

## Using a zebrafish model to quantify a cardiomyopathy phenotype in a causal novel gene

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### Introduction

Cardiomyopathies are primary myocardial disorders with a structurally and functionally abnormal heart muscle. Reaching an etiological diagnosis is important for prognosis and counselling on recurrence risks. Gene identification through WES still represents a major challenge, especially since the genetics is heterogeneous. However, proving that a certain gene is a novel cause may require lengthy functional studies, including animal models. We studied a small family with two siblings presenting with a novel, lethal CMP. It was characterized by fetal-onset restrictive CMP predominantly affecting the right ventricle and leading to irreversible heart failure and early death. This phenotype is unique and has not been reported in literature.

### Methods

Exome sequencing analysis was done in unaffected and affected siblings. Genomewide SNP typing and parametric linkage analysis was done in a recessive model. Embryos of wildtype, Tg(kdrl:EGFP)s843 and double transgenic Tg(gata1:DsRed2;kdrl:EGFP) zebrafish lines were used. The translational inhibition and splice site morpholino oligomer (MO) for zebrafish kif20a was injected into 1 to 2 cell stage embryos. Phenotypes were studied at 24, 30, 48, 72, 96 120 and 144 hours post fertilization (hpf). The efficiency of MO on inhibiting kif20a expression was checked by Western blot assay.

### Results

We identified two compound heterozygous functional variants in the KIF20A gene as the most likely cause in the family. In the zebrafish model, from day 2 onward a progressive cardiac phenotype was seen in 90% of the morphants with pooling of red blood cells proximal to the atrium, relative bradycardia, decreased cardiac function and cardiac oedema. Western blot showed a 74% protein reduction in kif20a ATG MO. To quantify cardiac function, confocal imaging of live embryos was done at 48, 72, 96 and 120 hpf. The atrium and ventricle was imaged separately and fractional shortening and heartrate is calculated. Histology is currently being performed.

### Conclusions

Several mutations in known and novel genes causing CHD and CMP are being identified as pathogenic since the advent of Next Generation Sequencing. Proving the causality is crucial. We show that a zebrafish model can be used reliably to quantify the phenotype and cardiac function in a novel gene causing cardiomyopathy.