations in the MYBPC3 gene encoding cardiac myosin-binding protein C (MYBPC3) are the most common genetic cause of hypertrophic cardiomyopathy (HCM), accounting for 30–40% of all HCM mutations. Most pathogenic mutations result in reduced functional MYBPC3 protein levels (haploinsufficiency) and can lead to left ventricular hypertrophy (LVH), diastolic dysfunction, cardiomyocyte disarray, heart failure and sudden cardiac death. The majority of MYBPC3 mutations arise via frameshift, nonsense, or conserved RNA splice site mutations on a single allele and result in protein truncation and reduction in total MYBPC3 protein levels. Restoration of MYBPC3 haploinsufficiency offers a viable therapeutic approach for the treatment of HCM.

While AAV currently represents the most tractable approach to a gene therapy treatment for MYBPC3 HCM, the size constraints of the AAV genome limits the design of therapeutic cargos. The large size of the MYBPC3 gene limits the use of gene regulatory elements commonly used to confer robust transgene expression. As such we have evaluated optimized AAV genomic cassettes utilizing novel promoters combined with cardiotropic capsids for optimal MYBPC3 expression. Optimized constructs were tested in our novel cardiotropic AAV capsid with systemic administration in WT mice. We identified one optimized construct that produced robust MYBPC3 expression in the heart.

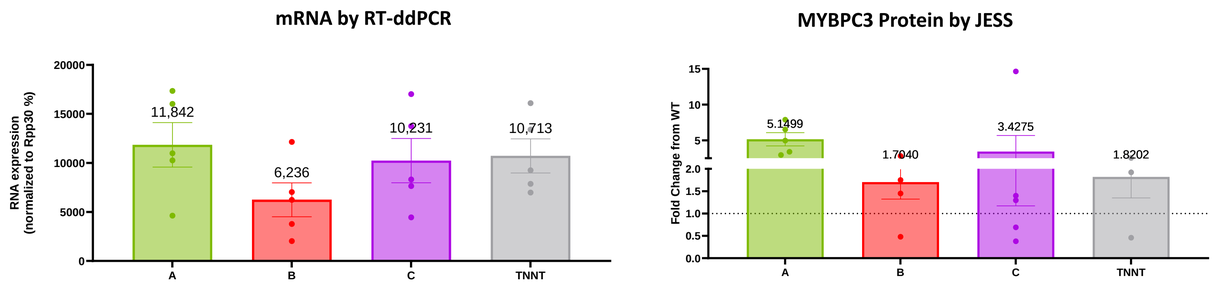
This optimized capsid and cargo were tested in the context of a MYBPC3-deficient murine model of disease. The MYBPC3-targeted knock-in (KI) mouse model carries the human c.772G4A MYBPC3 transition, which is one of the most frequent HCM mutations and is associated with a poor prognosis in patients (Vignier et al., 2009). Homozygous (-/-) KI mice exhibit systolic dysfunction starting from postnatal day 1 and develop severe left ventricular hypertrophy (LVH) by postnatal day 3, accompanied by a 90% decrease in MYBPC3 protein and RNA levels in the heart. In contrast, heterozygous (+/-) MYBPC3 KI mice (possessing one mutant allele and closely represent a majority of MYBPC3 familial HCM patient genetics) show a mild but progressive cardiac phenotype, with a 50% reduction in MYBPC3 protein and RNA expression in the heart, similar to findings in biopsies from patients with familial HCM. Here, we present, for the first time, therapeutic efficacy of our optimized capsid and cargo in both the severe homozygous (-/-) and mild heterozygous (+/-) MYBPC3 KI mice.

Identification of an optimized MYBPC3 therapeutic cargo

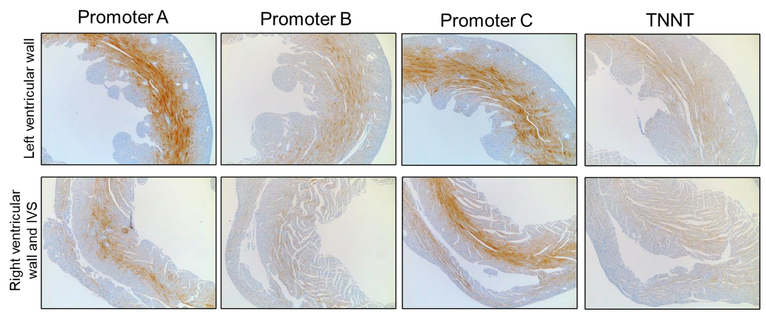
WT mice were injected IV with 2e13vg/kg of AAV expressing four different MYBPC3 constructs



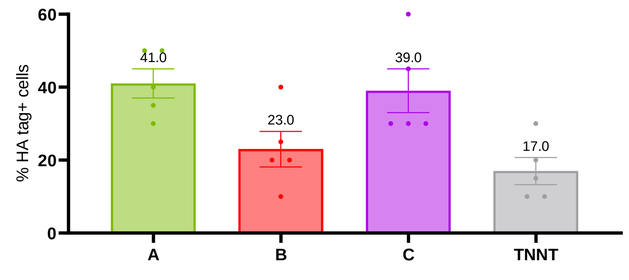
RNA expression and protein levels in the heart 28 days post injection



