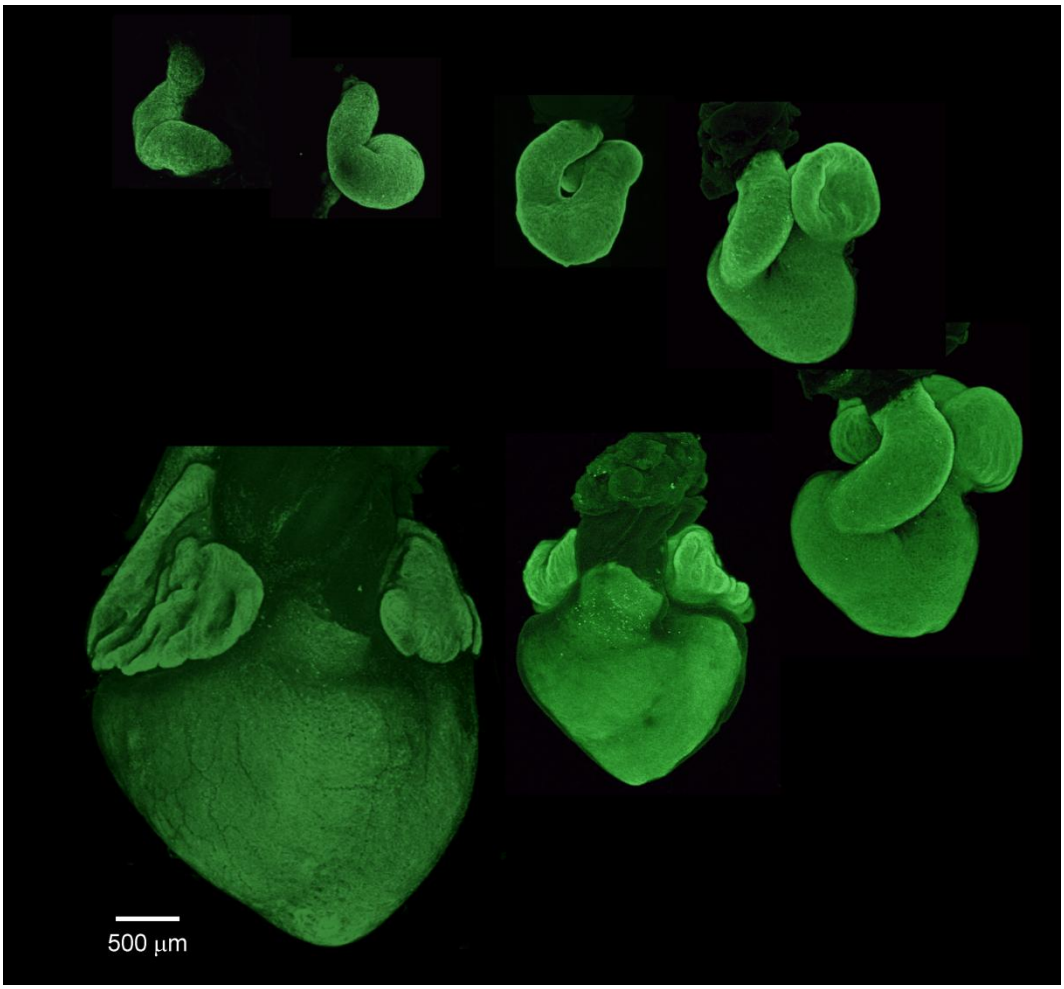
Cardiovascular Development Conference Amsterdam

November 4-6, 2015, Amsterdam, The Netherlands



Academic Medical Center, Department of Anatomy, Embryology and Physiology

and

European Society of Cardiology, Working Group on Development, Anatomy and Pathology

Welcome

The organizers would like to welcome you to the 2015 Amsterdam Cardiovascular Development Conference organized by the Department of Anatomy, Embryology and Physiology of the Academic Medical Center and the European Society of Cardiology, Working group on Development, Anatomy and Pathology. This conference builds on the success of the Weinstein Cardiovascular Development Conference organized in 2010 in Amsterdam and 2014 in Madrid, and the annual meetings of the Working group on Development, Anatomy and Pathology. The goal of our annual meeting is to provide a highly interactive forum for researchers in the field of cardiovascular development, anatomy and pathology in Europe. We are proud to announce that we have 99 participants from 17 different countries in Europe, Asia and the United States of America. Besides our Key Note speaker, Dr Olaf Bergmann, the participants have collectively submitted 67 abstracts from which 31 were selected by seven experts in the field to be presented in platform sessions and 36 abstracts in poster presentations. The organization would not have been possible without the financial support of the European Society of Cardiology, for which we are very grateful.
We hope that you will have a enjoyable and inspiring conference allowing you to renew longstanding collaboration, engage in new collaborations, and gain novel ideas to forward.

How to get to the meeting sites

From Schiphol to the IBIS City Stopera:
There are many options:

1. Take a train to "Amsterdam Central Station" (Amsterdam CS) and switch to the metro. Every metro that leaves Central Station is good. Leave the metro at "Waterlooplein" and walk along the Valkenburgerstraat. The IBIS hotel is at the left hand side at number 68. (Approx 500 meters)
2. Take a train that stops at "Amsterdam Bijlmer ArenA" or "Duivendrecht" and switch to the metro. Take a metro in the direction "Central Station". Leave the metro at "Waterlooplein" and walk along the Valkenburgerstraat. The IBIS hotel is at the left hand side at number 68. (Approx 500 meters)
3. A taxi is expensive between 75-100 euro, depending on the traffic. Only use a taxi that leave the official taxi stand. People that offer their service in the arrival hall are illegal taxi drivers.

From Schiphol to the Academic Medical Center (AMC):
To travel from Schiphol to the AMC on Wednesday morning November 4, it is best to use public transportation. A taxi is expensive between 75-100 euro, depending on the traffic. Every 10 minutes a train leaves Schiphol that stops at "Amsterdam Bijlmer ArenA" or "Duivendrecht". At either of these stations you switch to Metro in the direction of Gein. Leave the metro at "Holendrecht", the AMC is at your right hand. The entire trip takes approx. 45 minutes.

From the IBIS to the AMC.
Upon leaving the hotel turn right and walk to the metro station at Waterlooplein. Take a metro in the direction "Gein". Leave the metro at "Holendrecht", the AMC is at your right hand. From the IBIS to the department it will take approx. 45 minutes.

In the AMC.
The department of Anatomy, Embryology and Physiology is located in Building K on the second floor. To get there enter the AMC at the main entrance. Walk straight on till you arrive at the library. Turn right and take the first corridor at the left. Take the elevator to the second floor. Turn left upon leaving the elevator. At the statue of one the famous Amsterdam Anatomist Prof Tulp turn left. At the left side is the coffee room where we will convene.

From the IBIS to the ARTIS Conference Center
Upon leaving the hotel turn left.
After 100 meters turn right onto the Anne Frankstraat
After 200 meters turn right onto the Plantage Parklaan
After 200 meters turn left onto the Plantage Middenlaan
After 250 meters on the left site you have reached the ARTIS Conference Center.

To tailor your travel in Amsterdam and the Netherlands check the website:
http://9292.nl/en

Program Workshops

Wednesday November 4, 2015

Workshop on Congenital hearts
Faculty: Prof dr Bob Anderson, Dr Lucile Houyel, Dr Margot Bartelings, Dr Monique Jongbloed, Prof dr Roelof-Jan Oostra

13.00-14.00 Lunch
14.00-14.05 Opening (Roelof-Jan Oostra)
14.05-14.45 Video-assisted demonstration: Normal and abnormal morphology of the atrioventricular connections (Prof Bob Anderson)
14.45-15.05 Video-assisted demonstration: Ebstein's malformation (Dr Lucile Houyel)
15.05-15.30 Tea break
15.30-17.00 Hands-on session: Atrioventricular malformation and related anomalies (all faculty)

Workshop on Acquired heart disease.
Faculty: Prof dr Allard van der Wal, Prof dr Cristina Basso

13.00-14.00 Lunch
14.00-14.45 Opening (Allard van der Wall)
14.45-17.00 Hands-on workshop showing hearts from various acquired pathologies, like ischemic heart disease, valvular disease, cardiomyopathies.

Workshop on Analysis of qPCR data using LinRegPCR
Faculty: Dr Jan Ruijter, Dr Adrian Ruiz Villalba
Analysis of qPCR data using amplification curve analysis (LinRegPCR) and removal of between-plate variation (Factor-qPCR).

11.00-13.00 lectures
13.00-14.00 Lunch
14.00-17.00 Hands-on computer exercises.

All participants of the workshops and Faculty
17.00-18.00 Drinks in museum Vrolik

Evening on your own to enjoy Amsterdam

Program Conference

Thursday November 5, 2015

8.30 - 9.25	Registration and putting up posters	
9.25 - 9.30	Welcome	
	Session Cardiogenesis	
9.30 - 9.50	Kathryn Hentges	Linking the spliceosome to laterality: reversed cardiac looping in a mouse Prpf8 mutant
9.50 - 10.10	Martina Burczyk	M3 muscarinic receptors limit pacemaker potential during early cardiogenesis
10.10 - 10.30	Lucile Miquerol	Deciphering multiple roles of Nkx2-5 during ventricular compaction.
10.30 - 10.50	Carmen Lopez-Sanchez	Negative Fgf8-Bmp2 feed-back is regulated by miR-130 during early cardiac specification
10.50 - 11.10	Mayyasa Rammah	Dissecting the regulatory circuitry of complementary progenitor cell contributions to the arterial pole of the heart
11.10 - 11.40	**Coffee break**	
	Session Signalling	
11.40 - 12.10	Stefan Hoppler	Wnt signalling pathways regulate cardiomyocyte differentiation in Xenopus stem-cell-like explants and human Embryonic Stem Cell culture
12.10 - 12.30	Fabio Da Silva	The Wnt signaling regulator R-spondin 3 is essential for coronary artery formation in the developing heart
12.30 - 12.50	Karen Koefoed	SMURF Proteins in Human Heart Development and Cardiomyogenesis
12.50 - 14.00	**Lunch and posters**	
	Session Transcription	
14.00 - 14.20	Federico Tessadori	Tbx5a is required for normal cardiac looping morphogenesis in zebrafish
14.20 - 14.40	Gabriella Fulcoli	TBX1 and heart development: a critical role in the modulation of H3K4 monomethylation
14.40 - 15.00	Cornelis Boogerd	Endocardial Tbx20 promotes mesenchymal and myocardial cell movements required for septation
15.00 - 15.20	Srinivasan Sakthivel	Whole exome sequencing suggests a novel candidate gene for congenital heart disease
15.20 - 15.40	Seyed Javad Rasouli	The Role of Klf2 in Zebrafish Heart Development
15.40 - 16.10	**Coffee break**	
	Session Conduction	
16.10 - 16.30	Alena Kvasilova	The evolution of cardiac conduction system in the crocodilian heart
16.30 - 16.50	Bjarke Jensen	A specialized atrioventricular conduction system in the alligator heart offers a novel explanation for the evolution of the ventricular conduction system
16.50 - 17.10	Rajiv Mohan	Early specification and progressive restriction of the cardiac conduction system lineage during heart development
17.15 - 18.15	**Key Note**	
	Olaf Bergmann	Dynamics of cell generation and turnover in the human heart
18.15 - 22.00	**Posters, Drinks, Buffet**	

Friday November 6, 2015

8.30 - 9.00	Late Registration	
	Session Pathology	
9.00 - 9.30	Lucile Houyel	Inner architecture of the right ventricle: the role of the tricuspid valve.
9.30 - 9.40	Rodrigo Blanco	Coronay plaque disruption, thrombosis and inflammation in patients died of acute myocardial infarction
9.40 - 10.00	Athar Khalil	TBX5: The Missing Culprit Gene in Thalidomide Toxicity
10.00 - 10.20	Wan Chin Hsieh	Anatomy of Sinoatrial Nodal Artery: a meta-analysis and clinical review of it's anatomical characteristics
10.20 - 10.50	**Coffee break**	
	Session Techniques	
10.50 - 11.10	Adrián Ruiz-Villalba	Correct interpretation of qPCR results in studies of cardiac development requires validated reference genes and the identification of the amplified product
11.10 - 11.30	Silja Burkhard	RNA tomography allows spatial dissection of genome-wide expression profiles in the developing zebrafish heart tube.
11.30 - 11.50	Nikolai Klena	Interrogating the Complex Genetics of Congenital Heart Disease in Mice
11.50 - 13.50	**Lunch and posters**	
	Session Inflow tract and epicardium	
13.50 - 14.10	Laura Andrés-Delgado	BMP2 rescues PE formation in absence of heartbeat in zebrafish
14.10 - 14.30	Sjoerd Duim	The epicardium as modulator of the cardiac autonomic response during early development
14.30 - 14.50	Adriana Gomes	Septum transversum COUP-TFII expression regulates cardiac chamber growth, septation and vascularization
14.50 - 15.10	Marina Campione	Regionalized ANKRD1 expression is essential to finely regulate multiple aspects of cardiac remodeling and venous pole development
15.10 - 15.40	**Coffee Break**	
	Session Valves	
15.40 - 16.00	Chip Norris	Genetic and Developmental Etiology of Valvular Heart Disease
16.00 - 16.20	Frédéric Laurent	The HAND2 Cistrome in Mouse Embryonic Hearts Identifies its Target Genes During Endothelial-Mesenchymal Transition in the Atrioventricular Canal
16.20 - 16.40	Stuti Prakash	The role of Follistatin-like 1 in post-natal valve formation
16.40 - 17.00	Boudewijn Kruithof	Mechanobiology of the cardiac valves in an ex vivo flow model
17.00 - 19.00	**Farewell Drinks, taking down posters**	

Key note speaker:

Olaf Bergmann is an Assistant Professor at Karolinska Institutet. His research group focuses on dynamics and regulation of cellular renewal in various organ systems. He studied medicine at the Charité, Berlin, Germany and received his MD in 2006. His PhD was awarded from the Karolinska Institutet in 2010. Before he returned to the Karolinska Institutet in 2013 to establish his laboratory, he performed his postdoctoral work at the University of Lund, Sweden. He is a recipient of the Oskar Lapp Research Award (German Cardiac Society), and is currently a Ragnar Söderberg Fellow in medicine.

