# Investigating how the dysregulation of autophagy leads to abnormalities in heart development and disease.

*Chloe Frost*, Kate E Bailey, Joe Fenwick, Helen M Phillips. Institute of Genetic Medicine, Newcastle University.*

## Aims

The aim of the project was to delete the function of the autophagy gene *Atg16l1* throughout the developing mouse embryo and examine the consequences in the heart. We hypothesize that deletion of *Atg16l1* will prevent the formation of autophagosomes hence preventing autophagy to occur which may impact on how the heart develops.

## Introduction

Birth defects originating from the development of the heart are termed "Congenital Heart Defects" (CHDs) and are common, affecting approximately 8 in 1000 births[1]. Such defects can be life limiting and cause severe health issues later on in life. Although causative genes have been identified, there are many cases of CHD for which a disease causative gene has not yet been identified. Therefore, our aim was to investigate the possibility that disruption of the cellular process autophagy, is linked to CHD. Autophagy recycles damaged cellular components and has been implicated in other disease states such as cancer and neurodegeneration[2]. Autophagy has also been discovered to play a role in cardiac morphogenesis as when essential genes are knocked down in fish, an abnormal heart structure was seen with defects in cardiac looping and abnormal chamber morphology[3]. Autophagy requires the formation of a double membrane structure known as the autophagosome and is regulated by the formation of complexes of key autophagy proteins, including our gene of interest, Atg16l1 (Figure 1).

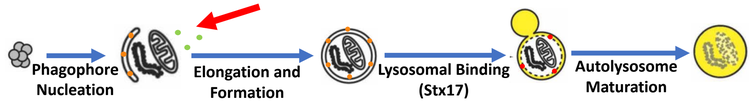


***Figure 1: A diagram showing the Autophagy Pathway.*** *Adapted from Mizushima 2007 [4]. A phagophore elongates and forms a autophagosome which then fuses with lysosome, maturing into an autolysosome which degrades the components inside.* ***Atg16l1*** *is a key component in the elongation of the phagophore to form a autophagosome. Red arrow indicates where in the process Atg16l1 is important.*

## Methods

*Atg16l1;Tm1a* transgenic mice were used to knock out the function of *Atg16l1* globally and *Atg16l1;Tm1c*$^{Tnt\text{-}Cre}$ mice allowed the conditional deletion of *Atg16l1* specifically in the embryonic cardiomyocytes in the heart. Embryos were collected and genotyped by PCR (Figure 3). For histological analysis of the hearts, the embryos were embedded into wax and sectioned then Haematoxylin & Eosin (H&E) stained so enabling visualisation of the structure of the heart (Figure 4). Immunohistochemistry was used for stx17 staining to allow visualisation of the autophagosomes (Figure 5).

## Results

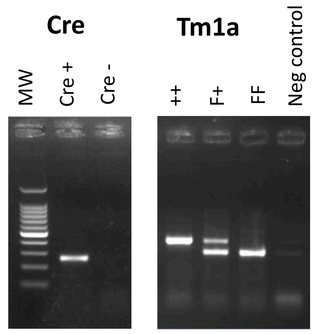


***Figure 3: Cre and tm1a PCR genotyping on DNA extracted from a limb images:*** *– MW – molecular weight ladder. Cre band seen at 280bp. Tm1a - Mutant (MT) FF band seen at 317bp, Heterozygous (contains one mutant allele and one normal allele – HET) F+ , Wild Type (normal – WT) ++ band seen at 430bp.* ***Mutant must be FF Cre +.***

