B6-Myh6-R404Q

Strain Name: C57BL/6JGpt-*Myh6*$^{em1Cin(R404Q)}$/Gpt
Strain Type: Knock-in
Strain ID: T051403
Background: C57BL/6JGpt

Description

The α-myosin heavy chain, also known as α MHC or MYH6, is a component of myosin. Myosin is composed of two highly similar heavy chains (MYH6, MYH7) and two pair of light chains, in which the heavy chain protein forms functional homodimers or heterodimers[1]. MYH6 and MYH7 expression was found to vary greatly across different species, MYH7 is the main isoform in the adult human cardiac ventricular myocytes, whereas MYH6 is the main isoform expressed in the adult murine ventricular myocardium[2]. Myosin is a motor protein that, together with the other proteins of the sarcomere, functions to regulate myocardial cell contraction, therefore, the abnormal structure of myosin can affect the contraction and function of heart[2]. Molecular genetic studies have demonstrated that the missense mutations (i.e. p.R403Q) of MYH7 can cause familial hypertrophic cardiomyopathy (FHC)[3].

Since MYH6 in mice is constitutively equivalent to MYH7 in humans, therefore, in order to simulate the mutation (p.R403Q) of MYH7 in human, we generated a FHC mouse model via introducing p.R404Q point mutation in mouse MYH6 protein, which is homologous to MYH7 p.R403Q mutation. B6-Myh6-R404Q strain is created on the C57BL/6JGpt background (strain number T051403) in Gempharmatech. Cardiac dysfunction and histopathology in this strain shows parallel phenotypes to human FHC, including myocardial hypertrophy, increased myocardial contraction and fibrosis. Therefore, B6-Myh6-R404Q strain is an ideal model for preclinical anti-FHC drug evaluation.

The B6-Myh6-R404Q strain was created at GemPharmatech using gene editing technology whereby a point mutation was introduced in exon 13, leading to Arg404Gln point mutation of MYH6 protein.

Strategy

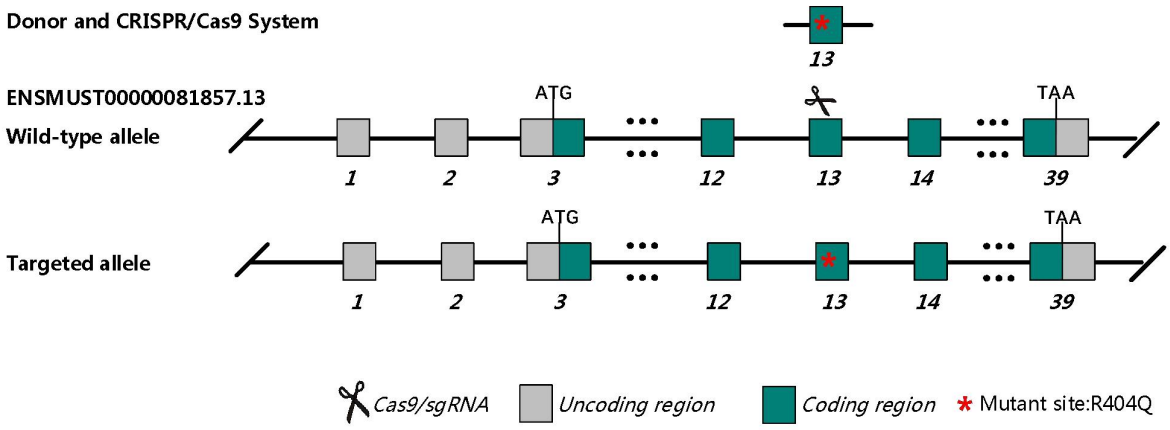


Fig 1. The B6-Myh6-R404Q strain strategy

A single point mutation was introduced into exon 13 of Myh6 gene via CRISPR/Cas9 technology.

