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SUPERNUMERARY OUTFLOW TRACT CUSHIONS IN A MODEL OF TRANSPOSITION OF THE GREAT ARTERIES

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Introduction Congenital heart defects are a leading cause of morbidity and mortality in new-borns; the most severe of which are abnormalities in the development of the outflow tract (OFT), such as transposition of the great arteries (TGA) and double outlet right ventricle (DORV). It has been previously suggested that TGA is caused by failed rotation of the OFT and/or abnormal spiralling of the two cushions that septate the OFT. Our aim was to investigate these possibilities using a murine model of TGA - the *Pitx2c* null (*Pitx2c*$^{-/-}$) mutant. Pitx2 is a transcription factor with a key role in left-right patterning and cardiac development. Homozygous knock-out of the c-isoform in mice causes predominantly TGA, but also DORV, and is lethal at birth. Heterozygotes are viable, fertile and have no gross structural abnormalities.

Method The *Pitx2c* mutant allele was generated as previously described by Liu et al. (2002). *Pitx2c*$^{+/-}$ and *Pitx2c*$^{-/-}$ embryos were collected at embryological ages E10.5, 11.5 and 12.5 days. The cardiothoracic region of each embryo was dissected and imaged using high resolution episcopic microscopy (HREM), an imaging technique allowing precise imaging of serial 2D surfaces throughout tissues which can then be compiled and reconstructed to provide accurate 3D visualisation of the sample. A computerised tracking pad was used to manually select regions of interest (ROIs) within serial images - the OFT cushions and outflow channels. Using the 'Amira' software these ROIs were assembled as 3D reconstructions of three replicate hearts of each genotype at each age; the morphologies of which were compared.

Results Hearts in the *Pitx2c*$^{+/-}$ were normal, with OFT channels that spiralled around one another in a left to right orientation, viewed distally. As previously described, *Pitx2c*$^{-/-}$ channels were straight, parallel and positioned dorso-ventrally distally; and the right ventricle, cushions and channels were each somewhat smaller. Unexpectedly, the *Pitx2c*$^{-/-}$ hearts also displayed novel endocardial cushion morphology. Not only were the cushions displaced in comparison to their *Pitx2c*$^{+/-}$ counter-parts, but two additional cushions were also found at 10.5 days. These cushions remodelled such that three remained at 11.5 days. At 12.5 days all *Pitx2c*$^{-/-}$ mice showed the typical morphology of the precursors of TGA or DORV.

Conclusions We report for the first time the finding of supernumery OFT cushions in a model of TGA. These additional cushions were transient and appeared to resolve into two abnormally placed cushions which create non-spiralled outflow channels. There are other apparent abnormalities in the outflows of the *Pitx2c*$^{-/-}$ model including rotation and size reductions, so the exact role of the additional cushions in the development of TGA requires further study.

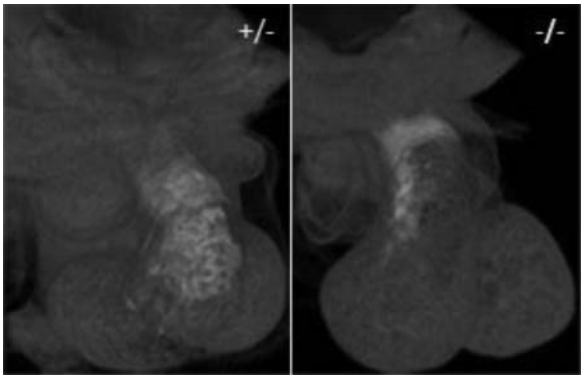

Figure 1 *Pitx2c* reconstructions (red=vessels, cushions: yellow=parietal, purple=septal, blue/green=additional cushions)

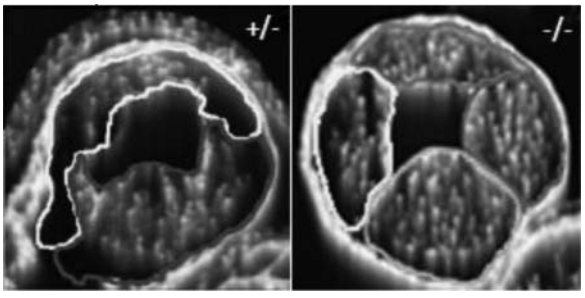

Figure 2 Cross-sections of *Pitx2c* OFTs