Title of the key note lecture:

Dynamics of cell generation and turnover in the heart

Cardiovascular diseases are not only the largest cause of death but also a major cause of functional impairment in the Western world. At the present time, the only replacement therapy for cardiovascular diseases is heart transplantation. Thus, the identification of alternative strategies to regain myocardial function after injury is highly desirable. Using retrospective ^{14}C dating, we have shown that the generation of new cardiomyocytes in humans is not restricted to development but instead continues throughout life. This finding opens up the possibility of augmenting cardiac regeneration in cardiac disease by revealing the underlying cellular and molecular mechanisms. I will discuss how cardiomyocytes contribute to physiological heart growth and homeostasis, and at which rates cardiomyocyte are exchanged during the course of aging and in cardiac disease.

Abstracts of platform sessions

In order of presentation

Linking the spliceosome to laterality: reversed cardiac looping in a mouse Prpf8 mutant

Michael Boylan, Gennadiy Tenin, Louise Stephen, Karen Mitchell and Kathryn Hentges
Faculty of Life Sciences, University of Manchester

Congenital heart defects are the most common non-infectious cause of infant mortality. A better understanding of the embryonic development of the mammalian heart is needed to identify the mechanisms underpinning congenital heart defects. One area for investigation is the establishment of left/right axis formation, as disruptions to this process can cause complex cardiac morphological abnormalities. One of the earliest readouts of laterality during development is the directional looping of the heart tube, as mutants with laterality defects often display reversed cardiac looping or unlooped hearts. To further our knowledge of the genes contributing to congenital heart defects, we employed a random chemical mutagenesis screen to identify recessive embryonic lethal mouse mutants with cardiac abnormalities, including laterality defects. One mutant isolated in this screen displayed a reversal of cardiac looping in approximately 50% of mutant embryos. Positional cloning revealed that the mutants have a missense mutation in the conserved spliceosomal gene Prpf8. Morpholino knockdown of zebrafish Prpf8 caused an increased incidence of cardiac looping defects. Additionally, laterality genes including nodal, lefty1, lefty2, and Pitx2 are aberrantly expressed in Prpf8 mouse mutants.
Notably, left-right axis formation is critically dependent on the function of cilia at the node. We have discovered that Prpf8 mutants have flattened nodes, shortened cilia and lack leftward fluid flow at the node, suggesting that nodal cilia function is impaired in Prpf8 mutant embryos. A role for Prpf8 in ciliogenesis in retinal pigment epithelial cells has recently been reported, and Prpf8 protein is localised to the cilium in RPE cells. This finding highlights the intriguing possibility that Prpf8 may have a direct role in ciliogenesis, although further experiments are needed to investigate the mechanism by which the Prpf8 missense mutation identified in our mouse model disrupts protein activity. We conclude that the mouse Prpf8 mutation impairs the function of cilia at the mouse node, thus disrupting left-right axis formation and causing cardiac laterality defects.

M3 muscarinic receptors limit pacemaker potential during early cardiogenesis

Burczyk MS[1], Casar Tena T[1], Burkhalter MD[2], Raad F[4], Matysik S[1], Wiese S[3], Forster M[1], Radenz M[1], Zimmermann WH[4], Kühl M[1], Philipp M[1]

[1]Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany

[2]Leibniz-Institut for Age Research - Fritz-Lipmann-Institut e.V. (FLI), Jena, Germany

[3]Core Unit Mass Spectrometry and Proteomics, Medical Faculty, Ulm University, Ulm, Germany

[4]Institute of Pharmacology, Heart Research Center Göttingen, University Medical Center Göttingen, Göttingen, Germany

Cardiac function depends greatly on G protein-coupled receptors such as beta adrenergic receptors, which regulate contractility and heart rate or muscarinic acetylcholine receptors, which control cardiac rhythmicity. Interestingly, muscarinic receptors are already expressed during stages of early cardiogenesis. Studies to elucidate a potential impact of muscarinic receptors on the developing heart, however, are rare. We show here that muscarinic receptors function already during early cardiogenesis by restricting pacemaker cell identity.

As a model we chose zebrafish because of their ease of manipulation as well as analysis. When treated with Tolterodine, an inhibitor of muscarinic M2 and M3 inhibitors, we observed morphological defects such as an elongated atrioventricular canal as well as functional impairments, namely atrial arrhythmia and atrioventricular block. Using subtype-specific receptor blockers we determined that these effects were caused by inhibition of M3 receptors rather than those of the M2 subtype. Upon closer analysis we further found that cells of atrioventricluar canal were lost, while the pacemaker cells were gained. Moreover, transcript analysis of isolated hearts demonstrated the initiation of a pacemaker program, while mass spectrometry analysis proved dysregulation of arrhythmogenic proteins. Most intriguingly, however, M3 inhibition during stages of cardiac progenitor specification and differentiation was sufficient to produce these phenotypes. This suggests that M3 receptors regulate the fate of the conduction system already on the progenitor level.

Deciphering multiple roles of Nkx2-5 during ventricular compaction.

Caroline Choquet[1], Thi Hong Minh Nguyen[2], Frank Kober[3], Monique Bernard[3], Nathalie Lalevée[2], Robert G. Kelly[1] and Lucile Miquerol[1]

[1]Aix-Marseille Université, IBDM CNRS UMR7288, Marseille, France.

[2]Aix-Marseille Université, INSERM, TAGC UMR_S 1090, Marseille, France.

[3]Aix-Marseille Université, CNRS, CRMBM UMR 7339, Marseille, France.

Left ventricular non-compaction (LVNC), characterized by prominent trabeculations and deep trabecular recesses in the ventricles, is the most common cardiomyopathy with a spectrum ranging from extreme normal variants to a pathological phenotype. In all forms, heart failure and sudden cardiac death represent the most severe complications related with non-compaction and arrhythmias. Ventricular trabeculae are a normal component of ventricular myocardium during embryonic development. Trabeculae are also known to contain the progenitor cells of the ventricular conduction system (VCS), which is composed of a complex network of interconnected Purkinje fibers controlling the rapid propagation of electrical activity in the ventricles. The transcription factor Nkx2-5 is a key regulator of cardiac development. Conduction disturbances and ventricular non-compaction are traits that have been observed in patients carrying *NKX2-5* mutations and in *Nkx2-5* mutant mice. However, the relationship between Purkinje fiber differentiation and trabecular compaction is unknown, despite recent evidence that abnormal conduction system morphology underlies conduction defects and arrhythmias. In order to dissect the role of this gene in the apparition of the pathological outcomes of LVNC, we specifically inactivated *Nkx2-5* in a time and tissue specific manner. *Nkx2-5* floxed mice were crossed with *Cx40-CreERT2* mice expressing inducible Cre recombinase in trabeculae and later in the Pukinje fiber network. Tamoxifen induced timed deletion was carried out before, during and after trabecular compaction to investigate the role of *Nkx2-5* during trabeculation and compaction as well as in the ventricular conduction system. Morphological and functional cardiac phenotypes in these different mutant mice were analysed by coupling non-invasive imaging techniques and immunofluorescent studies. Preliminary results have shown that the degree of non-compaction and VCS morphology differ according to the timing of inactivation, suggesting multiple roles for Nkx2-5 during trabecular development.

Negative Fgf8-Bmp2 feed-back is regulated by miR-130 during early cardiac specification

Carmen Lopez-Sanchez[1], Diego Franco[2], Fernando Bonet[2], Virginio Garcia-Lopez[3], Amelia Aranega[2], Virginio Garcia-Martinez[1]

[1]Human Anatomy and Embryology. Faculty of Medicine. University of Extremadura. 06006 Badajoz, Spain

[2]Cardiovascular Development Group, Department of Experimental Biology, University of Jaén, CU Las Lagunillas B3-362, 23071 Jaén, Spain

[3]Pharmacy Services. Minimally Invasive Surgery Centre, Cáceres, Spain

It is known that secreted proteins from the anterior lateral endoderm, FGF8 and BMP2, are involved in mesodermal cardiac differentiation, which determines the first cardiac field, defined by the expression of the earliest specific cardiac markers *Nkx-2.5* and *Gata4*. However, the molecular mechanisms responsible for early cardiac development still remain unclear. At present, microRNAs represent a novel layer of complexity in the regulatory networks controlling gene expression during cardiovascular development. This paper aims to study the role of miR130 during early cardiac specification.

Our model is focused on developing chick at gastrula stages. In order to identify those regulatory factors which are involved in cardiac specification, we conducted gain- and loss-of-function experiments in precardiac cells by administration of Fgf8, Bmp2 and miR130, through *in vitro* electroporation technique and soaked beads application. Embryos were subjected to *in situ* hybridization, immunohistochemistry and qPCR procedures. Our results reveal that Fgf8 suppresses, while Bmp2 induces, the expression of *Nkx-2.5* and *Gata4*. They also show that Fgf8 suppresses Bmp2, and vice versa. Additionally, we observed that Bmp2 regulates miR-130 -a putative microRNA that targets Erk1/2 (Mapk1) 3´UTR, recognizing its expression in precardiac cells which overlap with Erk1/2 pattern. Finally, we evidence that miR-130 is capable to inhibit Erk1/2 and *Fgf8*, resulting in an increase of *Bmp2*, *Nkx-2.5* and *Gata4.*

Our data present miR-130 as a necessary linkage in the control of Fgf8 signaling, mediated by Bmp2, establishing a negative feed-back loop responsible to achieve early cardiac specification.

This work has been partially financed with grants to research groups CTS005 (to VGM) from the Junta de Extremadura, with FEDER co-financing, and CVI-6556 (to DF) from the Junta de Andalucía Regional Council.

Dissecting the regulatory circuitry of complementary progenitor cell contributions to the arterial pole of the heart

Mayyasa Rammah, Magali Théveniau-Ruissy, Francesca Rochais and Robert G Kelly
Aix-Marseille University, Developmental Biology Institute of Marseilles, CNRS UMR7288, 13288, Marseilles, France.

Cardiac progenitor cells of the second heart field (SHF) contribute to the poles of the elongating embryonic heart and perturbation of SHF development leads to a spectrum of congenital heart defects in animal models and human patients. The SHF gives rise to the outflow tract (OFT) at the arterial pole of the heart that is later remodeled to form the myocardial base of the aorta and pulmonary trunk, the definitive left and right ventricular outlets. These myocardial domains are prefigured in the midgestation OFT and in the SHF, where the 22q11.2 deletion or DiGeorge syndrome gene *Tbx1* is required for development of the inferior OFT wall that gives rise to subpulmonary myocardium. We have identified a complementary Hes1-positive Tbx1-negative subpopulation of SHF cells that gives rise to the superior OFT wall and subaortic myocardium. Transcriptome analysis of superior and inferior OFT domains has identified Peroxisome proliferator activated receptor gamma (*Ppar*□) as among the most significantly enriched genes in future subpulmonary myocardium. Our experimental data and bioinformatic analysis reveal that Ppar□ is a *Tbx1*-dependent regulator of future subpulmonary myocardium and is required for SHF cell addition to the OFT. Furthermore, Ppar□ represses the transcriptional program of future subaortic myocardium. Genetic and explant approaches show that *Hes1*, a downstream target of Notch signaling, controls the molecular signature of future subaortic myocardium through transcriptional repression of *Ppar*□. Our results have begun to define the genetic regulatory networks controlling complementary progenitor cell contributions to the arterial pole of the developing heart relevant to our understanding of the origins of common congenital heart defects.

Wnt signalling pathways regulate cardiomyocyte differentiation in Xenopus stem-cell-like explants and human Embryonic Stem Cell culture

Silvia Mazzotta[1], Andreia S. Bernardo[2], Boni A. Afouda[1] and Stefan Hoppler[1]
1. Institute of Medical Sciences, Cardiovascular Research Programme, University of Aberdeen, Aberdeen AB25 2ZD, UK.
2. Systems Biology Division, MRC National Institute for Medical Research, Mill Hill, London, UK

Wnt signaling is an established key regulator of vertebrate embryonic cardiomyocyte differentiation, but its role at different stages appears complex and remains widely uncertain for human development. Remarkably, experimental activation followed by inhibition of canonical Wnt signaling had previously been shown to be essentially sufficient to drive human Embryonic Stem Cells (hESCs) towards primary cardiomyocyte differentiation.
In this study, we dissect the stage-specific role of canonical and noncanonical Wnt signaling during each developmental stage leading to early cardiac muscle commitment and initial differentiation, systematically analyzing the expression of endogenous Wnt signaling components; ligands, receptors, and the pathway activities *in vitro*, by using human Embryonic Stem Cells (hESCs) to model mesoderm, cardiogenic mesoderm development and subsequent primary cardiomyocyte differentiation.
Our findings not only widely confirm in human tissue previously proposed roles for canonical Wnt signaling in sequential stages of vertebrate heart development, but also reveal novel roles for the JNK-mediated noncanonical pathway in this process and we identify candidate Wnt signals and Wnt receptor genes mediating these roles of Wnt signaling in human heart muscle development.
We had previously established an accessible experimental system using stem-cell-like Xenopus explants to study conserved fundamental aspects of vertebrate cardiomyocyte development. In order to dissect Gene Regulatory Networks controlling vertebrate heart muscle development we have recently embarked on a detailed transcriptomics analysis (RNA-seq) of different stages leading to initial cardiomyocyte differentiation in this system.
As expected, we can detect waves of expression of genes associated with the anticipated embryonic stages in heart and heart muscle development. We are analysing the temporal sequence of gene expression of individual signalling, transcription factor and structural genes in order to formulate specific and testable hypothesis about the architecture of the Gene Regulatory Network controlling vertebrate heart muscle development. We are particularly interested in identifying how, when and where Wnt signalling functions in this Gene Regulatory Network.
(2286/2500 Chrs)

The Wnt signaling regulator R-spondin 3 is essential for coronary artery formation in the developing heart

Fabio Da Silva
University of Nice Sophia Antipolis - Institute Biology Valrose, Nice, France

Coronary arteries are essential to support the heart with oxygen and coronary diseases are the leading cause of death worldwide. Identifying the signaling pathways involved in the formation and specification of coronaries is therefore essential, as it could inspire novel regenerative treatments for cardiac diseases. The coronary arteries are derived from the vascular plexus of the heart that arises at E11.5 and is remodeled until the postnatal period. An integral part in this remodeling process is arterial venous differentiation. Arterial specification in the embryonic vasculature and postnatal vessels of the retina appears to require specific activation of the transcription factor SOX17. How coronary specification is acheived and – more importantly – which signaling molecules drive this process remains elusive. Here we identify R-spondin3 (Rspo3), a secreted activator of β – catenin signaling, as a crucial regulator of coronary artery differentiation. Rspo3 deficient embryos die early in development due to vascular defects in the placenta and conditional deletion of Rspo3 in the heart with the Islet1-Cre causes impaired development of the secondary heart field. However Rspo3 expression persists in angiogenic regions with high Wnt/β-catenin signaling in the heart. Temporal deletion of Rspo3 11.5 days post coitum with the ubiquitously expressed cCAGCreERT2 line leads to a complete absence of the coronary arteries and a drastic reduction in proliferation of the compact myocardium. Closer inspection of Rspo3 expression reveals it is specifically expressed in the cardiomyoblasts surrounding the first order branch vessels of the left and right coronary arteries, and that Sox17 is highly expressed in the endothelial cells of these vessels. Ablation of Rspo3 leads to decreased Wnt/Bcatenin signaling and, consequently, a significant reduction of Sox17 in these vessels. These results identify Rspo3 as a key regulator of arterial/venous differentiation in the first order branch vessels of the heart by controlling the expression of Sox17 in a Wnt/B-catenin-dependent fashion.