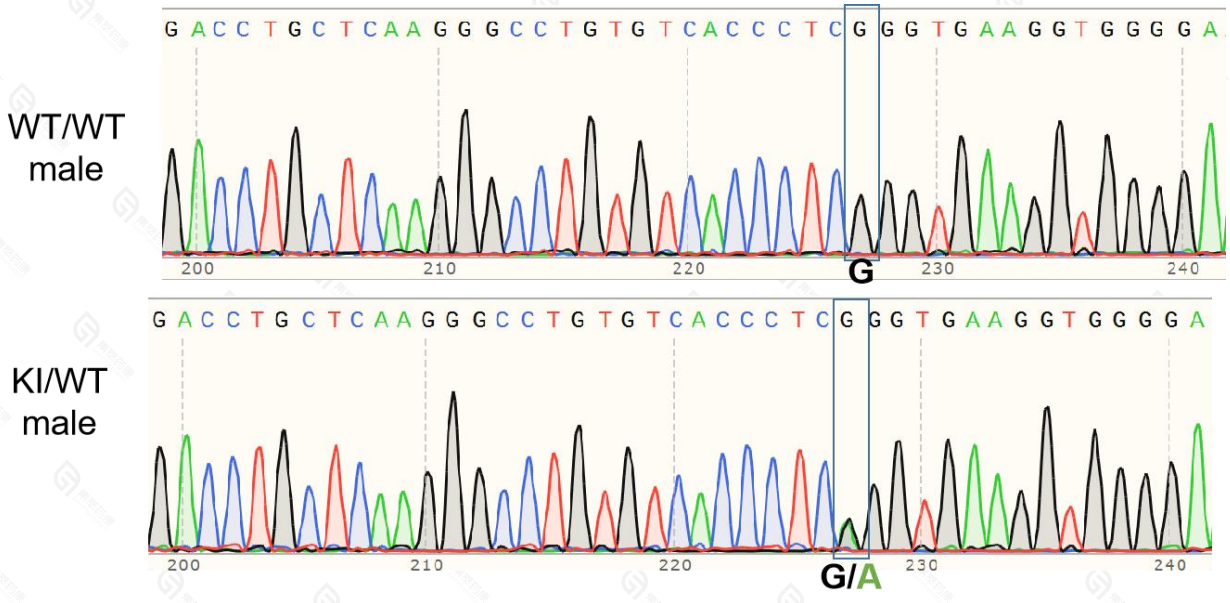
Applications

1.Anti-familial hypertrophic cardiomyopathy (FHC) drug screening and efficacy test.

2.Research on related diseases (such as myocardial hypertrophy and fibrosis) caused by defects of Myh6 (R404Q point mutation).

