A novel role for MICOS complex *CHCHD6* in establishing cardiac structure and function

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Abstract:

Hypoplastic left heart syndrome (HLHS) is a severe birth defect that accounts for up to 4% of congenital heart diseases. HLHS is thought to be a complex, multifactorial genetic disorder, however, the current and tentative list of identified associated genes is short and limits our ability to understand the genetic complexities and pathogenic mechanisms leading to this disease. **There is a need to uncover more genes that may contribute to the molecular, cellular, and morphological processes underlying HLHS**. A candidate HLHS gene list was generated by the Mayo Clinic based on whole genome sequencing of a patient with sporadic HLHS and their unaffected family. Cardiac-specific knockdown (KD) of MICOS (mitochondrial contact site and cristae organization system) complex *CHCHD3/6* in *Drosophila* resulted in drastically compromised heart contractility and altered filamentous (F-) actin, indicative of a novel and potentially critical role for CHCHD6 in the adult heart.

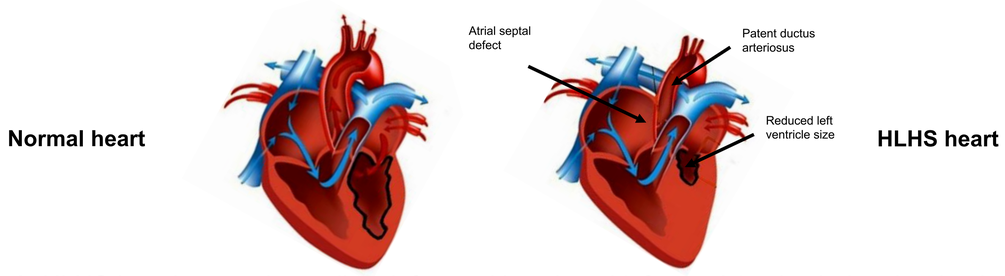

Figure 1: HLHS has three main cardiac defects with reduced left ventricle size being the dominant phenotype. As a result, HLHS patients have reduced systemic circulation and require a 3-step surgical procedure to restore the heart to working capacity (Image modified from news-medical.net).

Methodology:

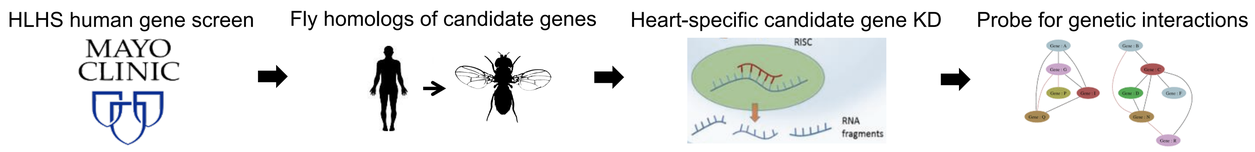

Figure 2: Pipeline to screen HLHS candidate genes. Once candidate genes are determined, genetic networks will be evaluated for possible gene combinations and pathways that could be linked to HLHS.

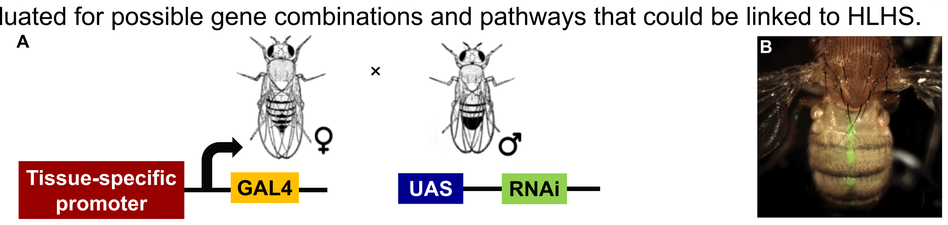

Figure 3: GAL4 UAS system for spatiotemporal KD via RNAi. **A)** Two distinct fly lines are crossed, one has a tissue-specific promoter for transcription of GAL4 and the other has GAL4 binding sites (UAS) for transcription of hairpin RNA to the gene of interest. Dicer-2 complex cleaves the hairpin RNA which is further degraded by RISC (RNAi silencing complex) for gene KD. **B)** GFP-labeled *Drosophila* heart, image courtesy of Geo Vogler.