SMURF Proteins in Human Heart Development and Cardiomyogenesis

Karen Koefoed[1,2], Josephine Skat Rørdam[1], Katrine Ajbro[1], Caroline Becker Warzecha[2], Maj Linea Vestergaard[1], Troels Askhøj Andersen[1], Kjeld Møllgård[1], Niels Tommerup[1], Søren Tvorup Christensen[2] and Lars Allan Larsen[1]

[1]Department of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark.

[2]Department of Biology, University of Copenhagen, Denmark.

TGFβ/BMP signaling regulates multiple events during heart development, including cardiomyogenesis, smooth muscle development, and formation of chambers and endocardial cushions. The E3 ubiquitin ligases SMURF1 and SMURF2 inhibit SMAD-dependent TGFβ/BMP signaling by targeting receptors and SMADs for proteasomal degradation.

Here we investigated the role of the two SMURF proteins in human heart development and *in vitro* differentiation of P19.CL6 stem cells into cardiomyocytes. Immunohistochemistry and qRT-PCR analyses on human embryonic and fetal heart tissues show that SMURF1 has a distinctive spatiotemporal expression pattern, which is associated with chamber formation and development of the conduction system.

To characterize the role of SMURF1 during cardiomyogenesis, we generated P19.CL6 cell lines with CRISPR/Cas9 mediated deletion of *Smurf1* followed by qRT-PCR analysis on the ability of cells to leave their pluripotent state and form clusters of beating cardiomyocytes. We find that Smurf1 knockout increases the rate of cardiomyogenesis, suggesting that Smurf1 functions as a negative regulator of cardiomyogenesis. In order to investigate the molecular mechanisms of SMURF1 function in more detail, we examined the function of SMURF1 in regulating TGFβ signaling at the primary cilium, which is required for proper heart development and known to regulate *in vitro* cardiomyogenesis in hESC and P19.CL6 cells. We find that both SMURF1 and SMURF2 localize to primary cilia, and RNAi-mediated knockdown of SMURF1 leads to increased phosphorylation of TGFβ-RI in the primary cilium as well as increased phosphorylation of SMAD proteins in cells stimulated with TGFβ-1.

In conclusion, we suggest that SMURF proteins regulate heart development possibly through the coordination of TGFβ/BMP signaling at the primary cilium. Further studies are underway to delineate the functions of SMURF1 and SMURF2 in heart development using knockout mice, and to understand whether the two SMURF proteins function redundantly to one another to regulate TGFβ/BMP signaling as well as other ciliary signaling pathways that control heart development.

Tbx5a is required for normal cardiac looping morphogenesis in zebrafish

Federico Tessadori[(1)], Fabian K. Kruse[(1)], Susanne C. van den Brink[(1)], Emily S. Noël[(1)], Malou van den Boogaard[(2)], Vincent Christoffels[(2)] and Jeroen Bakkers [(1,3)]

[(1)] Hubrecht Institute-KNAW & UMC Utrecht, Utrecht, the Netherlands

[(2)] Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

[(3)] Interuniversity Cardiology Institute of the Netherlands, Utrecht, the Netherlands

Within the first 26 hours of zebrafish embryonic development, cardiac progenitors cells form a cardiac disc, which rearranges into a linear heart tube; then driven leftward by asymmetric gene expression in a process named cardiac jogging. In the following 24 hours, the cardiac tube completes looping, which results in the formation of a functional ventricle and atrium, separated by the atrioventricular canal (AVC). The proper patterning and alignment of the cardiac segments is crucial to support the correct establishment of heart function.

In a forward genetic screen for genes involved in early cardiac morphogenesis, we identified *oudegracht* (*ogr*), a mutant defective for cardiac looping. Additionally, *ogr* exhibited slower heartbeat and peristaltic cardiac contractions. Positional cloning located the mutation responsible for *ogr* on the T-Box sequence of *tbx5a*, one of the zebrafish homologues to TBX5, whose mutations are associated with Holt-Oram syndrome in humans. *In vivo* and *in vitro* assays demonstrated that the truncated form of Tbx5a expressed by *ogr* retains no activity. Detailed analysis of the *ogr* phenotype at 2 dpf revealed an expansion of myocardial and endocardial AVC markers such as *bmp4, tbx2b* and *has2*, extended thickening of the cardiac wall and reduced expression of the chamber myocardium marker *nppa.* We have used 4D confocal imaging in combination with lineage tracing to identify the tissue rearrangements taking place during heart looping, and how these are affected in *ogr* mutants. We report here on our findings.

TBX1 and heart development: a critical role in the modulation of H3K4 monomethylation

F. Gabriella Fulcoli[1], Monica Franzese[2], Xiangyang Liu[3], Zhen Zhang[3], Claudia Angelini[2], Antonio Baldini[1,4]

1) CNR Institute of Genetics and Biophysics, Napoli, Italy. 2) Istituto per le Applicazioni del Calcolo, CNR, Napoli, Italy. 3) Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, Shanghai, China. 4) Dept. of Molecular Medicine and Medical Biotechnology, University of Naples Federico II.

Mouse studies and mutation searches in patients have identified *TBX1* gene haploinsufficiency as a cause of congenital heart disease in DiGeorge syndrome. Here, we found that TBX1 enhanced H3K4me1 deposition at a significant number of regions that it occupies throughout the genome. Treatment of cells with Tranylcypromine (TCP), an inhibitor of histone demethylases LSD1 and LSD2, rescued the expression of one third of the genes dysregulated by TBX1 suppression and it rebalanced H3K4me1 levels at many TBX1 occupied sites. *Lsd1* and/or *Lsd2* K/D also had a similar effect on a sample of target genes. In addition, we found that TCP treatment of *Tbx1* mouse mutants ameliorated significantly the cardiovascular phenotype. We investigated the mechanisms of phenotypic rescue and found that, in embryos, TCP treatment improved H3K4 monomethylation at approx. 300 genes affected by *Tbx1* haploinsufficiency. Remarkably, we found an overlap between genetic pathways perturbed in tissue culture and in embryos, and several of these pathways were rescued by TCP treatment. Furthermore, we found that the second heart field of TCP-treated mutant embryos had milder cell proliferation and cell polarization defects, compared to untreated embryos. These results indicate that H3K4 methylation is part of the mechanism of TBX1 function in heart development, and that epigenetic drugs can partially compensate for reduced dosage of TBX1.

Endocardial Tbx20 promotes mesenchymal and myocardial cell movements required for septation

CJJ Boogerd1,#, NJ Sakabe2, I Aneas2, MA nobrega2, S Evans1.

1) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla 92093, CA

2) Department of Human Genetics, University of Chicago, Chicago, IL 60637, USA.

cboogerd@ucsd.edu

Mutations in TBX20 are associated with congenital heart disease, including septal and valve defects, and double outlet right ventricle (DORV). However, tissue-specific mechanisms by which TBX20 contributes to congenital heart disease are not well understood. To investigate roles for Tbx20 in endocardium, we generatedTie2Cre;Tbx20 mutants. Mutants died at E14, with hearts displaying DORV, and hypomorphic dorsal mesenchymal protrusion (DMP) and cushions, leading to multiple septation defects. Although endothelial mesenchymal transition occurred in mutant cushions, mutant mesenchymal cells did not disperse normally. Non-cell autonomous roles of endocardial Tbx20 were evidenced by decreased myocardialization of outflow tract and failure of DMP formation. Microarray analysis of endocardial lineages demonstrated decreased expression of genes associated with extracellular matrix and mesenchymal phenotype in mutants, with Versican emerging as a likely candidate to explain observed phenotypes. Intersection of ChIP-Seq, ATAC-Seq and microarray datasets with existing chromatin-loop maps identified a potential long-range endocardial lineage enhancer of Versican bound by Tbx20. Enhancer assays in transgenic mice confirmed expression of this enhancer in endocardial lineages. Our work highlights a role for Tbx20 in endocardially-derived mesenchyme in coordinating cell autonomous and non-cell autonomous behaviors required for normal septation. Additionally, our data provides new insight into transcription factor codes regulating endocardial lineage gene expression.

Whole exome sequencing suggests a novel candidate gene for congenital heart disease

Srinivasan Sakthivel[1], Troels Askhøj Andersen[1], Sabrina Gade Ellesøe[2], Jose M. G. Izarzugaza[3], Natasja Spring Ehlers[3], Maren Mönnich[1], Suzanne van Geloven[1], Muhammad Farooq1, Søren Tvorup Christensen[4], Søren Brunak[2,3], Lars Allan Larsen[1]

[1] Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark. [2] NNF Center for Protein Research, University of Copenhagen, Copenhagen, Denmark. [3] Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark. [4] Department of Biology, University of Copenhagen, Copenhagen, Denmark.

Congenital heart defects (CHD) are the most common type of birth malformation and result in major mortality worldwide, affecting 8 in 1000 live births. The etiology for these malformations remains unknown, but genetic factors play an important role. We identified a Danish family presenting autosomal dominant inherited CHD with reduced penetrance. Whole exome sequencing led to the identification of a heterozygous mutation (c.1937_G>A) affecting a conserved Arginine residue (p.Arg646Gln) encoded in the *PLEKHA6* gene. The mutation segregated with CHD in the family, was not present in 2000 Danish controls, and displays a very low frequency in the ExAC database of 64K exomes (MAF of 8.25e-06). *PLEKHA6* encodes a protein of unknown function, with gene ontology annotations related to phospholipid binding. The gene shows a high expression in embryonic hearts and is up-regulated during differentiation of stem cells into cardiomyocytes in the P19.CL6 stem cell model. Whole mount in-situ hybridization in 2dpf wild-type zebrafish embryos show *plekha6* expression in multiple organs and tissues, including brain, gut and heart.

Surprisingly, knockdown of *PLEKHA6* in P19.CL6 cells increases the cells ability to differentiate into cardiomyocytes, suggesting an inhibitory function of PLEKHA6 during cardiomyogenesis. We have initiated *plekha6* gene knock-out in zebrafish embryos by injection of cas9 mRNA and *plekha6* specific guide RNAs into fertilized oocytes. The injections results in embryos mosaic for mutations in plekha6. Preliminary data suggest a dosage sensitive effect on embryonic development, which include cardiac defects. Experiments with lower doses of guide RNA and different plekha6 guide RNAs are ongoing.

The Role of Klf2 in Zebrafish Heart Development

S. Javad Rasouli and Didier Y. R. Stainier
Dept.III- Developmental Genetics, Max Planck Institute for Heart and Lung Research, Ludwigstraße 43, 61231 Bad Nauheim, Germany

Biological flows are required for vertebrate organogenesis. For example, cardiovascular development, hematopoiesis and kidney morphogenesis are some of the processes affected by fluid-dependent mechanical stresses [1-3]. Shear stress generated by blood flow can regulate hemodynamic responses that are necessary for cardiovascular processes such as trabeculation, atrioventricular valve leaflet formation and blood vessel maturation, but the molecular and genetic bases for this regulation are poorly understood [3-5].
In addition, several recent studies have reported that *Klf2*, a flow responsive gene, is required for cardiovascular development; however the detailed role and effectors of Klf2 are not well understood at this time [2-6]. Since the lack of KLF2 function in mouse results in embryonic lethality, a detailed understanding of its role in cardiovascular development is a difficult challenge [7]. To overcome this challenge, we use the zebrafish (*Danio rerio*), as a complementary model organism for developmental genetic studies, especially of the cardiovascular system [8-10].
To avoid using morpholinos which have been shown to induce off-target effects [11], we used the TALEN technology to mutate both *klf2* paralogues in zebrafish. Surprisingly, our results reveal that neither *klf2a*$^{-/-}$*Δ10* nor *klf2b*$^{-/-}$*Δ8* mutants show an obvious phenotype. Therefore, we recently generated *klf2a;klf2b* double mutants to examine whether the lack of both paralogues affects zebrafish development and cause significant cardiovascular defects.
We are now using imaging tools to better analyze the potential phenotypes in *klf2a;klf2b* double mutants, and our preliminary data obtained from *klf2a;klf2b* double heterozygous incrosses indicate that *klf2a*$^{-/-}$*;klf2b*$^{-/-}$ embryos exhibit cardiac defects. We are currently conducting additional experiments to better understand these defects. Understanding the role of Klf2 in embryonic heart development should improve our knowledge about processes leading to congenital heart defects.
Literature:

[1] Serluca, F.C., I.A. Drummond, and M.C. Fishman. **2002**. 12(6): 492-97.
[2] Pardanaud, L. and A. Eichmann, *Stem cells*. **2009**. 459(7250): p. 1068-69.
[3] Slough, J., L. Cooney, and M. Brueckner. **2008**. 237(9): 2304-14.
[4] Vermot, J., et al. **2009**. 7(11): p. e1000246.
[5] Wang, L., et al. **2011**. 118(15): 4102-10.
[6] Oates, A.C., et al. **2001**. 98(6): 1792-801.
[7] Lee, J.S., et al. **2006**. 11(6): 845-857.
[8] Stainier, D.Y. **2001.** 2(1): 39-48.
[9] Alexander, J., D.Y. Stainier, and D. Yelon. **1998**. 22(3): 288-99.
[10] Staudt, D. and D. Stainier. **2012**. 46: 397-418.
[11] Gerety, S.S. and D.G. Wilkinson. **2011**. 350(2): 279-89.