Data support

1. The point mutation detection of B6-Myh6-R404Q



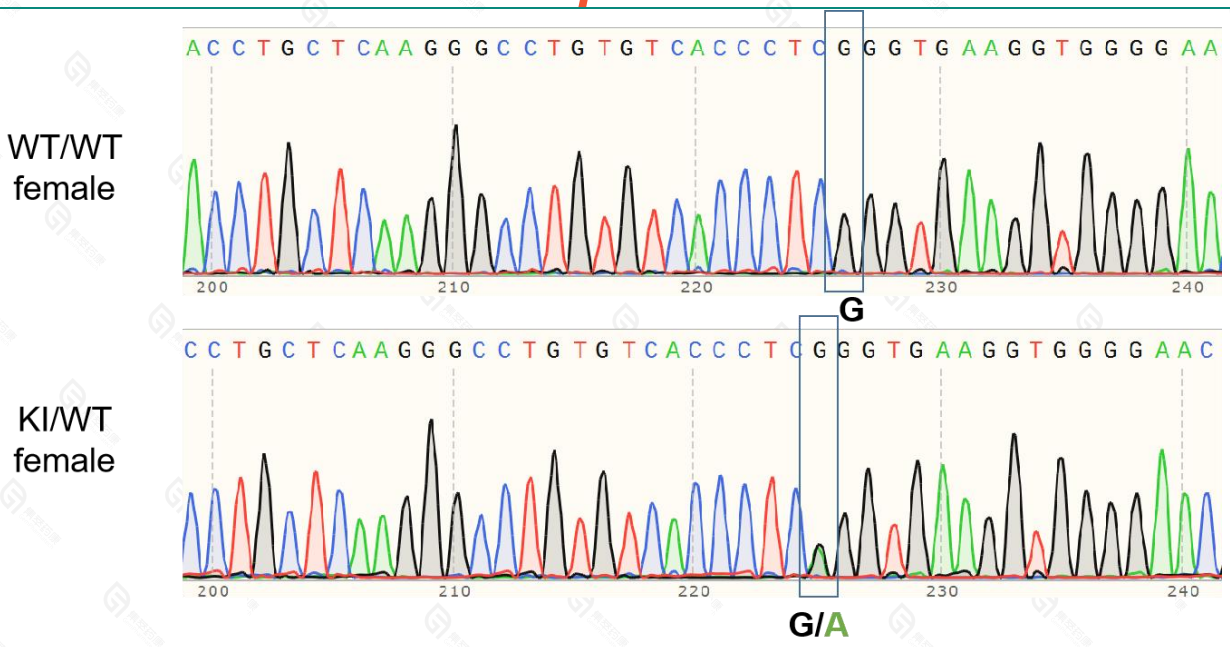
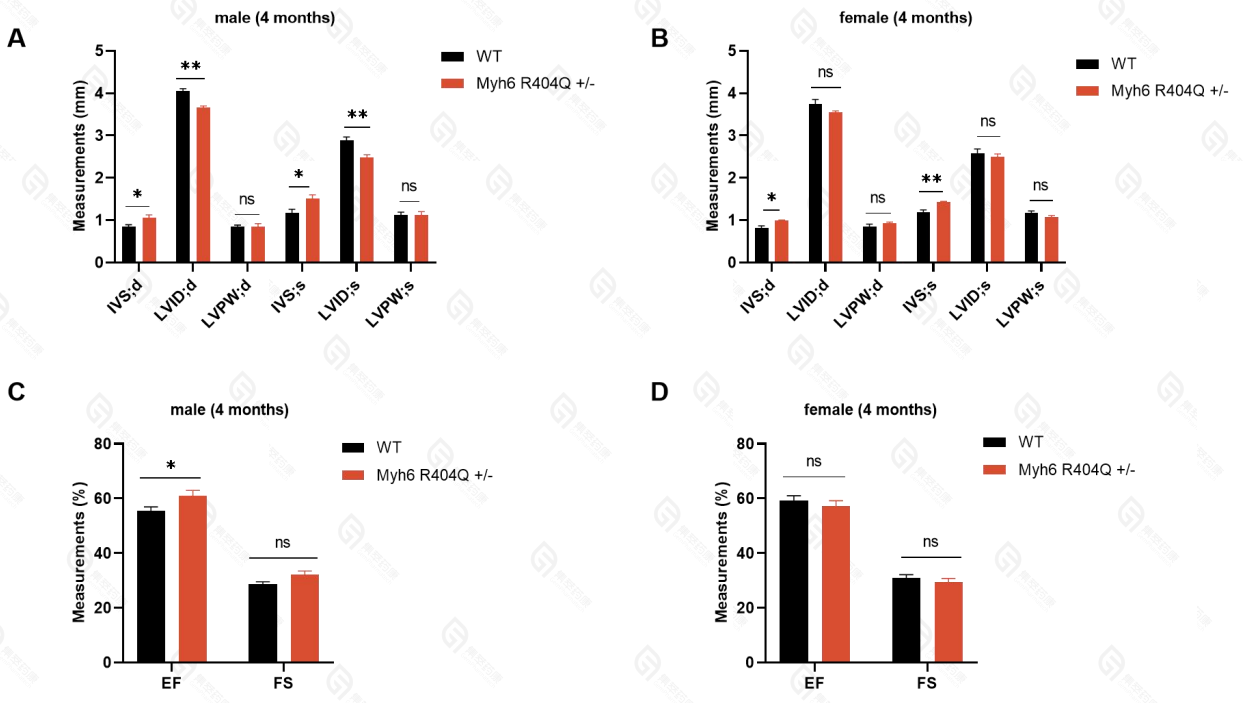


Fig 2. The point mutation detection of B6-Myh6-R404Q

Mouse tails from WT and Myh6 R404Q $^{+/-}$ were collected and fragment containing R404Q mutation site was amplified specifically by RT-PCR. Sanger sequencing was used to detect the amplified fragments. The results (double peak readout in blue box) indicated expression of the mutated allele in B6-Myh6-R404Q heterozygous mice.