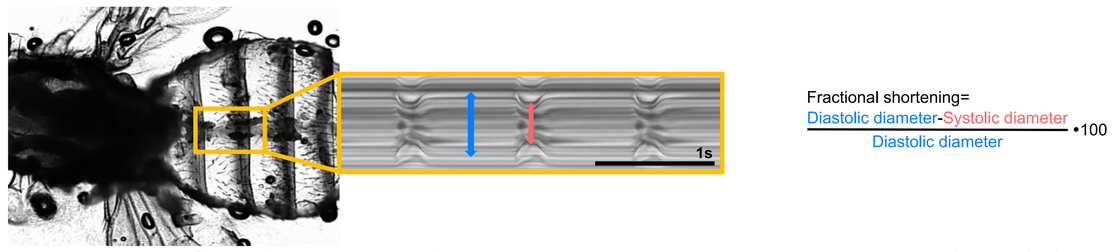

Figure 4: *in situ* prep of the *Drosophila* heart and a corresponding m-mode depicts a single-pixel slice of the heart plotted over time. SOHA calculates cardiac-relevant parameters such as contractility and rhythmicity.

Results:

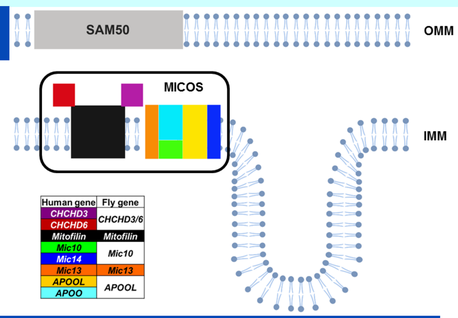

Figure 5: Illustrated model of the human MICOS complex in the inner mitochondrial membrane (IMM) which functions to maintain cristae morphology and respiratory complex assembly. *CHCHD6* KD has striking cristae phenotypes in various systems including yeast, cell lines, mouse, and *Drosophila* where the cristae junctions are altered or absent. SAM50 has been shown to interact with the MICOS complex (Image based on Guarani et al., 2015).

Cardiac-specific KD of *CHCHD3/6* leads to reduced fractional shortening (contractility) and altered F-actin

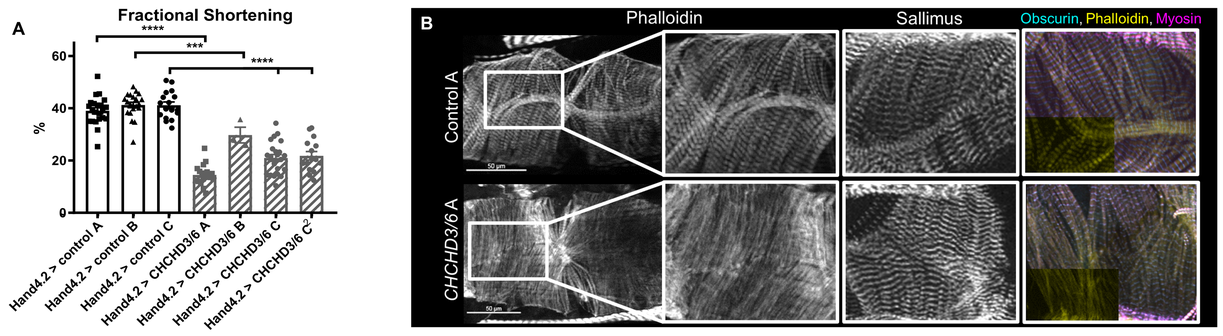

Figure 6: A) 1-week old heart-specific *CHCHD3/6* KD flies have reduced fractional shortening. Unpaired two-tailed t-test, ***p≤ 0.001, ****p≤ 0.0001, error bars= SEM. **B)** Cardiac tissue from *Hand*$^{4.2}$>*CHCHD3/6* flies have altered F-actin (Phalloidin) where the Z-lines are not well defined. Additional sarcomeric components including Sallimus (TITIN-like protein in humans), Myosin, and Obscurin (M-band marker) did not exhibit as striking of structural defects.