The evolution of cardiac conduction system in the crocodilian heart

Alena Kvasilova[1], Martina Gregorovicova[2], Bjarke Jensen[3,4], Vincent Christoffels[4], David Sedmera[1, 2]

[1] Institute of Anatomy, First Faculty of Medicine, Charles University, Czech Republic
[2] Institute of Physiology, The Czech Academy of Sciences
[3] Department of Bioscience-Zoophysiology, Aarhus University, Denmark
[4] Anatomy, Embryology & Physiology, AMC, The Netherlands

The crocodilians have a completely septated heart, similar to birds and mammals, thus occupying a unique position among reptiles. In avian and mammalian development, the functional and morphological emergence of the bundle branches correlates with ventricular septation; we have therefore tested the hypothesis that evolution of the specialized conduction system is linked to ventricular septation, rather than homoiothermy, as postulated by Davies.

We studied a group of 11 embryos of the Siamese and Mugger Crocodile between 3 and 84 days of incubation, and serial early embryonic sections with HNK-1 and Hematoxylin staining of the Nile Crocodile, using optical mapping, ultrasound biomicroscopy, histo- and immunohistochemistry, in situ hybridization and 3D reconstruction. We focused on the nature of myocardial atrioventricular continuity and development of the annulus fibrosus in the crocodilian heart.

At the early, pre-septation stage (12 days of incubation), the ventricular activation pattern progressed in a left-to-right/base-to-apex sweep, similar to that observed at the early stages of avian and mammalian cardiogenesis and in unseptated reptilian (Anole) hearts. In two post-septation stages investigated (45 and 84 days), the epicardial activation patterns showed apex-to-base activation, indicative of presence of a preferential conduction pathway within the ventricles. In two hearts (50 days of incubation) we saw an epicardial breakthrough of circular shape near the right ventricular apex. Immunohistochemistry showed HNK-1 positivity in the pacemaker area and in the interventricular septum, similar to the situation in the chick and rat embryonic hearts where it is used as a marker of the developing conduction system. In situ hybridization for crocodilian Cx40 transcripts (VCS marker) showed, however, uniform expression in the entire trabeculated ventricular myocardium.

We therefore conclude that late embryonic crocodilian hearts possess a preferential conduction pathway somewhat similar to one present in the hearts of homoiotherms. Its emergence correlates with ventricular septation and presence of some conduction system markers.

Supported by Ministry of Education PRVOUK P35/LF/5 and institutional grant RVO: 67985823 (The Czech Academy of Sciences).

A specialized atrioventricular conduction system in the alligator heart offers a novel explanation for the evolution of the ventricular conduction system

Bjarke Jensen1, Bastiaan J Boukens1-2*, Dane A Crossley II3, Christopher R. Gloschat2, Ruth M Elsey4, David Sedmera5, Igor Efimov2, Vincent M Christoffels1*
these authors contributed equally
Affiliations : 1Department of Anatomy, Embryology, and Physiology, Heart Failure Research Center, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands. 2Department of Biomedical Engineering, George Washington University, DC, USA. 3Department of Biological Sciences, University of North Texas, Denton, TX 76210, USA. 4Rockefeller Wildlife Refuge, Louisiana Department of Wildlife and Fisheries, Grand Chenier, LA, 70643, USA. 5Institute of Anatomy, First Medical Faculty, Charles University, Prague, Czech Republic

Background and aim : Hearts of mammals and birds have a specialized conduction system comprising an atrioventricular bundle and Purkinje fiber network enabling rapid, apex-first activation of both ventricles. The single ventricle hearts of ectothermic vertebrates (fish, amphibians, reptiles) lack these components, suggesting they evolved in parallel with endothermy. Crocodilians, however, are the only ectothermic vertebrates with two ventricles and a full ventricular septum, the structure in which the atrioventricular bundle resides in endotherms. Here we investigated the presence of an atrioventricular bundle and Purkinje fiber network in crocodilians.
Methods and results : Markers of the atrioventricular bundle in mammals and chicken, the transcription factors Tbx3 and Tbx5, were expressed dorsally in the crocodilian septum. Cuts through this tentative atrioventricular bundle tissue led to propagation block, indicating its requirement for impulse conduction to the ventricles. Markers of the mammalian ventricular Purkinje fiber network, Cntn2 and Gja5, were widely expressed in the entire trabecular ventricular wall. Optical mapping of action potentials revealed breakthrough of the activating impulse halfway between the base and apex at the level of the Tbx3-expressing atrioventricular bundle.
Conclusions : We conclude that crocodilians have a specialized atrioventricular bundle, and suggest the ventricular septum and atrioventricular bundle evolved in parallel. The entire trabeculated wall of ectothermic vertebrates corresponds to the Purkinje fiber network, which is miniscule in mammals and birds because of the evolution of a thick compact ventricul

Early specification and progressive restriction of the cardiac conduction system lineage during heart development

Rajiv Mohan, Mathilda Mommersteeg, Vincent Wakker, Arie Verkerk, Vincent Christoffels

The adult cardiac conduction system (CCS), comprising the sinus node, atrioventricular node and junction, the atrioventricular bundle and bundle branches, is composed of specialized cardiomyocytes that orchestrate the rhythm of the heart. In the adult heart, they are the only cells with a pacemaker phenotype, capable to generate spontaneous action potentials, a feature that is absent in the working myocardium (WM) of the chambers. It is unclear from which progenitors the adult CCS components originate during embryogenesis and precisely when the CCS lineage becomes specified. The adult CCS components specifically express T-box transcription factor Tbx3, which is expressed early in heart development and is required for the correct development and functioning of the CCS. We hypothesize that 1) the Tbx3+ cardiomyocytes in the embryo function as a CCS framework and 2) they are the progenitors of the adult CCS.

To address these issues, transgenic mouse lines have been generated expressing either fluorescent protein Venus or tamoxifen-inducible Cre under control of the *Tbx3* locus. Expression and electrophysiological analyses of the Venus-positive cardiomyocytes revealed that Tbx3 expression specifically marks cardiomyocytes with a pacemaker phenotype throughout development from as early as embryonic day 10.5, briefly after the initiation of chamber differentiation. To investigate the origin of the mature CCS, Tbx3+ cells were pulse labeled at subsequent stages of development using the tamoxifen-inducible Cre system, and the fate of their progeny was assessed in the late fetal heart when the CSS components have been well formed. Interestingly, as early as embryonic day 7.5-8.5, just after gastrulation, Tbx3+ cells contribute to all components of the CCS except for the sinus node. One day later, the heart still a tubular structure, the Tbx3+ population contributes to all CCS components. The embryonic Tbx3+ population also contributed to WM cardiomyocytes in the chambers. Both the contribution and the distance from the CCS to the labeled WM cells progressively decreased with labeling Tbx3+ cells at later stages of embryonic development.

We conclude that 1) the Tbx3+ cardiomyocytes in the early embryonic heart function as the embryonic CCS, 2) the mature CCS components originate from the Tbx3+ cardiomyocytes in the early developing heart, and 3) Tbx3+ cardiomyocytes initially are bipotent, giving rise to CCS and WM cardiomyocytes, but become progressively restricted to the CCS lineage.

Inner architecture of the right ventricle: the role of the tricuspid valve.

Lucile Houyel, MD Bettina Bessières, MD Meriem Mostefa-Kara, MD Daniela Laux, MD Marie Gonzales, MD
Marie-Lannelongue Hospital, Le Plessis-Robinson, France

A major anatomic characteristic of the right ventricle, in addition to coarse and few apical trabeculations, is the presence of muscular bands arranged in a semicircular fashion: parietal band and subpulmonary conus (grouped under the term ventriculo-infundibular fold, VIF), septal band (SB), moderator band (MB). The embryologic origin of SB, MB and anterior papillary muscle of the tricuspid valve (APM) is still controversial: tricuspid myocardial primordium (tricuspid gully), or condensation of the right ventricular trabeculations.
Material and methods: In order to determine if the presence of the muscular bands of the right ventricle could be related with the degree of development of the tricuspid valve, we reviewed 32 postnatal and 26 fetal human heart specimens with tricuspid atresia (TA) from the anatomic collection of the French Reference Center for Complex Congenital Heart Defects.
Results: Forty-two hearts had ventriculo-arterial concordance, 14 had D-transposition. There were 52 muscular TA (including 6 without any right ventricular cavity), with a dimple in the floor of the right atrium, and 6 membranous TA with imperforate valvar tissue. All 52 hearts with a right ventricular cavity had a well-developed VIF. A rudimentary SB (with demonstrable limbs in 3) was present in 6/46 muscular TA vs 6/6 membranous TA ($p=0.000$), rudimentary MB in 3/46 muscular TA vs 6/6 membranous TA ($p=0.000$), rudimentary APM in 3/46 muscular TA vs 3/6 membranous TA ($p<0.02$). Left juxtaposition of the atrial appendages was found in 2% muscular TA vs 33% membranous TA ($p=0.02$).
Conclusion: SB and MB are absent in the vast majority of hearts with muscular TA, but are present in all hearts with membranous TA, while VIF is always present. These anatomic findings support the hypothesis of a dual embryologic origin for the muscular bands of the right ventricle: the VIF is reminiscent of the inner curvature of the heart, while the SB, the MB and the APM develop later, from the muscular tricuspid primordium, itself developed from the posterior part of the primary fold, and thus belong to the right ventricular inlet.

CORONARY PLAQUE DISRUPTION, THROMBOSIS AND INFLAMMATION IN PATIENTS DIED OF ACUTE MYOCARDIAL INFARCTION

Authors: Rodrigo Blanco[1], Ricardo Sarmiento[1], Coloma Parisi[2], Sebastian Fandiño[2], Federico Blanco[1], Maria Florencia Raffaelli[2], Gerardo Gigena[1], Miguel Riccitelli[1].

1, Department of Cardiology. 2, Department of Pathology. Hospital "Dr. Cosme Argerich". Buenos Aires. Argentina.

Postal address: Juncal 4631, 13A (CP:1425). E-mail address : rodrigoiblanco@yahoo.com.ar

Objective: to evaluate the presence and distribution of vulnerable plaques, thrombosis and inflammatory infiltrates in patients who died of acute myocardial infarction (AMI) and compare with patients who died of non-coronary heart disease.

Methods: we analyzed the coronary arteries from 82 patients who died of AMI and 30 patients who died of non-coronary heart disease (Control group) using light microscopy. The time from symptom onset to death was < 5 days for all cases and the autopsies were performed within 6 hours after death. The vessels were cut transversely at 2-mm intervals and arterial sections were stained with hematoxylin and eosin. The presence of thrombus, intraplaque hemorrhage, endothelial rupture and inflammatory infiltrates were registered. A p value <0.05 was considered statistically significant.

Results: In patients who died of AMI, we found thrombus in 70.7% of the culprit arteries and in 26.2% of non-culprit arteries (p < 0.001). Intraplaque hemorrhage was found in 64.6% of culprit arteries and in 37.8% of non-culprit arteries (p < 0.001); endothelial rupture in 24.4% of culprit arteries and in 3.6% of non-culprit arteries (p < 0.001). There was no difference in the presence of inflammatory infiltrates (68.3% versus 59.1%, p=0,16). Comparing non-culprit arteries with coronary arteries of patients died of non-coronary heart disease, the presence of thrombus was significantly higher (26.2% vs.14.4%; p<0.03), as well as the presence of intraplaque hemorrhage (37.8% vs.7,.7%; p<0.001) and inflammatory infiltrates (59.1% vs. 21.1%; p <0.001). (Table1)

Conclusions: Our histopathologic study found that coronary plaque instability is present in more than one coronary artery in AMI, in the culprit and non culprit vessels, and shows a widespread inflammatory phenomenon that involves the entire coronary tree.

Table 1.	Culprit arteries (n=82)	Non-culprit arteries (n=164)	P	Control group arteries (n=90)	P*
Thrombus (%)	58 (70,7)	43 (26,2)	<0,001	13 (14,4)	0,03
Intraplaque hemorrhage (%)	53 (64,6)	62 (37,8)	<0,001	7 (7,7)	<0,001
Endothelial rupture (%)	20 (24,4)	6 (3,6)	<0,001	1 (1,1)	0,2
Inflammatory infiltrates (%)	56 (68,3)	97 (59,1)	0,16	19 (21,1)	<0,001

* Non culprit arteries vs Control group arteries.

TBX5: The Missing Culprit Gene in Thalidomide Toxicity

Athar Khalil, Nehme El-Hachem, Mazen Kurban, Georges Nemer
American University of Beirut, Beirut, Lebanon

Holt-Oram syndrome (HOS) is a rare autosomal dominant disease associated mainly with upper limb malformation and congenital heart defects (CHD) caused by haploinsufficiency of the T-box transcription factor 5 (TBX5). Congenital heart disease (CHD) is a leading cause of death, with an incidence of approximately 6–8 in 1,000 live births. Only 13 % of all CHD cases are thought to be inherited and the rest are sporadic in nature. Some CHD are caused by environmental factors and teratogens like thalidomide. Thalidomide was synthesized in 1957 and marked as a sedative drug that was used by pregnant women to prevent morning sickness but it caused severe malformations in the newborns similar to those detected in HOS patients, thus it was removed from the market in 1961. Previous studies showed that TBX5 transcription was reduced as a response to thalidomide detected by semi-quantitative RT-PCRs on RNA extracted from wing buds of chicken embryos.