2. Analysis of echocardiography parameter



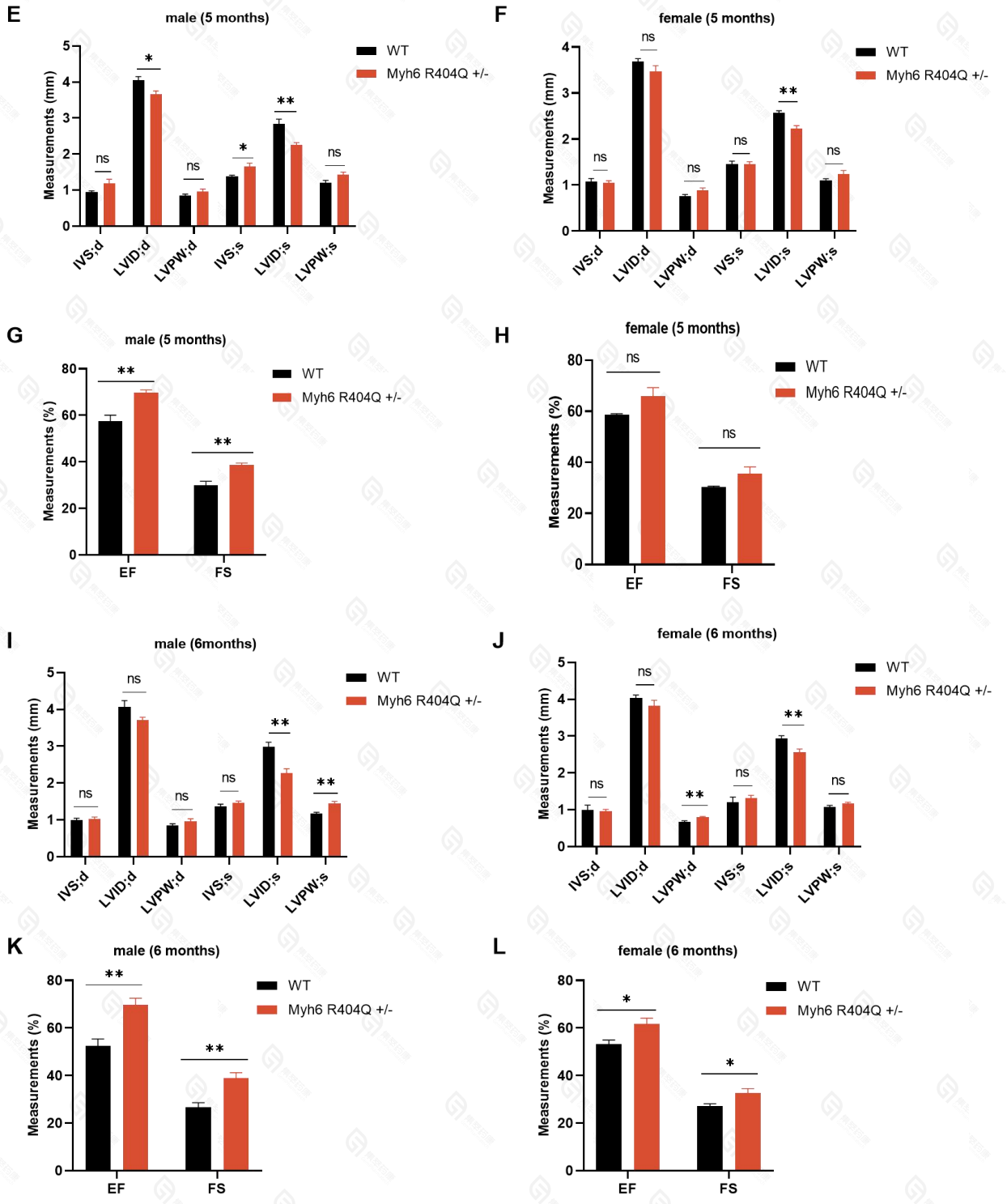


Fig 3. Echocardiography analysis of B6-Myh6-R404Q heterozygous mice

A-L: Echocardiography data at the age of 4, 5, 6 months (n=5). Compared with WT mice, B6-Myh6-R404Q heterozygous mice developed left ventricle hypertrophy and increased contractile function at 4, 5, 6 months of age. The myocardial hypertrophy of male mice was more obvious than female mice. Values are expressed as mean ± SEM. Comparison between groups involved unpaired two-tailed Student's t test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