Promoter A showed a robust increase in MYBPC3 protein levels in the myocardium of WT mice 28 days post AAV injection



Immunohistochemical (IHC) staining of mouse heart sections with HA antibody showing HA-positive staining (brown) in the myocardium. Images taken at 2.5x

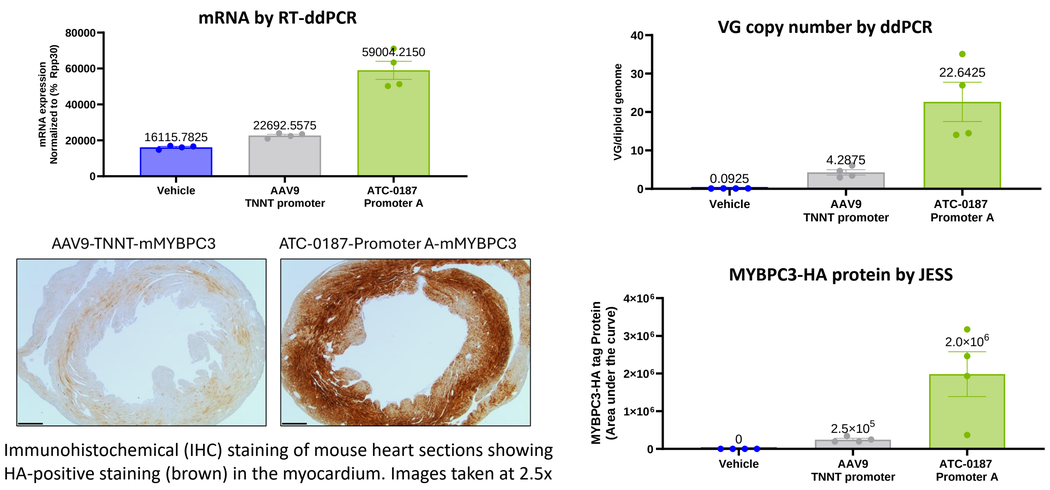


To quantify the fraction of positive cells in the heart, individual scores were assigned for each heart section for both 10 and 20X magnification fields of view (FOV). Individual FOV scores and individual and group means were recorded

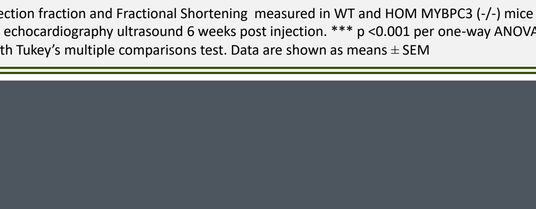
Affinia cardiotropic capsid (ATC-0187) and optimized MYBPC3 construct shows superior expression and biodistribution compared to AAV9-TNNT

WT mice were injected IV with 1e14vg/kg of ATC-0187 expressing an optimized MYBPC3 construct or AAV9-TNNT-MYBPC3

RNA expression and protein levels in the heart 28 days post injection

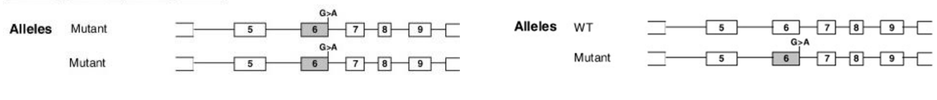


AAV9-TNNT-mMYBPC3 ATC-0187-Promoter A-mMYBPC3

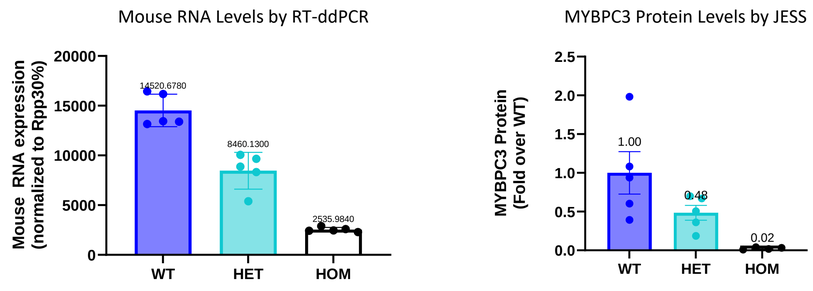


Immunohistochemical (IHC) staining of mouse heart sections showing HA-positive staining (brown) in the myocardium. Images taken at 2.5x

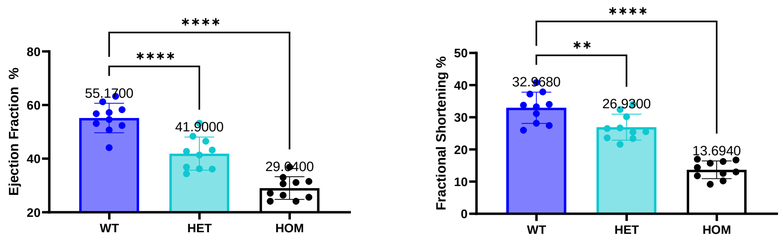
Characterization of an MYBPC3 knock-in mouse model carrying a human point mutation