We aimed to investigate the effect of thalidomide on TBX5, suggesting an interaction between them as the *in-silico* docking prediction showed. We used the electric mobility shift assay (EMSA) to confirm that thalidomide decreases the binding affinity between TBX5 protein and consensus sequence of T-box. While thalidomide didn't affect the cellular localization or the protein stability of TBX5 as indicated by immuno-fluorescence and western blot respectively. Suppressed expression activity of

Vascular endothelial growth factor (VEGF) and atrial natriuretic factor (ANF) promoter was obtained in the presence of thalidomide assessed by luciferase assay. While thalidomide was neither able to suppress the interaction of TBX5 with GATA4 presented by VEGF promoter expression, nor affected this interaction on the protein level as shown by co-immunoprecipitation assay. Thalidomide could significantly suppress cellular proliferation and migration of embryonal rhabdomyosarcoma cells as indicated by the MTT and wound healing assays respectively, but it did not affect the endogenous expression TBX5 in this cells line.

These results were the first to show that thalidomide bind specifically to TBX5 on its DNA binding domain suppressing its transcriptional properties. Also we were the first to show the antiproliferative and antiangiogenic effect of thalidomide on embryonal rhabdomyosarcoma. Revealing the two faces of thalidomide; one related to its teratogenic mechanism of action and a second one related to its benefits in cancer treatment.

Anatomy of Sinoatrial Nodal Artery: a meta-analysis and clinical review of it's anatomical characteristics

Brandon Michael Henry 1 Jens Vikse 1, Joyeeta Roy 1, Piravin Kumar Ramakrishnan 1, Wan Chin Hsieh 2, Jerzy A. Walocha, M.D.,Ph.D. 1, Krzysztof A. Tomaszewski, M.D.,Ph.D. 1
1 International Evidence-Based Anatomy Working Group, Department of Anatomy, Jagiellonian University Medical College, Krakow, Poland / 2 First Faculty of Medicine, Charles University in Prague, Czech Republic

Introduction
The Sinoatrial Nodal Artery (SANa) supplies blood to the Sinoatrial Node (SAN),which is a highly variable vessel . Due to its variability and susceptibility to iatrogenic injury, we are aimed at review the normal anatomy of the SANa and the prevalence of its anatomical variations.
Methods
We performed an extensive search through June 2015 of the major electronic databases: PubMed, Scopus, EMBASE, ScienceDirect, Web of Science, and the China National Knowledge Infrastructure (CNKI) to identify all articles reporting anatomical data on the SANa .There are no lower date limit or language restrictions applied. In addition, the references of all included articles were extensively searched. Data regarding the anatomy of artery were extracted and pooled into a meta-analysis using MetaXL version 2.0.
Results
Total sixty-six studies (n=21455 hearts) were included in the meta-analysis. Most commonly the SANa arose as a single vessel with a pooled prevalence of 95.5% (95%CI:93.6-96.9). Duplication and triplication of the artery were also observed with pooled prevalence of 4.3% (95%CI:2.8-6.0) and 0.3% (95%CI:0-0.7), respectively. The most common origin of the SANa was from the right coronary artery (RCA), found in 68.0% (95%CI:55.6-68.9) of cases, followed by origin from the left circumflex artery, and origin from the left coronary artery with pooled prevalence of 22.1% (95%CI:15.0-26.2) and 2.7 (95%CI:0.7-5.2), respectively. A retrocaval course of the SANa was the most common course of the artery with a pooled prevalence of 47.1% (95%CI:36.0-55.5). The pooled prevalence of an S-shaped SANa was 7.6% (95%CI:2.9-14.1).
Conclusion
The most common SANa type is running as a single vessel, originating from the RCA, then taking a retrocaval course to reach the SAN. In order to prevent iatrogenic injury during cardiac interventions, it is essential to understand its normal and variant anatomy.

Correct interpretation of qPCR results in studies of cardiac development requires validated reference genes and the identification of the amplified product

Ruiz-Villalba Adrián, PhD[1,2], Mattiotti Andrea, MSc[1], Cano-Ballesteros Sara, MSc[3], Gunst Quinn D, Ing[1], van den Hoff Maurice J, PhD[1], Ruijter Jan M, PhD[1]

(1) Department of Anatomy, Embryology and Physiology, Academic Medical Center, Amsterdam, The Netherlands
(2) Foundation for Applied Medical Research, University of Navarra, Pamplona, Spain
(3) Department of Animal Biology, University of Málaga, Málaga, Spain

Aims. Optimization of qPCR assays in studies of cardiac development
Background. Quantitative PCR allows the precise measurement of DNA concentrations and is considered to be straightforward and trouble free. However, the specificity of the PCR product and the reproducibility after normalization of the data can be a concern, especially in studies related to cardiac development.
Methods and results. Unexpected, non-specific PCR products were observed to occur in validated PCR assays. Their occurrence was quantified using 93 targets from Wnt signalling pathways in five different regions of chicken embryonic heart. In addition, the reproducibility of the gene expression levels was analysed using two different strategies of normalization: reference genes vs global mean. In parallel, the stability of potential reference genes was studied on 68 different samples, derived from cardiac development to pathological mouse adult hearts, using the *qbase+* software.
Conclusions. qPCR artefacts and the specific product cannot be identified based on the amplification curves, but require a melting curve analysis and size determination on gel. Especially low-input qPCR studies, when 'not expressed' is a possible outcome of the assay, have to be carefully evaluated. Two genes were found to be the most stable reference genes and can be used to analyse changes in gene expression in most, if not all, different cardiac research fields in mice.

RNA tomography allows spatial dissection of genome-wide expression profiles in the developing zebrafish heart tube

Silja Burkhard[1]; Jan Philipp Junker[1]; Emily Noël[1]; Alexander van Oudenaarden[1]; Jeroen Bakkers[1]

[1] Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, The Netherlands

The developing heart tube undergoes rapid morphological and functional changes during the initial stages of embryonic development. The transition from the tubular heart stage at 24hpf to the fully functional 2-chambered heart at early larval stages (72hpf) is accompanied by gradual regionalization of gene expression. Progressive spatial restriction of gene expression has been shown for many major regulators of heart morphogenesis. The assessment of these dynamic changes in transcription has thus far been limited to expression analysis on a single gene level by methods such as in situ hybridization or immunohistochemistry. As an unbiased approach to dissect gene expression patterns during regionalization of the developing heart and to identify novel genes involved, we have taken advantage of RNA tomography. This recently described method provides transcriptome-wide expression data in a highly spatially restricted manner (Junker et al., 2014, Cell).

Embryonic hearts of different developmental stages were dissected and embedded in tissue freezing medium for cryo-sectioning along the anterior-posterior axis. RNA was isolated from each individual 10µm section and processed for RNA library preparation. The libraries were sequenced using the Illumina NextSeq platform.

We have obtained genome-wide transcription information allowing for the spatial dissection of expression profiles in the developing heart tube. Regions of specific gene expression within the heart will allow for the identification of novel genes involved in the pattering of the heart. Furthermore, using this approach in the analysis of cardiac mutant zebrafish lines can provide information on the effect of single gene mutations on genome-wide transcription levels.

Interrogating the Complex Genetics of Congenital Heart Disease in Mice

Nikolai T. Klena, George C. Gabriel, Xiaoqin Liu, You Li, Molly C. Schwartz, Torrin B. Weida, Madhavi Ganapathiraju and Cecilia W. Lo
University of Pittsburgh, Pittsburgh, USA

Congenital heart disease (CHD) is among the most common birth defects, but despite its prevalence, its etiology is still not well understood. To interrogate the genetic etiology of CHD, we undertook a large-scale forward genetic screen in ENU mutagenized mice to recover recessive mutations causing CHD. Using fetal echocardiography for cardiovascular phenotyping, we ultrasound interrogated 100,000 mice from over 3,000 pedigrees, and recovered >250 mutant mouse lines exhibiting a broad spectrum of CHD, including the first mouse models of hypoplastic left heart syndrome. Mutation recovery using whole mouse exome sequencing analysis identified 147 CHD causing mutations in 95 genes. Unexpectedly 47 (52%) of the genes are cilia related, pointing to a central role for cilia in CHD pathogenesis. Interestingly, many of the mutations encode proteins that are either direct protein-protein interactors or are part of the same multiprotein complexes. This included proteins that are components of the cilia transition zone and cilia inversin compartment, suggesting that an interactome network may provide the genomic context for CHD pathogenesis. This model would also lend itself to a more complex genetic model of disease, with multiple genes in varying dosages contributing to disease via non-Mendelian interactions. To test this complex multigenic model, we examined the overall prevalence of mutations (heterozygous/homozygous) in the 96 CHD genes within the 147 mouse exome datasets. This analysis showed a higher incidence for mutations in these 96 genes then would be expected by random chance, thus suggesting they might also act as modifiers. To further experimentally assay for multigenic interactions, we conducted intercrosses between mutant lines harboring the pathogenic CHD causing *Anks6* and *Nek8* mutations, two genes encoding proteins that are direct interacting partners localized to the cilia inversin compartment. Mice heterozygous for either mutation exhibited no phenotype. However, the analysis of 26 double heterozygous *Anks6/Nek8* offspring revealed 13 (50%) with the same mutant phenotypes as observed in the homozygous *Anks6* or *Nek8* mutants. These findings of epistasis demonstrate digenic interactions can indeed play a role in the more complex genetics of CHD pathogenesis. They also suggest genes identified to cause CHD in a recessive mode of inheritance may contribute to more complex genetic model of disease that may be more relevant to human CHD pathogenesis.

BMP2 rescues PE formation in absence of heartbeat in zebrafish

Laura Andrés-Delgado, Claudia Quiñonez-Silvero, Marina Peralta, Maria Galardi-Castilla and Nadia Mercader
CNIC, Madrid, Spain

During embryonic development, the Proepicardium (PE) arises as a group of cells evaginating from the pericardium close to the venous pole of the embryonic heart tube. PE cells are the progenitor cells giving rise to the epicardial layer of the heart, as well as contributing to vascular smooth muscle cells from the coronary vasculature and intracardiac fibroblasts among others. We recently found that PE cell release is driven by pericardiac fluid advections generated by the heartbeat. Our studies also suggested that PE formation is influenced by the beating heart. It is unclear how the heartbeat is controlling this event and which signaling pathways might act downstream or in concert with mechanical forces controlling PE outgrowth. It has been previously described that BMP signaling is required for PE specification. Moreover, in the chick, PE cell adhesion to the myocardial surface has been shown to depend on the chemoattractant action of myocardial BMP2 on PE cells. In this project we investigated the coordinated effect of the secrete molecule bone morphogenetic protein 2 (BMP2) and mechanical forces (the heartbeat) on PE formation.
Using in vivo imaging we determined that in absence of a heartbeat a transient PE cluster emerges, which does regress without contributing to epicardial layer formation. Overexpression of BMP2 increases the number of PE and epicardial cells in the zebrafish.. By contrast, overexpression of the BMP pathway inhibitor Noggin prevents PE formation. Importantly, ectopic BMP2 expression was able to rescue PE formation in the absence of a heartbeat, using 2,3-Butanedione Monoxime treatments, but not in a genetic model of absent heartbeat *(silent heart mutants)*.
In conclusion, BMP signaling is necessary but not sufficient for PE formation, possibly acting downstream of flow force triggered effects.

The epicardium as modulator of the cardiac autonomic response during early development

Sjoerd N. Duim[2†], Tim P. Kelder[1†], Rebecca Vicente-Steijn[1,4,5], Anna M.D. Végh[2], Boudewijn P.T. Kruithof[2], Anke M. Smits[2], Thomas C. van Bavel[1,2], Noortje A. M. Bax[3], Martin J. Schalij[4], Adriana C. Gittenberger-de Groot[1,4], Marco C. DeRuiter[1], Marie-José Goumans[2*], Monique R.M. Jongbloed[1,4*]

†,* authors contributed equally

1. Department of Anatomy & Embryology, Leiden University Medical Center, Leiden, The Netherlands;
2. Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands;
3. Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands;
4. Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands.
5. ICIN Netherlands Heart Institute, Utrecht, The Netherlands.

Background

Modulation of heart rate, contraction force and conduction velocity is controlled by the cardiac autonomic nervous system (cANS) and is essential for proper cardiac functioning. The neurotransmitter epinephrine stimulates cardiac output by binding to beta (β) adrenergic receptors. Previous work showed that before establishment of the cANS the embryonic chicken heart is capable of responding to epinephrine by increasing the heart rate. The aim of this study was to unravel the role of the epicardium in modulating the early autonomic response to epinephrine.

Results

Immunofluorescence analysis revealed that the epicardium transiently expressed neural markers during early development. Specifically, epicardial cells expressed tubulin beta-3 chain (TUBB3) and neural cell adhesion molecule (NCAM). In addition, the epicardium also expressed beta 2 adrenergic receptor (β2AR) which ensures binding of epinephrine. To investigate the possible functional role of the epicardium in the modulation of the early autonomic response to epinephrine ex-ovo micro-electrode recordings were performed. A significant reduced response of the heart rate to epinephrine was found after inhibition of the epicardial outgrowth at HH24. The role of the epicardium was confirmed by the absence of a response to epinephrine in HH15 heart, when there is no epicardium present yet. Interestingly, the hampered epicardial covering of the sinus venosus correlates with the disturbed response to epinephrine.

Conclusion

This study suggests a transient role for the epicardium as an autonomic modulator during early cardiac development.

Septum transversum COUP-TFII expression regulates cardiac chamber growth, septation and vascularization

Pires-Gomes, A. Guadix, J.A. Cano-Ballesteros, S. Peréz-Pomares, J.M.
University of Málaga, Málaga, Spain

The septum transversum (ST) is a fold of the lateral mesodermal which separates prospective thoracic and abdominal cavities. It contains various populations of cells which have been shown to contribute to coronary vessel development via the proepicardium (PE). As a matter of fact, coronary development and ventricular wall growth and maturation are two inextricably related phenomena. Coronary formation is tightly regulated by a variety of juxtacrine and paracrine signals, some of which are derived from the epicardium, i.e. the outermost tissue layer of the heart. Disruption of such signaling pathways has a direct an impact on both coronary and compact ventricular myocardium development.