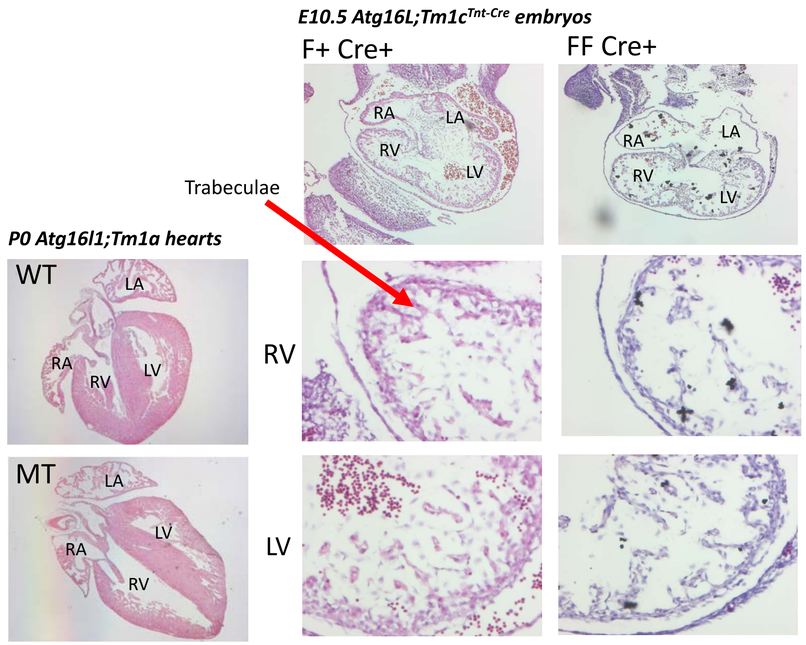


***Figure 4: H&E Staining of E10.5 Atg16L;Tm1c$^{Tnt\text{-}Cre}$ embryos and P0 Atg16l1;Tm1a hearts.*** *RA– right atria. LA – Left atria. RV – Right ventricle. LV – Left ventricle. Tm1a images at x2.5 magnification. Top tm1c-tnt images at magnification x5. Ventricle tm1c images taken at magnification x20.*

H&E staining showed that mutants developed less trabeculae and formed thinner ventricular walls this can be observed in both the tm1a and tm1c-tnt images. This could indicate a development issue which could be related to CHDs and future functional issues. Thinner walls could mean an increased likelihood of perforations in the heart lining potentially causing a ventricular septal defect.

Having identified structural differences in the Atg16l1;Tm1a mutant embryonic hearts, we next investigated the presence of autophagosomes using the Stx17 antibody. Staining suggested that there was a reduced number of Stx17+ve autophagosomes. This is expected as the Atg16l1 protein is involved in the elongation of phagophores to form the autophagosomes which are required for autophagy.

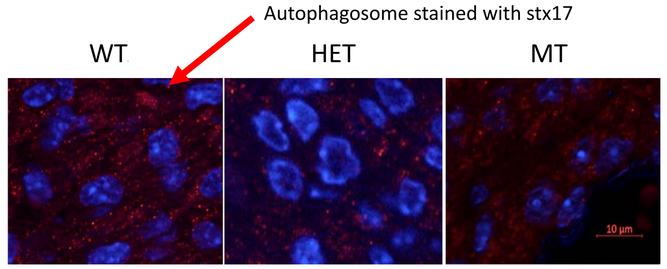


***Figure 5: Immunohistochemistry stx17 staining of P0 Atg16l1;Tm1a hearts****: Right ventricle imaged at x63 magnification. The red spots are the stained stx17 which indicate the autophagosome number. We stained the surrounding cells with DAPI. n = 9 observing 3 mutants.*

## Discussion

In conclusion when the *Atg16l1* gene in the autophagic pathway was knocked out, the embryo hearts developed thinner ventricular walls, which could cause future functional issues. Both tm1a and tm1c-tnt appeared to have the same phenotype which means we can deduce *Atg16l1* has an important role in cardiomyocytes. There also appeared to be less autophagic activity in the mutant mice this should be the case as we knocked out the *Atg16l1* gene which is crucial for the formation of autophagosomes. In future work I would need to measure autophagy flux to quantitatively confirm that autophagy is inhibited in mutant mice.

## References

[1] NHS, Congenital Heart Disease, cited Wednesday 16th October 2019, https://www.nhs.uk/conditions/congenital-heart-disease/
[2] Saha S, Panigrahi DP, Patil S, Bhutia SK, Autophagy in health and disease: A comprehensive review, Biomedicine and Pharmacotherapy, August 2018:104;485-495. doi: 10.1016/j.biopha.2018.05.007
[3]Lee E, Koo Y, Ng A, Wei Y, Luby-Phelps K, Juraszek A, Xavier RJ, Cleaver O, Levine B, Amatruda JF, Autophagy is essential for cardiac morphogenesis during vertebrae development, Autophagy, April 2014:10(4);572-87, DOI: 10.4161/auto.27649.
[4] Mizushima N, Autophagy: process and function, Genes & development: 2007;21(22):2861-73, DOI: 10.1101/gad.1599207