HOM and HET KI mice have 80 and 50% of WT MYBPC3 mRNA and protein



At 2 weeks of age HOM KI mice have severe cardiac function deficit; HET mice have an intermediate cardiac phenotype by ultrasound

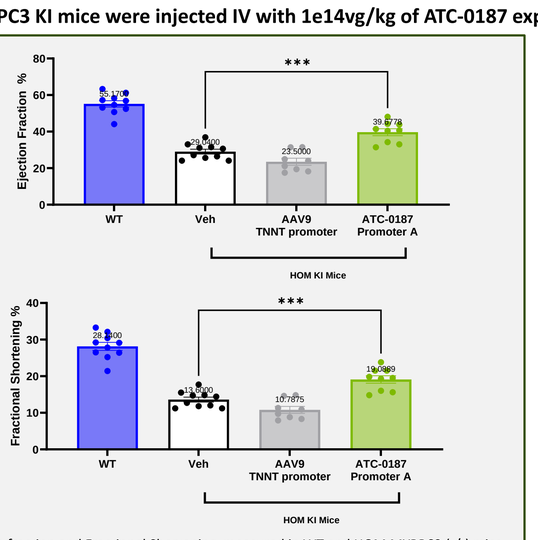


** p <0.01,****p <0.0001 per one-way ANOVA with Tukey's multiple comparisons test. Data are shown as means ± SEM

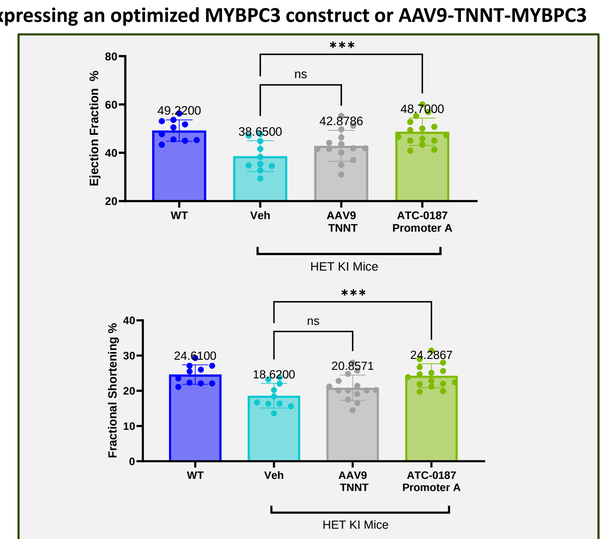
ATC-0187-MYBPC3 treatment improves cardiac function in HOM and HET KI mice

Echocardiography of WT, HET and HOM MYBPC3 KI mice

MYBPC3 KI mice were injected IV with 1e14vg/kg of ATC-0187 expressing an optimized MYBPC3 construct or AAV9-TNNT-MYBPC3



Ejection fraction and Fractional Shortening measured in WT and HOM MYBPC3 (-/-) mice by echocardiography ultrasound 6 weeks post injection. *** p <0.001 per one-way ANOVA with Tukey's multiple comparisons test. Data are shown as means ± SEM



Ejection fraction and Fractional Shortening measured in WT and HET MYBPC3 (+/-) mice by echocardiography ultrasound 11 weeks post injection. *** p <0.001 per one-way ANOVA with Tukey's multiple comparisons test. Data are shown as means ± SEM

Summary and conclusions

- We have identified a novel cardiotropic capsid and optimized MYBPC3 therapeutic cargo that can achieve therapeutic levels of MYBPC3 in the heart and lead to improvement of cardiac function in a clinically relevant animal model of genetic HCM
- Utilizing a next-generation capsid (ATC-0187) with enhanced cardiac tropism, we demonstrated that IV delivery of our optimized construct significantly improved cardiovascular function in both homozygous (-/-) and heterozygous (+/-) MYBPC KI mice
- No adverse in-life observations or test article related histopathologic findings were observed in heart, skeletal muscle or liver
- In contrast, AAV9-TNNT-MYBPC3 showed no efficacy at equivalent doses in either homozygous (-/-) and heterozygous (+/-) MYBPC KI mice
- This gene therapy approach offers a promising strategy for treating MYBPC3 hypertrophic cardiomyopathy, and a highly cardiotropic capsid may serve as a novel therapeutic option for the safe and effective delivery of cardiac proteins for treatment of cardiovascular disease

References:
Vignier N, Schlossarek S, Fraysse B, Mearini G, Kramer E, Pointu H, Mougenot N, Guiard J, Reimer R, Hohenberg H, Schwartz K, Vernet M, Eschenhagen T, Carrier L. Nonsense-mediated mrna decay and ubiquitin-proteasome system regulate cardiac myosin-binding protein c mutant levels in cardiomyopathic mice. *Circ Res.* 2009;105:239–248.

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