COUP-TFII has been described to play a major role in heart development. *COUP-TFII* systemic mutant displays embryonic lethality (around E9.5) and display defects in systemic angiogenesis and cardiac inflow development. Since the mechanisms underlying the contribution of ST cells to coronary vessel and cardiac inflow development are not well known, we have aimed at characterizing the role of *COUP-TFII* in the ST/PE.

Our results confirm that *COUP-TFII* cardiac expression in E9.5 mouse embryos is extensive in the ST, the primitive sino-atrial chamber myocardium and some early endocardial cells. Using transgenic *Wt1*-Cre and G2*Gata4*-Cre mouse lines, we have traced (ROSA26-YFP) the fate of these subpopulations of the cardiac inflow, including PE-derived cells, and found a clear COUP-TFII expression in significant subsets of *Wt1* and G2*Gata4* cell lineages. Then we conditionally deleted *COUP-TFII* in these two cell populations (*Wt1*-Cre;*COUP-TFII*$^{fl/fl}$; or G2Gata4Cre;*COUP-TFII*$^{fl/fl}$). At E9.5, the expression of *COUP-TFII* in Wt1Cre;*COUP-TFII*$^{fl/fl}$ mice was found to be reduced in the cardiac inflow and the sino-atrial myocardium. Older mutant embryos display a complex phenotype, showing anomalous left atrium morphogenesis and positioning, hypoplastic ventricular compact myocardium and reduced lumenization of coronary vessels (Notch1, EphrinB2) leading to their death around E15. The phenotype of the G2Gata4Cre;*COUP-TFII*$^{fl/fl}$ is even more severe, leading to embryonic death around E12.5.

Surprisingly, atrial and ventricular septal defects (E14.5) were also found in these mutants. A careful inspection of these animals revealed decreased *COUP-TFII* expression in the dorsal mesenchymal protrusion, a ST-independent, secondary heart field-related cell population required for proper cardiac septation.

These results, taken together, suggest that COUP-TFII has an important role in coordinating the developmental functions of cells from the ST. Interfering with normal ST/PE contribution to the forming heart might impair coronary blood vessels and cardiac inflow development.

Regionalized ANKRD1 expression is essential to finely regulate multiple aspects of cardiac remodeling and venous pole development.

Marina Campione1, Giulia S. Ganzetti2, Virginia Cora1, Simone Tiso1, Steven B. Bleyl4, Laura Monti3, Ileana Badi3, Raffaella Cinquetti3, Giulia Chiesa2, Alessandra Tessari1, Federico Caicci5, Lucia Manni5, Annalisa Grimaldi3, Roberto Taramelli3, Cinzia Parolini2 and Francesco Acquati3.

1 CNR- Neuroscience Institute, Dept. of Biomedical Sciences, University of Padova, Italy; 2 Dept. of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy; 3 Dept. of Theoretical and Applied Sciences and Biotechnology and Life Sciences, Università degli Studi dell'Insubria, Varese, Italy; 4Dept. of Pediatrics, University of Utah, Salt Lake City, Utah, USA; 5 Dep. of Biology, University of Padova, Italy.

The acquisition of correct cardiac anatomical organization is the result of tightly regulated developmental steps, therefore even minor mistakes in these chain of events invariably results in congenital heart disease (CHD). To date, a huge number of gene mutations have been identified in CHD patients, however our understanding of their cellular role in the developing myocardium is often poor.

Cardiac Ankirin Repeat Protein (CARP), encoded by the *Ankrd1* gene, is a multitasking mechanosensor protein involved in physiological and pathological remodeling of ventricular myocardium. A missense mutation (T116M), leading to increased stability and expression of the protein, has been identified in total abnormal pulmonary venous return (TAPVR) patients, thus suggesting a role of the gene also in cardiac venous pole development.

Here we show that Ankrd1 expression is highly regionalized during mouse heart development.

Myocardial overexpression of ANKRD1 wt or T116M resulted in reduced viability due to complex cardiac morphogenetic defects including abnormal pulmonary venous connections, as visualized by Amira-3D reconstructions at mid fetal stage. Irregular cardiomyocyte organization and increased intercellular space observed in E13.5 transgenic hearts suggested that ANKRD1 can act as a signaling molecule whose overexpression progressively affects the cellular properties of cardiomyocytes.

E10.5 ANKRD1 transgenic hearts were more compressed along the antero-posterior and dorso-ventral axis and presented strong impairment in cardiac developmental remodeling, including rotation and cranial expansion of the sinoatrial region which is critically required for correct venous pole development.

Our results uncover for the first time ANKRD1 as a crucial modulator of heart development, whose regionalized expression is required to define the final shape and relative position of the cardiac components. Additionally, they support the role of Ankrd1 as a critical signaling molecule which finely modulates the cardiomyocyte cellular properties, thereby eventually influencing cardiac shape.

We propose that ANKRD1 mutations in humans lead to TAPVR as a result of developmental impairment in early venous pole remodeling steps. Moreover, regionalized expression of the gene in the developing myocardium could link ANKRD1 mutations to additional forms of CHD.

Genetic and Developmental Etiology of Valvular Heart Disease

Katelynn Toomer, Kimberly Sauls, Susan Slaugenhaupt, David Milan, Robert Levine, Chip Norris
Medical University of South Carolina, Charleston, USA

Cardiac valve diseases are common clinical problems that affect >10% of the human population and result in more than 16,000 surgical cases each year. The etiologies of valve diseases remain poorly understood. Through an international consortium focused on valvular heart disease, we now have compelling genetic and functional evidence that significantly advances our understanding of valve disease pathogenesis. Genome wide association studies (GWAS), linkage and capture sequencing of large families with inherited valve disease have yielded a potential unifying theory for the etiology of mitral valve prolapse (MVP) and bicuspid aortic valve disease (BAV). The results of the genetic and functional studies will be presented in the context of these human valve diseases. Additionally, mouse and zebrafish data that has led to novel mechanistic understanding for valve development as well as disease pathogenesis will be discussed.

The HAND2 Cistrome in Mouse Embryonic Hearts Identifies its Target Genes During Endothelial-Mesenchymal Transition in the Atrioventricular Canal

Frédéric Laurent[1], Javier Lopez-Rios[1], Marco Osterwalder[1,2], Iros Barozzi[2], Dario Speziale[1], Jennifer Akiyama[2], Joy Lincoln[3], Axel Visel[2], Aimée Zuniga[1] and Rolf Zeller[1]

[1]Developmental Genetics, Department of Biomedicine, University of Basel, CH- 4058 Basel, Switzerland

[2]Genomics Division, MS 84-171, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

[3]Center for Cardiovascular and Pulmonary Research, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA

During the embryonic development of the mammalian heart, the formation of the cardiac valves is a critical step towards the establishment of an unidirectional blood flow. Valves formation takes place in the outflow tract (OFT) and in the atrioventricular canal (AVC), where the cells of the endothelial lining of the heart, the endocardium, undergo endothelial-mesenchymal transition (EndMT) and proliferate to form cardiac cushions that will be remodeled into cardiac valves. Among the transcription factors that direct the proliferation and fates of cardiac progenitor cells, the basic helix-loop-helix protein HAND2 plays a critical role during the differentiation of second heart field (SHF) derived structures (OFT and right ventricle). Indeed, *Hand2*-deficient mouse embryos display severe right ventricle hypoplasia and die prior to embryonic day E10.5. The direct targets and gene regulatory networks controlled by HAND2 during heart morphogenesis have remained elusive thus far. Using mice expressing a 3xFLAG epitope-tagged HAND2 protein, we studied the spatio-temporal distribution of HAND2 and performed ChIP-Seq analysis to determine the range of its target sequences (cistrome) in E10.5 embryonic hearts. In addition to the identification of HAND2 target genes in the SHF, we have established that HAND2 directly controls a network of genes that regulate EndMT in the AVC. Indeed, the endocardial cells of *Hand2*-deficient embryos fail to undergo EndMT both *in vivo* and in an *in vitro* AVC explant culture system. As the expression of *Snai1*, a key regulator of EndMT, is absent from *Hand2*-deficient endocardial cells, adenoviruses were used to re-express SNAI1 in explant cultures, which results in an increased number of cells undergoing EndMT *in vitro*. Furthermore, we generated transgenic reporter mouse embryos for *cis*-regulatory modules (CRMs) directly bound by endogenous HAND2 chromatin complexes in the *Snai1* genomic landscape. We found that one of these CRMs is active in the cardiac cushions in both the OFT and the AVC. In addition, this CRM recapitulates most of the endogenous *Snai1* embryonic expression at E10.5. Altogether, our study establishes that HAND2 is an important regulator of *Snai1* and other EndMT genes in the endocardium of the atrioventricular canal.

The role of Follistatin-like 1 in post-natal valve formation.

Stuti Prakash[1], Lorena Flores Ruiz[3], Quinn D Gunst[1], Jan M Ruijter[1], Jose Luis De La Pompa[2], Luis J Jimenez Borreguero[3], Maurice van den Hoff[1].

[1]Department of Anatomy, Embryology and Physiology, Academic Medical Center, Amsterdam, The Netherlands, [2]Program of Cardiovascular Developmental Biology, Department of Cardiovascular Development and Repair, [3]Department of Epidemiology, Atherothrombosis and Image, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Heart AV valves are highly conserved and dynamic structures composed of cells derived from different lineages and a highly organized extracellular matrix. Dysregulation of the cellular contribution and ECM organization leads to valve disease which is a leading cause of morbidity and mortality in adults.

Follistatin-like 1 (Fstl1) is a secreted glycoprotein expressed mostly in the non-myocardial component of the heart and is induced in response to injuries that promote myocardial hypertrophy and eventually heart failure. Fstl1 is thought to be an extracellular regulator of BMP signalling.

To investigate the role of Fstl1 in the endocardial/endothelial cell lineage, we have created a conditional Tie2Cre knock-out mouse of Fstl1. Knock out mice are born healthy but start to die from two week onwards, 50% of the mice have died by three weeks, 90% by two months and all are dead by three months. Autopsy showed the hearts to be enlarged. Morphological examination showed that the ventricular walls are hypertrophic and the AV valves are long, thick and billowing.

Next we performed echocardiographic analysis using the Visual Sonics Vevo 2100 imaging system to establish the hemodynamic changes in these Fstl1 conditional knock-out mice from the first until the 23rd day after birth. This analysis showed that heart function was normal upto 10 days after birth. Subsequently, the heart was found to be enlarged whereas mitral valve regurgitation was identified from 14 days after birth onwards. Immunofluorescent and in situ hybridization analysis showed an upregulation of extracellular matrix proteins and embryonic valvular genes. Evaluating the local activity of BMP and TGFβ signaling using immunofluorescent staining and antibodies directed to P-Smad1/5 and P-Smad2/3, respectively, showed an upregulation in the endocardial and mesenchymal cells of the valves.

Taken together, our data suggest that absence of Fstl1 from the endothelial/endocardial lineage results in a dysregulation of TGFβ/BMP signalling and in turn in very long and thick incompetent AV valves. The analysis, furthermore, suggest that these valves can be considered to be myxomatous valves.

Mechanobiology of the cardiac valves in an ex vivo flow model

Boudewijn P.T. Kruithof[1], Samuel C. Lieber[3], Marianna Kruithof-de Julio[2], Vinciane Gaussin[4], Marie-José Goumans[1]

Departments of [1]Molecular Cell Biology and [2]Urology, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Mechanical Engineering, New Jersey Institute of Technology, Newark, NJ, USA; [4]Cardiovascular Research Institute, Department of Cell Biology and Molecular Medicine, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey, USA;

Heart valve disease is a major cause of mortality and morbidity in the Western population. Mechanical stimuli are suggested to play a role in valvular disease, however, little is known about their influences on the cellular and molecular mechanisms underlying heart valve disease. This is mainly due to the absence of a culture model, which allows manipulation of the environmental conditions of the valve in its normal position in the heart. We have adapted the existing Miniature Tissue Culture System and have cultured the mouse aortic valve under conditions in which the aortic valve continuously experiences mechanical forces similar as during peak systole (laminar and disturbed shear stress at ventricular and aortic sites respectively) or peak diastole (stretch and pressure at ventricular and aortic sites respectively). Site-specific and/or culture condition-dependent responses were observed including thickening of the leaflets, proliferation and activation of the valvular interstitial cells, remodeling of the extracellular matrix and activation of signaling pathways. Using lineage-tracing, endothelial to mesenchymal transformation of the endothelial cells of the valves was observed. Using our ex vivo flow model we have shown that specific mechanical stimuli regulate distinct cellular and molecular processes involved in valvular disease. This model could therefore provide major insights in the mechanisms underlying the initiation and progression of heart valve disease.

Abstracts of poster session

01: Mir-27 distinctly regulates muscle-enriched transcription factors and growth factors in cardiac and skeletal muscle cells

Estefania Lozano-Velasco, Jennifer Galiano-Torres, Alvaro Jodar-Garcia, Isabel Lopez-Navarrete, Amelia E Aranega, Diego Franco
Cardiovascular Research Group, Department of Experimental Biology, University of Jaén, Jaén, Spain

microRNAs are non-coding RNA that exert post-transcriptional regulatory mechanisms by either blocking transcript translation or promoting mRNA degradation. Over the last decade, novel insights have been gained on the functional role of distinct microRNAs in cardiovascular development and disease. We have previously reported a discrete number of microRNAs that are differentially expressed during cardiogenesis. In particular, we demonstrated that miR-27, which is overtly expressed in the developing heart, targets *Mef2c* muscle-enriched transcription factor. More recently, a role for miR-27 regulating Pax3 has been reported in skeletal muscle. In this study we sought to get further insights into the regulatory mechanisms exerted by miR-27 in cardiac and skeletal muscle cells. We assayed whether distinct muscle-enriched transcription factors and growth factors predicted to be targeted by miR-27 are deregulated after miR-27 overexpression in three distinct cell types, i.e. Sol8 skeletal myoblast, HL-1 cardiomyocytes and 3T3 fibroblasts, respectively. qPCR analyses demonstrated that miR-27 overexpression deregulated *Runx1* and *Mef2c* expression in both cardiac and skeletal muscle cells, in line with previous reports. In contrast, *Mstn*, *Myocd*, *Mdf1*, which are also predicted to be targeted by miR-27, display different deregulated patterns in cardiac as compared to skeletal muscle cells, while *Mef2d* displayed no significant differences. Similarly, miR-27 over-expression lead to no significant differences for *Tgfbr1*, *Tgfbr3* and *Bmpr1a*, while *Egfr1* and *Fgf1* showed different deregulated patterns in cardiac vs skeletal muscle cells. Overall these results demonstrate that miR-27 can selectively up-regulate and down-regulate a discrete number of target mRNAs in a cell-type specific manner. A mechanistic working hypothesis as how this is exerted will be presented.

