**MECHANICAL REGULATION OF CONGENITAL HEART DEFECTS (CHD)**

**Hemodynamics in CHD**

**European Commission Seventh Framework (FP7) People Programme Marie Curie Actions**

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**Project Budget: 100,000 Euros**

**Project Duration: April 2011 – April 2015**

**Summary description of the project objectives**

This study focuses on a significantly important health problem, congenital heart defects (CHDs). Our aim is a better understanding of the formation of these defects in utero for generating new novel treatment strategies.

CHDs refer to a wide class of heart defects at birth. Formation of these defects in utero during pregnancy is closely related to the development of the heart which is briefly explained here. The heart is the first functional organ to develop in the embryo, convecting nutrients to surrounding tissues to facilitate growth. As the embryo grows, the heart transforms from a linear valve-less tube to a multi-chambered structure complete with 4 fibrous valves. Changes in pressure, strain and wall shear stress (WSS) accompany cardiac morphogenesis and orchestrate molecular and cellular responses that help coordinate downstream tissue changes. CHDs form when cardiac morphogenetic processes are disrupted. These defects affect 1–2% of newborn children and are the leading cause of death in infants under 1 year of age. CHDs represent the single largest class of birth defects and account for approximately 25% of all human congenital abnormalities. Despite their prevalence, the etiology of many CHDs remains unknown. While clinical and experimental research has identified multiple genetic mutations that participate in the formation of CHDs, they fail to fully account for the disease phenotype. A recent study suggested that, point mutations in heart forming genes may contribute to only approximately 10% of CHDs. On the other hand, in addition to genetic factors, environmental factors such as drug exposure and hemodynamic patterning (i.e. how the blood flows through the heart) were shown to contribute to the development of CHDs. Mechanical perturbation of blood flow can induce diseased phenotypes.

Due to the difficulties to access human fetuses, embryonic animal models have been used to study cardiac development and to better understand the origins of CHDs. Vertebrate species, in particular, are favorite models since developmental processes are highly conserved among them. Typically studied models include the mouse, the zebrafish, and the chicken (or in general avian) embryos. Due to the availability of knockouts, mouse models are typically used to assess the effects of genes on cardiac development and cardiovascular disease. Mouse embryos, however, are not ideal models to study the effects of hemodynamics on cardiac development because they are very difficult to image and manipulate inside their mother’s womb, and cannot develop beyond early stages outside the womb. Zebrafish and avian embryonic models have therefore been more extensively used to monitor hemodynamic conditions during development. Chick embryos, in particular, are often used as a biological model of cardiac development because of several reasons: 1) The embryo develops in a planar orientation on top of the egg yolk, enabling a variety of imaging and local microsurgical options to alter blood flow, 2) The cardiogenic period of the chick is longer than other species (fish, frog, mouse) enabling more detailed spatiotemporal analysis, 3) Chicks tolerate microsurgical treatments well and are of limited ethical concern, and 4) the chick embryonic heart develops similarly to the human embryonic heart (similarities include the formation of a four chambered heart with four valves, as in human, left side of chicken heart works in systemic circulation and right side works in pulmonary circulation, two pump systems working in parallel).

The present study is designed to alter blood flow through developing embryonic chicken hearts in a clinically relevant way and investigate subsequent abnormal heart development. The motivation here is based on previous clinical observations: In most CHD cases, blood flow and morphology of structures are abnormal in ether only left or in only right side of the heart. Therefore we create disturbed blood flows on either left or on right side of the embryonic hearts at early stages of heart development. We then quantify morphological (i.e. chamber and valve sizes) and functional (i.e. blood flow velocities through valves) changes and characterize disturbed hemodynamics via computational fluid dynamics (CFD) methods. We look for correlations of abnormal morphology with disturbed hemodynamic forces.

*By characterizing the disturbed hemodynamics due to clinically relevant interventions, our objective is to correlate the abnormalities in the hemodynamics with the abnormalities in morphology following the interference. This information is critical to understand CHD development and hence for the generation of future clinical therapies for CHDs.*

**Description of the work performed since the beginning of the project**

Two experimental techniques were utilized in this study. These are left atrial ligation (LAL) and right atrial ligation (RAL). LAL is performed at embryonic day (ED) 4 (i.e. total embryonic development for chicken embryos last 21 days). At that stage, heart has not fully septated into left and right sections. LAL disturbs blood flow in left side (i.e. systemic circulation) of the heart. Therefore, LAL study will be relevant to the CHDs affecting systemic circulation like Hypoplastic Left Heart syndrome. The other technique that was employed at ED5 is RAL. RAL disturbs blood flow in the right side (i.e. pulmonary circulation) of the heart. Therefore, RAL study will be relevant to the CHDs affecting pulmonary circulation like Tetralogy of Fallot.