3. Pathology analysis

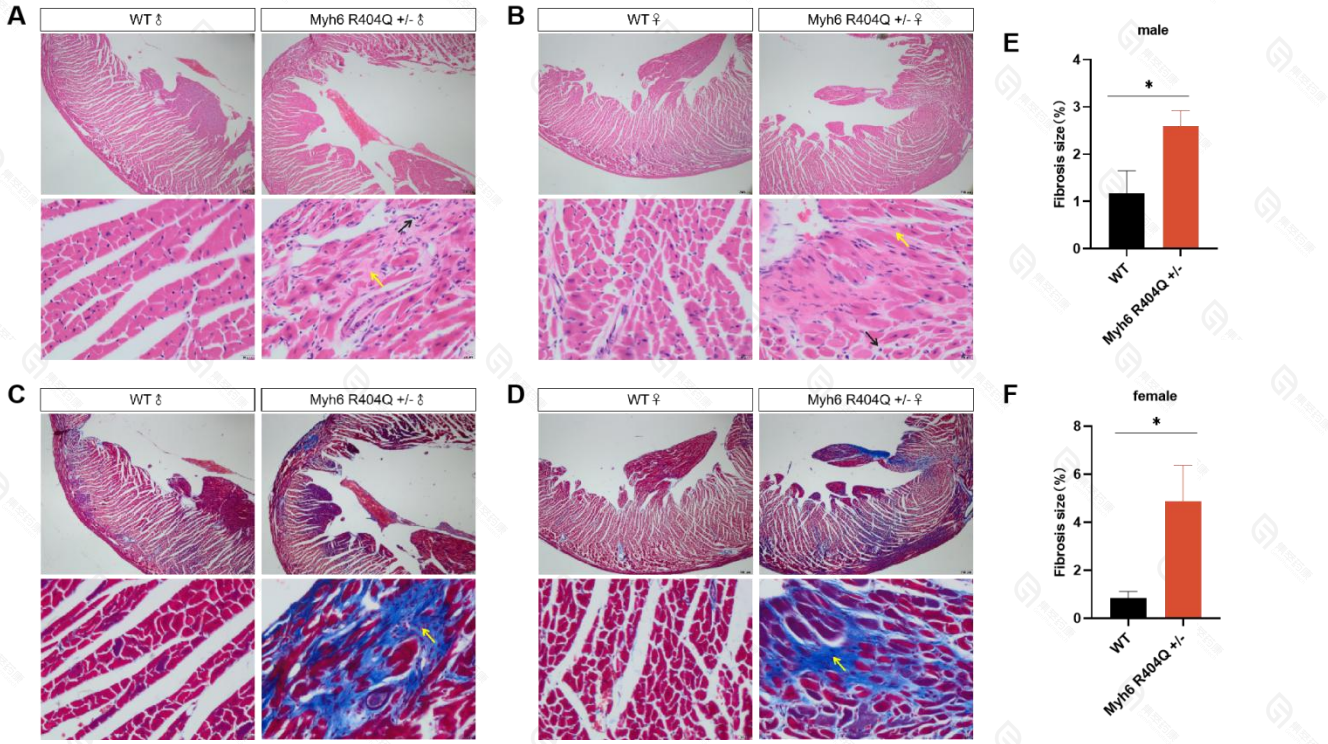


Fig 4. Pathology analysis of B6-Myh6-R404Q heterozygous mice

Histopathologic section of the hearts from WT and B6-Myh6-R404Q heterozygous mice was stained with hematoxylin and eosin (A and B) or Masson trichrome (C and D), (n=5). Compared with WT mice, fibrosis (yellow arrow) and inflammatory cell infiltration (black arrow) were observed in B6-Myh6-R404Q heterozygous at the age of 5 months (A and B), and significant heart fibrosis was observed in B6-Myh6-R404Q heterozygous individuals compared with WT littermates (C and D). E and F: Quantification results of fibrosis size analyzed by ImageJ. A-D: Scale bar, 200 μm in the first row, 20 μm in the second row. All data represent as mean ± SEM. Comparison between groups involved unpaired two-tailed Student's t test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

References

1. England J, Loughna S. Heavy and light roles: myosin in the morphogenesis of the heart. Cell Mol Life Sci. 2013 Apr;70(7):1221-39.
2. Ntelios D, Meditskou S, et al. α-Myosin heavy chain (MYH6) in hypertrophic cardiomyopathy: Prominent expression in areas with vacuolar degeneration of myocardial cells. Pathol Int. 2022 May;72(5):308-310.
3. Geisterfer-Lowrance AA, Christe M, e al. A mouse model of familial hypertrophic cardiomyopathy. Science. 1996 May 3;272(5262):731-4.