02: The ssessment of efficiency of treatment with nebivolol (nebilet) and lerkanidipine (lercamen) of patients with arterial hypertension on the background of Ischemic Heart Disease.

G.Abuladze MD, PhD, N.Jinjolia MD PhD, G.Uchava, M. Nebieridze PhD
LTD ADAPTI angiocardiology clinic, Tbilisi, Georgia

Purpose of study was to investigate the clinical efficiency of treatment with nebivolol 5mg and lerkanidipine in patients with moderate and high Arterial Hypertension on thre background of IHD.
Methods : 128pt were investigated (68 man and 60 women with age over 50 years) having moderate and high forms of AH. The pt were divided in two groups. Igr(n=66pt) were taken nebilet and lercamen. IIgr-were taken lercamen. Observation was lasted for 6 months. During each visit in every month were monitoring arterial pressure, heart rate, ECG, Echocardiography dates.
Results: All patients in Igr managed to reach targeted AH level. IIgr only-72%.If the targeted level of pressure wasn't reached it was added thiazides in 1.5-2.5% . In Igr also earlier was reached the target HR than in II gr, also at the end of the study was noted decrease ischemic episodes in Igr by 82.3% while in IIgr (with lercamen treatment) by 48.5%. The use of cardio selective beta- blocator nebilet together with Ca-antagonist lercamen was based on hemodynamic and metabolic inter relations. Based on Echocardiography investigation after 6 month in Ig was recorded increase LV EF by 6% ($p<0.05$), besides was noted significantly reduction LV septal wall thickness, significant reduction of left ventricular mass index. In addition was recorded improvement of LF diastolic function in the same group in the result of treatment compared to IIgr.(decrease LA diameter, $p<0.01$, improved LV trans mitral flow parameters: E/A ratio mean significantly increased ,mean DT and IVRT significantly decreased from baseline values $p<0.05$. Pulm. Venous systolic wave velocity increased, atrial PV component decreased $p<0.001$)
Conclusion: After 6 months of treatment in Igr was noted superior clinical efficiency to achieve targeted arterial pressure , improvement LV systolic and diastolic function, reduction LV wall thickness, anti ischemic effect compared with isolated use of lercamen. Such

combination with nebilet and lercamen is one of the most effective method of treatment of moderate and high AH in pt with IHD.

03: Influence of long-term therapy with losartan, valsartan and eprosartan on left ventricular hypertrophy and nt-probnp level in hypertensives

L. Popescu, N. Sapojnic, A. Caraus
Institute of Cardiology , Hypertension, Chisinau, Moldova, Republic of Moldova

Background: Hypertension continues to be the strongest risk factor for increased cardiovascular morbidity and mortality. The left ventricular hypertrophy (LVH) and diastolic dysfunction represent an "association of interests" that lead to development of heart failure (HF). Thus, there is a need for a well tailored therapy of hypertensive phenotype of HF with preserved ejection fraction, before it reaches to the final stage of cardiovascular continuum.
Objective: To compare the effects of an angiotensin receptor blockers-based regimen with valsartan, eprosartan or losartan on LVH and NT-proBNP levels in hypertensives with preserved LV systolic function.
Methods: 154 hypertensive patients (56,5% of men; mean±SD age 53,6±0,5 years) with concentric LVH remodeling (LV mass >115 g/m2 and 95 g/m2 in men and women, respectively), were randomly assigned to treatment with valsartan, eprosartan, losartan over a period of 24 months. Echocardiography, ambulatory blood pressure monitoring and NT-proBNP assessments were performed at baseline and after 24 months treatment.
Results: The baseline ambulatory blood pressure levels were similar among the groups and reductions were similar. At baseline, the LV mass index was comparable in all groups, but after the treatment, the reduction was more evident in the eprosartan group (-35 g/m2, $p<0,001$), and there was positive correlation of changes in ambulatory SBP with LV mass index ($r=0,5$; $p<0,001$). The NT-proBNP levels more decreased in the eprosartan group (-45,6%, $p<0,001$), while in the valsartan and losartan group the reduction was comparable (-28,9% and 26,5%, respectively, $p<0,01$).
Conclusion: The findings of this study showed that all three agents progressively reduced LVH, but the reduction was significantly greater in the eprosartan group. Additionally, eprosartan was associated with a greater reduction of NT-proBNP levels.

04: Reciprocal repression between Fgf8 and miR-133 regulates cardiac induction through Bmp2 signaling

Carmen Lopez-Sanchez[1], Diego Franco[2], Fernando Bonet[2], Virginio Garcia-Lopez[3], Amelia Aranega[2], Virginio Garcia-Martinez[1]
[1]Human Anatomy and Embryology. Faculty of Medicine. University of Extremadura. 06006 Badajoz, Spain
[2]Cardiovascular Development Group, Department of Experimental Biology, University of Jaén, CU Las Lagunillas B3-362, 23071 Jaén, Spain
[3]Pharmacy Services. Minimally Invasive Surgery Centre, Cáceres, Spain

Fgf8 constitutes a crucial factor involved in MAPK/ERK signaling pathway. Also, FGF receptors have demonstrated to play a significant role during the early steps in the Fgf8/ERK signaling. There are previous studies where FGFR1 has been identified in the endoderm underlying the precardiac mesoderm. Furthermore, it has been reported that cardiomyocyte proliferation is suppressed after miR-133 overexpression, and that miR-133 is also involved in late stages of mouse cardiac development as well as in molecular mechanisms regulating adult cardiovascular diseases.
We show in chick embryos miR-133 expression within the precardiac areas, a pattern which is comparable to that of FGFR1. Interestingly, miR-133 overexpression experiments resulted in a decrease of *Fgf8* expression, whereas we observed an increase of *Bmp2* and subsequently of cardiac specific markers *Nkx-2.5* and *Gata4*. Our loss-of-function experiments -through Fgf8 siRNA electroporation- showed an increase of miR-133 expression. After our Bmp2 experiments, we observed that miR-133 is upstream-regulated by Bmp2. All those results suggest that miR-133 constitutes a crucial linkage in the crosstalk between Fgf8 and Bmp2 signaling by regulating the Fgf8/ERK pathway during cardiac induction.

This work has been partially financed with grants to research groups CTS005 (to VGM) from the Junta de Extremadura, with FEDER co-financing, and CVI-6556 (to DF) from the Junta de Andalucía Regional Council.

05: AngiomiR-126 expression is regulated by Arid3b during early chick vascular development

Juan Jose Sanz-Ezquerro[1,2], Veronica Uribe[2], Virginio Garcia-Lopez[3], Virginio Garcia-Martinez[4], Carmen Lopez-Sanchez[4]
[1]Centro Nacional de Biotecnología (CSIC), Madrid, Spain.
[2]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.
[3]Pharmacy Services. Minimally Invasive Surgery Centre, Cáceres, Spain
[4]Faculty of Medicine. University of Extremadura. Badajoz, Spain

It has been reported that Arid3b, a member of the conserved ARID family of transcription factors, is essential in control of cell growth, differentiation, and development. Its implication in heart and limb development has suggested a role in regulating cell movements and rearrangements. However, its precise roles are poorly understood. Here, we show in chick embryos that *Arid3b* is expressed within the pre-cardiovascular areas, including primitive streak cells, endocardial tubes and dorsal aorta. In order to gain insight into the role of this molecular factor, we have carried out a series of experiments based on gain- and loss-of-function through *in vitro* electroporation of wild-type and dominant-negative *cArid3b*, respectively. Our results reveal that Arid3b overexpression increases, while the dominant-negative represses, the endothelial-specific miR-126 expression. This "angiomiR" represses Spred-1 expression, a negative regulator of Ras/MAP kinase pathway, which is activated by angiogenic growth factors, such as VEGF (vascular endothelial growth factor) and FGF (fibroblast growth factor), responsible for endothelial cells proliferation, migration and adhesion. Therefore, Arid3b functions as a necessary endothelial-specific modulator in developmental angiogenesis.

This work has been partially financed with grant to research group CTS005 (to VGM) from the Junta de Extremadura, with FEDER co-financing.

06: ANALYSIS OF MEIS TRANSCRIPTION FACTORS IN CARDIOVASCULAR DEVELOPMENT AND HOMEOSTASIS.

N. Muñoz-Martín, L. Carramolino, M. González-Lázaro, A. López-Delgado, V. Cadenas and M. Torres.
Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

Approximately 1% of live births present congenital heart disease (CHD) and around 20% of these are related with outflow tract defects (OFT). The heart is the first functional organ during embryogenesis and its formation is tightly regulated. The main sources of progenitors that contribute to cardiac development are the first (FHF) and second heart field (SHF), the proepicardium and the cardiac neural crest. Interestingly the OFT specifically derives from SHF progenitors.
Transcription factors (TFs) are the principle regulators of cardiac development. Meis1 and Meis2 are TFs that belong to the TALE family homeodomain proteins. They are highly conserved among species, and during the development of various organs, their expression pattern is quite similar. Mice lacking Meis1 die at ED14.5-15.5 presenting haematopoietic, eye and heart defects. In addition, Meis1 function affects neonatal cardiomyocytes (CMs) proliferation and cardiac regeneration ability.
We have already determined the expression pattern of Meis in early development and performed lineage tracing to see the contribution of Meis1+ precursors to the heart. Our results indicate transient Meis1 expression in the FHF and persistent expression of both genes in SHF progenitors, which is downregulated in differentiating CMs in the cardiac tube. Since these observations suggest functional redundancy, we started the combined deletion of both genes. We observed cardiac malformations in SHF derivatives in all conditions studied, with increasing severity as more copies of Meis1 and Meis2 are eliminated. These data confirm a redundant role for Meis1 and 2 in SHF development.
We are also interested in the function of Meis in differentiated CMs. We are analysing the expression pattern of these TFs in the heart at different stages and, we are generating Meis1/2 loss of function models using alpha-MHC-Cre. Furthermore, we are generating an inducible loss of function model in the myocardium (Meis1/2$^{f/f}$;alpha-MHC-merCremer$^{+/-}$) to study whether the proliferative/regenerative capacity of neonatal CMs is modified after the deletion of both genes.
In summary, due to redundancy, the full description of Meis roles during cardiac development and homeostasis requires deletion of both genes, which we present here for the first time. We hope our results increase the knowledge regarding CHD origin and may improve understanding the mechanisms that govern cardiac regenerative ability.

07:ZIC2 IS A BIFUNCTIONAL REGULATOR OF _NODAL_ AT THE EMBRYONIC NODE REQUIRED FOR SPECIFICATION OF LEFT SIDED CARDIOGENIC MESODERM

Iain M. Dykes, Dorota Szumska, Linta Kuncheria, Rathi Puliyadi, Costis Papanayotou, Jurgen E. Schneider, Jérôme Collignon , Shoumo Bhattacharya

Left-sided expression of the NODAL-PITX2 pathway is critical for normal development of the heart and viscera. We show that mice lacking the transcription factor ZIC2 fail to acquire left-sided identity in the mesoderm, indicated by right pulmonary and atrial isomerism, dextropositioning of organs and vascular defects. This phenotype arises from a failure to express *Nodal* at the embryonic node, leading to consequent absence of *Nodal-Pitx2c* expression in the left lateral plate mesoderm. ZIC2 directly regulates *Nodal,* recognising a motif present in the HBE and PEE enhancers, and in *Lefty1* and *Shh*. These sites are associated with deacetylated histone in embryonic stem cells (ESCs), yet *in vitro* assays indicate ZIC2 can also mediate transcriptional activation of HBE from sites not bound in ESCs. These data suggest that an interaction of ZIC2 with additional factors determines the transcriptional outcome of binding in a context-dependent manner, and that a balance between activation and repression determines HBE activity. In support of this hypothesis we demonstrate that GLI1 and ZIC2 interact in a non-additive manner at HBE. We propose a model in which ZIC2 acts as a switch, co-operating with a signal downstream of nodal flow to activate left-sided *Nodal* expression at the node.

08: SOX17 expression in the endocardial precursor cells regulates the mouse heart tube development

Rie Saba[1], *Keiko Kitajima[2], Hidekazu Ishida[1], Ioannis Kokkinopoulos[1], Yoshiakira Kanai[3], Masami Azuma-Kanai[4], Peter Koopman[5], Chikara Meno[2], Ken Suzuki[1] and Kenta Yashiro[1]*
[1]Translational Medicine and Therapeutics, William Harvey Research Institute, Queen Mary University of London, UK, [2]Department of Developmental Biology, Graduate School of Medical Sciences, Kyushu University, Japan, [3]Center for Experimental Animal, Tokyo Medical and Dental University, Tokyo 113-8510, Japan, [4]Department of Veterinary Anatomy, The University of Tokyo, Japan, [5]Division of Molecular Genetics and Development, Institute for Molecular Bioscience, The University of Queensland, Australia

The endocardium is the singular endothelial component in the innermost layer of the heart. In the mouse embryogenesis, it appears as the endocardial layer at embryonic day E8.5, at the same time of the heart tube formation, and contributes to cardiogenesis by playing many crucial roles in the heart tube patterning and chamber, valve and coronary vessel formation. It is considered that the endocardial cell lineage is derived from *Nkx2-5*-positive cardiac progenitor cells (CPCs), however the molecular mechanism of its differentiation in the early phase remains to be elucidated. In this study, we show that *Sox17* is expressed by the 20-30% of *Nkx2-5*-positive CPCs in the mouse embryo from E7.5 to E8.5, and that these cells are committed to the endocardium. Gain of function study revealed that Sox17 is insufficient to convert the fate of CPCs but can bias it toward the endocardium. Mesodermal Sox17-depletion resulted in the defective heart tube looping and trabeculation. Single cell-microarray expression profiling of *Sox17*-null heart tube showed that misregulation of both endocardial and myocardial transcriptome had already been induced at E8.5. These results let us conclude that SOX17 expression in the endocardial precursor cells is essential to regulate the differentiation of the endocardium during the heart tube development.