*In the first period of the project*, we investigated the functional and morphological adaptation of the heart under disturbed hemodynamics in either left (via LAL) or right side (via RAL) of the heart. For this purpose, blood flow velocities were measured via ultrasound and 3-D geometries were gathered via micro-CT. Blood flow velocities were used to calculate parameters of heart function, which were peak velocity (an indicator for shear stress levels) and average velocity (an indicator of cardiac output) through the atrioventricular (AV) canal. To quantify morphological changes following LAL and RAL, we measured left and right AV valve orifice areas as well as left and right ventricle volumes from micro-CT scans at ED7 (at ED7, heart is septated into left and right sections). AV valve orifice area sizes indicate if the valve developed normally. Bigger orifice indicates overdevelopment and smaller orifice indicates underdevelopment. Similarly ventricular volumes show if the chamber is over or under developed.

*In the second project term*, we generated computational fluid dynamics (CFD) models to characterize hemodynamic environment in the heart following LAL and RAL. Model geometries are formed from micro-CT images whereas ultrasound measured blood flow velocities were used as inlet velocity boundary conditions in these models. Our models are at ED4 and ED7 for LAL, and at ED5 and ED7 for RAL. *Our aim is to unravel correlations of abnormal morphology with disturbed hemodynamic forces* *following the interference.*

**Description of the main results achieved**

*In the first term of the project*, we investigated the functional and morphological adaptation of the heart under disturbed hemodynamics in either left (via LAL) or right side (via RAL) of the heart. We have found that, LAL results in an immediate decrease in shear stress levels in AV canal but does not cause an immediate alteration in cardiac work at ED4. At ED7 (i.e. at that stage, heart is septated into left and right sections), left AV canal experiences decreased shear stress with same level of cardiac work compared to controls whereas right side experiences similar levels of shear stress at that stage. RAL on the other hand does not cause an immediate change in shear stress levels and cardiac work at ED5. However, when heart is septated at ED7, shear stress levels and cardiac work is decreased in right side but not on the left side. To quantify morphological changes following LAL and RAL, we measured left and right AV valve orifice areas as well as left and right ventricle volumes from micro-CT scans at ED7. We found that, LAL results in slight underdevelopment of left AV valve and slight overdevelopment of right AV valve at ED7, whereas RAL results in severe overdevelopment of left AV valve and slight underdevelopment of right AV valve at ED7. For ventricular volumes, LAL decreases left ventricle volume and increases right ventricle volume, whereas RAL does not affect left ventricle volume and decreases right ventricle volume at ED7.

*In the second project term*, we generated CFD models to characterize hemodynamic environment in the heart following LAL and RAL. Model geometries are formed from micro-CT images whereas ultrasound measured blood flow velocities were used as inlet velocity boundary conditions in these models. Our models are at ED4 and ED7 for LAL, and at ED5 and ED7 for RAL. Our aim is to unravel correlations of abnormal morphology with disturbed hemodynamic forces.

Below are the summary and significance of the results from our CFD simulations:

Summary of Overall CFD Results

- According to our CFD results, LAL results in an immediate decrease in left atrial inflow rate and an immediate increase in right atrial inflow rate at ED4 as expected. However, outflow rates through left and right sides are relatively unchanged for LAL embryos compared to controls. This surprising finding is contrary to what had been suggested in previous studies, which was the assumption of immediate redirection of blood to the right side of the heart following LAL. WSS levels, on the other hand are decreased in left side of the AV canal and are relatively unchanged in the right side at ED4.

- RAL results in an immediate decrease in right atrial inflow rate and an immediate increase in left atrial inflow rate at ED5 as expected. RAL also results in an immediate decrease in right ventricular outflow rate and an immediate increase in left ventricular outflow rate at ED5. Interestingly, following RAL, WSS levels are increased in both left and right sides of the AV canal at ED5.

- At ED7, the left AV canal of LAL embryos are exposed to higher WSS compared to controls, while in RAL embryos, WSS levels are also higher in the left AV canal but to a lesser extent compared to controls. In the right AV canal on the other hand, for LAL embryos, WSS levels increased at ED7, and these values decreased for RAL embryos compared to controls.

Significance of Overall CFD Results

Based on the results summarized above, correlations of disturbed hemodynamic forces and abnormal morphology are as follows:

* Left AV valve (mitral) development is very sensitive to WSS (decreased WSS at ED4 due to LAL decreases valve orifice size at ED7, and increased WSS at ED5 due to RAL increases valve orifice size)
* Left ventricle development is very sensitive to left atrial inflow rate (decreased left atrial inflow at ED4 due to LAL decreases left ventricular volume at ED7, and less pronounced difference at left atrial inflow rate at ED5 due to RAL alters left ventricle volume slightly)
* Right ventricle development is very sensitive to right atrial inflow rate (increased left atrial inflow at ED4 due to LAL increases right ventricular volume at ED7, and decreased right atrial inflow at ED5 due to RAL decreases right ventricular volume).
* We did not see any correlation between WSS level at right AV valve at ED4 or ED5 with valve size at ED7.