The adult *CHCHD3/6* KD phenotypes are cardiomyocyte-specific and relevant at early adult stages

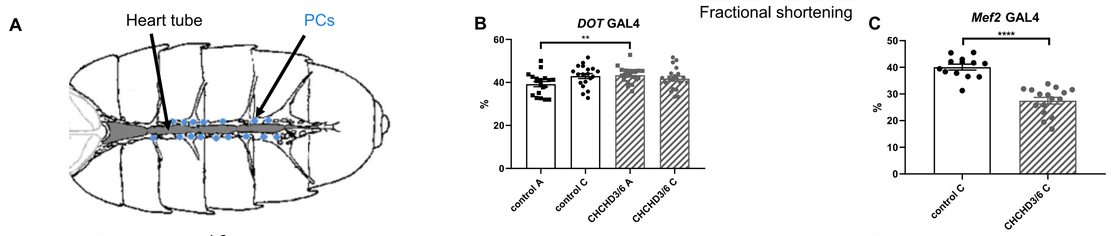

Figure 7: A) The *Hand*$^{4.2}$ GAL4 driver line is specific to CMs and pericardial cells (PCs), which aid in excretion (Image modified from Xie et al., 2013). **B)** *DOT* GAL4 KD of *CHCHD3/6* in the PCs did not cause reduced fractional shortening. **C)** *CHCHD3/6* KD with a *Mef2* GAL4 driver (all muscle cell driver, not in PCs) caused a significant reduction in fractional shortening and no change in F-actin (not shown) in the C fly line while the A fly line was pupal lethal. Unpaired two-tailed t-test, **p≤ 0.01, ****p≤ 0.0001, error bars= SEM.

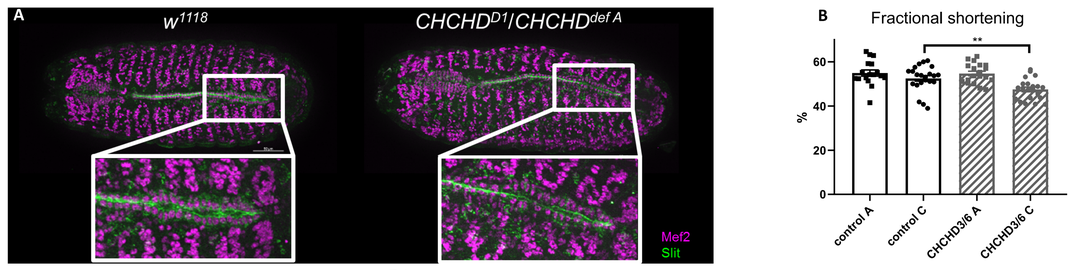

Figure 8: A) Stage 16 embryos (*CHCHD*^{D1} LOF crossed to *CHCHD* deficiency line) stained for Mef2 and Slit (secreted lumen protein). *CHCHD3/6* LOF embryos do not have overt cardiac-specific defects compared to *w*^{1118} control. **B)** Fractional shortening measured from early pupae using a *Hand*$^{4.2}$, tdtK (cardiac RFP) driver. Early pupae do not have as significant changes in fractional shortening as in adults, suggesting *CHCHD3/6* is particularly important after this developmental stage. Unpaired two-tailed t-test, **p≤ 0.01, error bars= SEM.

