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Pathological Consequences of Altered Hemodynamics During Heart Valve Development.

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As per the Centers for Disease Control and Prevention (CDC), congenital heart defects (CHDs) affect about 40,000 babies each year, and are the most common type of birth defects. Defective heart valves are the most prevalent of all CHDs, due to the fact that the structure and function of cardiac valves – atrioventricular (AV) and outflow tract (OFT) valves – are primarily determined during early embryogenesis. Congenital valvular diseases may remain asymptomatic for a long period of time and manifest in adulthood thereby predisposing the patient to valve disease later in life. Treatment of diseased valves often requires valve replacement surgery. Furthermore, most of the replaced aortic valves have congenital abnormalities associated with them; thus strengthening the argument that several adult valve diseases can be attributed to abnormal valve development. This makes it imperative to decipher the mechanisms of valvulogenesis.

Epithelial-mesenchymal-transition (EMT) is a tightly regulated process that plays an important role in embryonic development. During valvulogenesis, EMT occurs in a subset of AV and OFT cells which then adopt a migratory phenotype and populate the CJ. EMT is very active during development of cardiac valves, however, once mature valves have formed, this process decreases significantly, functioning at a basal level to produce mesenchyme for valve maintenance. Failure of EMT down regulation in mature valves results in excess ECM and fibrous, stiffer diseased valves.

Hemodynamic stimuli (such as shear stress and pressure) play an important role in embryonic cardiogenesis. By employing a 3D *in vitro* culture system, we have previously shown that shear stress influences the expression and deposition of fibrous ECM proteins in both AV [1] and OFT cushion explants [2]. Furthermore, several research groups have shown that altering blood flow through the embryonic heart leads to anomalies in the structure and function of valves. Using advanced imaging techniques researchers have better understood the effects of altering hemodynamic loads through embryonic avian hearts on valve development. However, the effect of altered hemodynamics at the molecular level has not been completely understood. Establishing the molecular biology of regulation of valvulogenesis by blood flow is vital to develop therapeutics to treat/prevent CHDs. To fill this void in the field of valvulogenesis, we have developed a novel *ex ovo* (*in vivo*) banded chicken embryonic system to alter intracardiac blood flow and assess its effect on genetic regulation of valve development [3]. We hypothesize that altering intracardiac hemodynamic loads through the chicken embryonic heart influences both EMT and ECM production resulting in abnormal cardiac valve development.

In the present study, Hamilton and Hamburger stage 17 chicken embryos were randomly divided into two groups: (1) Banded – OFT was partially constricted at the OFT/ventricle junction (OVJ) **Figure 1** and (2) Control – embryos not subjected to banding. Both banded and control embryos were then

allowed to develop for an additional 24 h after which all analyses were performed. The success of banding was confirmed by 2D ultrasound imaging (with the ultrasound probe being placed at the OVJ). As expected, the flow velocity was higher in the banded compared to the control embryos, a finding that was also mirrored by computational fluid dynamics; which further showed that this constriction site also experienced a concomitant increase in shear stress. Importantly, there were no differences in heart rate and volumetric flow rate, indicating that our banding procedure did not compromise systemic cardiovascular physiology. Using hematoxylin and eosin (HE) stained sections, the OFT was 3D reconstructed using AMIRA software from which cushion volume was determined. HE stained OFT sections were also analyzed to determine the number of mesenchymal cells invading the CJ. The cushion volume and the number of mesenchymal cells in the cushion tissue were significantly lower in the OFT from banded compared to control hearts. This could potentially lead to the formation of abnormal hypocellular OFT valves. Furthermore, OFT banding led to significant changes in transcript levels of genes involved in mechanotransduction, ECM production e.g. Collagen1, EMT signaling, e.g. TGFßR3 and cell migration e.g. MMP2 – processes which are important for formation of healthy valve tissue. Immunocytochemistry was done on sections of banded and control heart for many of the same genes. Similar results were seen in the changes in expression and localization of proteins mentioned above. To further investigate the genetic consequences of banding on cardiac development, microarray analysis was performed and revealed differential expression of more than 130 genes in OFT from banded vs. control embryos including those directly involved in valve development such as transglutaminase 2. Using RTPCR, we have begun to validate the expression changes in a number of genes indicated in our microarray with the desire to investigate the differential expression and localization of those proteins in both OFT and AV tissue. In conclusion, we have demonstrated, for the first time that altering hemodynamics through the embryonic chick heart by partial OFT constriction caused changes at the mRNA and protein levels in OFT and AV cushions which could lead to formation of diseased valves [4].

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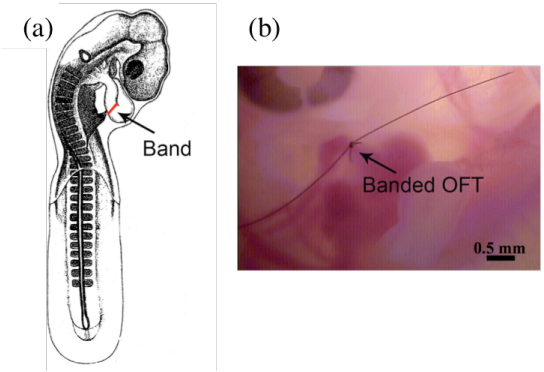

Figure 1. (a) Schematic showing banding site. (b) *Ex ovo* banded model imaged 24 h after banding

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