**The potential impact and use of the results**

To contribute to a better understanding of the CHD formation, the project aims to highlight the relation of disturbed hemodynamic forces and abnormal morphology during heart development. For this purpose, clinically relevant heart defects were generated on chicken embryos via microsurgery, and subsequent heart development were investigated via imaging and CFD modeling. We have found that, both blood flow rates and WSS levels affect AV valve and ventricular chamber developments. Left and right sides of the heart however respond differently to flow induced forces. This is an expected finding since left side works as a part of systemic circulation and under higher loads compared to right side which is a part of pulmonary circulation.

Specifically, our results show that flow constriction through the heart affect the development of the respective ventricle on the side of the constriction. The ventricle on the opposite side of the constriction overdevelops due to hemodynamic alteration. This is a very critical finding and will have an impact for future clinical therapies: to induce development for an underdeveloped ventricle, inflow constriction at the side of this ventricle should be removed. Alternatively, an inflow constriction can be induced to the opposite side of the underdeveloped ventricle. In utero surgery trials are becoming more common for especially for the deadly diseases like Hypoplastic Left Heart Syndrome. The encouraging results from the current study will form basis for potential new treatments.

We have found that, left AV valve (mitral) development is very sensitive to WSS whereas right AV valve (pulmonary) is not. This is also a critical finding which is evaluated in terms of the fact that CHDs related to aortic valve is much more common than CHDs related to pulmonary valve. Therefore, most likely WSS abnormalities affecting to the pulmonary valve during development can be tolerated whereas the abnormalities at the aortic valve result in a defected valve at birth. This encouraging finding is expected to form a base for in utero treatment strategies to restore WSS levels in left AV canal to treat aortic valve defects.

**Career development and re-integration of the researcher**

By the approval of the project in 2011, Dogus University limited the teaching load of the researcher with two classes per semester as promised in the proposal, enabling the researcher to focus on research activities. With the research experience gained from the FP7 study, the researcher was able to secure two more grants from national scientific agency of Turkey (TUBITAK) with a total budget of about 175,000 euros. More recently, the researcher has received a highly prestigious award from the Turkish Academy of Sciences (TUBA) entitled as “Young Scientist Outstanding Achievement Award (GEBIP)”. The researcher became a member of the academy which enables him to attend research and career development activities by the academy. Dr. Yalcin received a fund of 20,000 Euros for three years to be spent on his research as part of this award.

<http://www.tuba.gov.tr/winners/gebip-kazananlar-listesi/prog_id/12/mid/121/mid/180/>

Thanks to the IRG and these TUBITAK grants, the researcher secured his position in the university. He has recently applied for the promotion to associate professorship.

Again thanks to these research grants, researcher formed research collaborations with other research groups. A Bilateral Research Co-operation Agreement was signed and is currently effective with Cardiovascular Developmental Bioengineering Laboratory at Biomedical Engineering Department of **Cornell University,** USA (www.butcherlab.com), on generating in vivo and computational models of congenital heart defects. Another Bilateral Research Co-operation Agreement was signed and is currently effective with Laboratory for Tissue Engineering at the Department of Cardiac Surgery of **Klinikum der Universität München,** Germany (http://www.herzklinik-muenchen.de/en/home/), on generating computational models of flows through heart valve scaffolds, xenografts and homografts.

**State of the art**

This project outlines a systematic approach to study the embryonic development of heart defects using animal models. Imaging modalities of echocardiography and micro-CT were combined elegantly with computational modeling to investigate biological mechanisms responsible for disease progression. As far as we know, this is the first study which characterized disturbed hemodynamic forces and related this information to clinically relevant morphological abnormalities. As stated in the current review paper by Midgett and Rugonyi, (2014, Frontiers in Physiology, Congenital heart malformations induced by hemodynamic altering surgical interventions, doi: 10.3389/fphys.2014.00287), there are only few studies who has focused on hemodynamics following surgical interventions on animals**.** Therefore, we believe that, our study will be a pioneer study in the field. The methodology developed in the study to investigate development of heart disease is readily applicable to the investigation of other cardiac diseases like atherosclerosis. The project is a good example of combining biological analysis with engineering analysis techniques.

*Results from this study are announced via the project website below:*

[*http://staff.dogus.edu.tr/cagatayyalcin/?page\_id=33*](http://staff.dogus.edu.tr/cagatayyalcin/?page_id=33)

*Dr. Yalcin’s publications information can be accessed from the following website:*

[*https://scholar.google.com.tr/citations?hl=en&user=QtMeO8wAAAAJ*](https://scholar.google.com.tr/citations?hl=en&user=QtMeO8wAAAAJ)