Cardiac KD of other MICOS and related genes have reduced fractional shortening, however F-actin appears normal

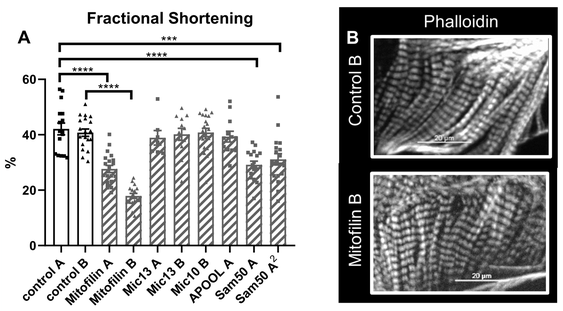

Figure 9: A) Fractional shortening measurements from *Hand*$^{4.2}$ KD of individual subunits of the MICOS complex and *SAM50*. 3-week old *Mitofilin* KD and *SAM50* KD female flies have reduced fractional shortening. Unpaired two-tailed t-test, ***p≤ 0.001, ****p≤ 0.0001, error bars= SEM. **B)** 1-week old female fly hearts with *Hand*$^{4.2}$ GAL4 cardiac-specific KD of *Mitofilin* were imaged for F-actin (stained with Phalloidin). No other MICOS complex subunit exhibited *CHCHD3/6*-like F-actin staining when knocked down cardiac specifically (not shown).

All muscle KD of *CHCHD3/6* leads to mitochondrial dysfunction

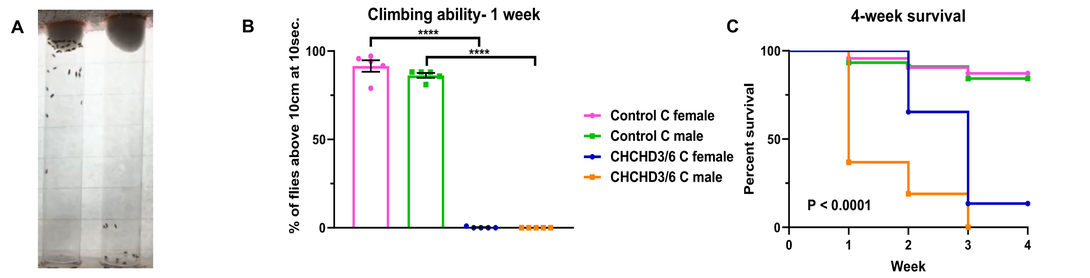

Figure 10: A) Climbing ability was assessed by counting the number of flies that reached 10 cm by 10 seconds. **B)** MitoGFP; *Mef2* GAL4 (muscle-specific) *CHCHD3/6* KD flies had reduced climbing ability; control C f= 178 and m= 125, CHCHD3/6 C f= 154 and m= 112; unpaired two-tailed t-test, ****p≤ 0.0001, error bars= SEM. **C)** *CHCHD3/6* KD males and females had reduced survivability; Mantel-Cox test.

Additional MICOS variants were found in HLHS patients

Gene	*CHCHD6*	*CHCHD3*	*Mic10*	*Mitofilin*	*SAM50*	*APOO*
HLHS Proband	11H, 158H, 228H	179H (*de novo*), 199H, 287H, PCGC	8H	126H, 145H	41H, **87H**, 156H, 292H, **363H**	**87H**, **363H** (X-linked)
Fly gene	*CHCHD3/6*		*Mic10*	*Mitofilin*	*Sam50*	*APOOL*

Figure 11: 183 HLHS probands were screened for MICOS variants. Multiple MICOS variants were found, including two patients (87H and 363H) which harbored both *SAM50* and *APOO* variants. Interestingly, one HLHS patient from the pediatric cardiac genomics consortium databank also has a mutation in *CHCHD3*.

Summary:

- *CHCHD6* was found as a candidate HLHS gene using patient-specific data
- When *CHCHD3/6* was knocked down heart-specifically in *Drosophila*, there was a significant reduction in fractional shortening and remodeling of F-actin in the heart
- These cardiac phenotypes are relevant at early adult stages of development
- Climbing ability and viability were reduced when *CHCHD3/6* was knocked down using a muscle-specific driver

Outlook: We are continuing to examine interactions between *CHCHD3/6* and other emerging candidate genes to identify novel gene functions and pathways likely to contribute to HLHS. Further validation of novel candidate genes, genetic interactions, and causal pathways can possibly lead to the targeted prevention of HLHS, post-natal risk of heart failure in HLHS, as well as other congenital heart diseases.

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