09: Specific roles for miR-15, miR-199, miR-23, miR-130 and miR-106 during early developing chick angiogenesis

Carmen Lopez-Sanchez[1], Diego Franco[2], Fernando Bonet[2], Angel Dueñas[2], Amelia Aranega[2], Virginio Garcia-Lopez[3], Ana Ortiz[1], Virginio Garcia-Martinez[1]
[1]Human Anatomy and Embryology Faculty of Medicine University of Extremadura 06006 Badajoz Spain
[2]Cardiovascular Development Group Department of Experimental Biology University of Jaén CU Las Lagunillas B3-362 23071 Jaén Spain
[3]Pharmacy Services Minimally Invasive Surgery Centre Cáceres Spain

It is known that several microRNAs play essential roles in distinct and diverse biological contexts, such as early cardiovascular development. By means of ISH with microRNA-specific LNA-probes, we performed a detailed analysis of five microRNAs -miR-15, miR-199, miR-23, miR-130 and miR-106- which display increased expression levels during early cardiovascular development in chick embryos. Our results reveal their expression pattern from early

gastrula stage, first in the primitive streak and subsequently in the vasculogenic and cardiogenic areas. It is also known that several microRNAs represent crucial factors involved in MAPK/ERK signaling pathway, which has been demonstrated to be essential for initial cardiovascular differentiation. Additionally, by means of bioinformatic analysis through Target Scan prediction, we identified five putative microRNAs that target several 3´UTR links, sites broadly conserved among vertebrates, in this signaling pathway. Based on the above data, we focused on those molecular factors during early angiogenesis. Our proposed model establishes the presence of two differentiated groups of microRNAs: pro-angiogenic and anti-angiogenic, both linked to the various steps previously described in the MAPK/ERK signaling pathway, specifically through VEGF activation. Thus, miR-23 and miR-199 could act as pro-angiogenic factors by repressing their target protein SPROUTY2 (a RAF modulator) and SEMA6A (a VEGFR2 modulator), respectively. On the other hand, miR-15 (by repressing its target proteins VEGF and RAF), and both miR-130 and miR-106 (by repressing their target protein ERK) could modulate the pathway in a repressive manner. We are currently developing experimental strategies to test with working hypothesis.
This work has been financed with grants to research groups CTS005 (to VGM) from the Junta de Extremadura, with FEDER co-financing, and CVI-6556 (to DF) from the Junta de Andalucía Regional Council

10: THE ROLE OF FOLLISTATIN-LIKE 1 IN AV CONDUCTION

Andrea Mattiotti, Quinn D Gunst, Marc Sylva, Bas J Boukens, Jan M Ruijter, Maurice JB van den Hoff.
Department of Anatomy, Embryology and Physiology, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Follistatin-like 1 (Fstl1) is a secreted glycoprotein suggested to be involved in several signaling pathways, including sodium channel activity. Disruption of Fstl1 results in a variety of developmental defects, especially in connective tissue of multiple organs, including heart. Fstl1 full knockout mouse ($Fstl1^{KO/KO}$) die just after birth for collapse of the trachea.
In situ hybridization (ISH) analysis shows that Fstl1 is expressed in and surrounding the cardiac conduction system (CCS). In order to study the role of Fstl1 in development of the CCS, we create an endocardium Fstl1 conditional knockout mouse ($Fstl1^{cKO/KO\ ;\ Tie2\text{-}cre}$).
At neonatal day 1 (ND1), $Fstl1^{cKO/KO\ ;\ Tie2\text{-}cre}$ shows a significant increase in the PR and RR intervals compared with their littermate controls ($Fstl1^{cKO/WT\ ;\ Tie2\text{-}cre}$).
By ISH using probes against Hcn4, Cx40 and cTnI we evaluated the morphology and size of AV node (Hcn4+, Cx40-) and AV bundle (Hcn4+, Cx40+). No significant differences were observed. Next we assessed the cells forming the fibrous insulation (Col3a1+) and the deposit of extracellular matrix (Pico Sirius Red staining) surrounded the AV node and bundle. Again no significant differences were observed between Fstl1-endoKO and control.
These results indicate that anatomical defects do not underlie the effect of Fstl1 on the in delay the PR and RR intervals in CCS. Molecular analysis of gene expression of ion channels and their activity is indicated to unveil the role of endocardial Fstl1 on conduction in the CCS.

11: Unraveling the molecular and epigenetic mechanisms by which retinoic acid receptors regulate genes in the developing mouse heart

Sonia Stefanovic[1], Karen Niederreither[2], Muriel Rhinn[2], Vincent M Christoffels[3] and Stéphane Zaffran[1]*
[1] Genetics & Development of Cardiac Defects, Inserm UMR_S910, Timone Medical School, University of Aix Marseille, sonia.stefanovic@univ-amu.fr
[2] Institute of Genetics & Molecular & Cellular Biology, Cnrs UMR 7104, Inserm U964, Strasbourg
[3] Department of Anatomy, Embryology & Physiology, Academic Medical Center, University of Amsterdam

**Presenting author, recipient of a Marie-Curie Fellowship Award*
Defects in myocardial lineages of the venous pole of the heart can result in various congenital malformations such as misconnection of the pulmonary and caval veins, incomplete septation of atrial chambers and abnormal rhythms. Retinoic acid (RA) signalling is preferentially active in the venous pole of the embryonic heart. The formation of the venous pole depends on RA, the active derivative of vitamin A, by acting as a diffusible activator of nuclear receptors (RA receptors, RARs). The retinaldehyde dehydrogenase 2 (Raldh2) is responsible for RA synthesis during early development. Mutation of *Raldh2* results in embryonic lethality with venous pole patterning defects. In the presence of RA, RARs bind RA response elements and recruit HATs. In the absence of RA, RARs can actively

repress gene transcription by recruiting HDACs that promote chromatin compaction and gene repression. Whether this can be the case in the context of heart development is currently unknown. Though functional analysis of RARs in developing heart provided important insights, it has been very difficult to distinguish direct and indirect regulation of gene expression. Moreover, frequent redundancy in receptor functions precluded their complete functional characterization. Here, we propose to study in details how the RARs take part in the transcriptional network for the development of distinct myocardial lineages in the venous pole region. RA target candidate genes will be identified by RNA sequencing on total RNA extracted from FACS sorted myocytes deficient for *Raldh2* or exposed to RA in utero. Secondly, an inducible conditional knockout mouse line of *Raldh2* will be used to delete *Raldh2* in venous pole myocardial progenitors. To identify the direct target genes of RARs in the developing heart, we will determine the binding sites of RXR□ on a genome-wide scale by ChIP-seq from hearts of wild type embryos. This study proposes to map RA response elements, which will be compared with loci associated with susceptibility to heart diseases.

12: EPICARDIAL FAT THICKNESS IN METABOLIC SYNDROME AND EARLY VASCULAR DAMAGE

O. N. Korneeva, O.M. Drapkina, V. T. Ivashkin
I.M. Sechenov First Moscow State Medical University, Moscow, Russia

Background: Increased visceral adiposity is considered the hallmark of the metabolic syndrome (MS). Epicardial adipose tissue has been implicated in the pathogenesis of coronary atherosclerosis.
Aim: To evaluate in patients with MS the threshold values of echocardiographic epicardial fat thickness and their association with a) metabolic and clinical parameters, b) early atherosclerotic vascular damage, by carotid intima media thickness (IMT).
Methods: Epicardial fat thickness was evaluated by transthoracic echocardiogram in 41 consecutive patients with MS and in 20 controls without MS.
Results: Patients with MS had significantly higher epicardial fat thickness than controls (4.95±2.6 and 2.69±1.8 mm, p=0.01). Considering as increased values higher than 2.7 mm (median of controls) we evaluated variables associated with increased epicardial fat. Age, BMI, waist circumference, fasting glucose, HOMA-IR, IMT were significantly higher in subjects with increased epicardial fat than without, while the echocardiographic diastolic function index early/atrial peak flow was significantly lower. At multivariate analysis HOMA-IR remained the independent variable associated with epicardial fat (p=0.04, OR1.8, 95% CI1.037-3.58). **Conclusion:** In conclusion patients with MS had higher value of epicardial fat thickness than controls, the increased epicardial fat values were associated with insulin-resistant and with early vascular damage. Epicardial fat measurement, an easy diagnostic tool to define visceral and cardiac adiposity could be proposed to better predict the cardiovascular risk.

13: Conditional deletion of Wt1 in cardiomyocytes causes severe defects in the developing heart

Carmona, R., Cañete, A., Ariza. L. and Muñoz-Chápuli, R.
Department of Animal Biology, University of Málaga and Andalusian Center for Nanomedicine and Biotechnology (BIONAND), Málaga, Spain.

The Wilms' Tumor-1 gene (*Wt1*) encodes a zinc-finger transcription factor involved in the development of several organs and in tumorigenesis. In normal development, *Wt1* is transiently expressed in several mesodermal tissues, namely in mesothelial and submesothelial cells as well as in the septum transversum, epicardium and epicardial-derived cells. Wt1 knockout embryos show defective kidneys, gonads, spleen and adrenal glands and die at mid-gestation due to heart abnormalities, which include thin myocardial wall and vascular defects.
We have used a Wt1GFP knock-in line (Hosen et al., Leukemia 200, 7 21:1783-91), in which the exon 1 of one Wt1 allele has been replaced by the GFP sequence, as an independent, real-time reporter of the Wt1 expression. By Cre/LoxP technology, we have also generated two lines of transgenic mice: a lineage tracing system (mWt1/IRES/GFP-Cre; $ROSA^{YFP}$) and a conditional deletion of WT1 gene in cells expressing cardiac Troponin T (c-TnT^{Cre}; $Wt1^{LoxP/LoxP}$). The TnT^{Cre} promoter drives expression of Cre recombinase in the cardiomyocyte lineage beginning at E7.5 and its expression has been reported in all myocardium at E10.5 (Jiao et al., Genes Dev. 2003, 1;17:2362-7).
By lineage tracing, we have found a population of YFP+ cardiomyocytes at E8.5, before the formation of the epicardial layer. These cardiomyocytes are located in the common ventricle

and sinus venosus. *Wt1* promoter activation was confirmed by GFP expression in the Wt1GFP knock-in line.
The conditional deletion of Wt1 in TnT+ cells caused severe damage in the developing heart, particularly muscular defects in the interventricular septum and ventricular wall, as well as defective sinus venosus formation. These embryos did not survive after birth.
The results suggest an expression of the *Wt1* gene in a subpopulation of cardiomyocytes during early development of the heart. It is uncertain if this expression involves the production of functional Wt1 protein, but it seems essential for cardiac development.

14: Left juxtaposition of the atrial appendages: where are the pectinate muscles?
Houyel L (1), Laux D (1), Mostefa-Kara M (2), Gonzales M (3), Bessières B (2). (1) Centre Chirurgical Marie-Lannelongue-M3C, Le Plessis-Robinson, France. (2) Necker-M3C, Université Paris-Descartes, Paris, France. (3) Hôpital Trousseau, Université Pierre et Marie Curie, Paris, France.)

Left juxtaposition of the atrial appendages (L-JAA) is a rare anomaly in which the two atrial appendages are located to the left of the great arteries. Although several anatomic studies have been published, the internal architecture of the right atrium in L-JAA was not well described.
Material and methods: We reviewed 21 postnatal and 5 fetal human heart specimens with L-JAA from the anatomic collection of the French Reference Center for Complex Congenital Heart Defects. Special attention was paid on the extent of the pectinate muscles inside the right atrium. All heart specimens had normal atrial situs, concordant atrioventricular and discordant ventriculoarterial connections: 20 D-transposition, 6 L-transposition. None had heterotaxy. There were 11 transposition of the great arteries, 8 double outlet right ventricle, 3 double inlet left ventricle, 4 tricuspid atresia. The conus was always abnormal, bilateral in 22, subaortic in 4.
Results: Pectinate muscles were confined inside the right atrial appendage (RAA) in 20/26 hearts (77%, group 1) and spilled out it, although not extending to the crux, in 6 (group 2). In 9 hearts of group 1 and 4 of group 2, there was a small accessory RAA in normal position with pectinate muscles inside it (p=ns). The only significant difference between the 2 groups was the incidence of hypoplastic right ventricle (65% in group 1 vs 0 in group 2, $p<0.03$)
Conclusion: In 77% of L-JAA, the pectinate muscles do not extend to the crux and can be completely absent of the right atrial wall. This suggests that the pectinate muscles are constitutive of the morphologically right atrium only if the RAA is in normal position, and raises several questions concerning the development of the atria. In other terms, the extent of the pectinate muscles (to the crux of the heart or not) might be determined by the situation of the RAA relative to the great vessels, and thus might not be an intrinsic property of the morphologically right atrium. Normal atrial situs and absence of heterotaxy could indicate that outpouching of the atrial appendages from atrial walls might occur after the establishment of the left-right asymmetry, as assessed in one of the rare experimental models of L-JAA. Further experimental studies are warranted to elucidate this anatomic and embryologic enigma.

15: Effect of Hypoxia on Gene Expression in the Chick Embryonic Heart
Naňka O. (1), Krejčí E. (1), Barth JL. (3), Sedmera D.(1, 2)
(1) Institute of Anatomy, Charles University First Faculty of Medicine, Prague, Czech Republic
(2) Institute of Physiology, Academy of Sciences of the Czech Republic, Czech Republic
(3) Regenerative Medicine and Cell Biology, Medical University of South Carolina

Our previous results demonstrate that normal embryonic development involves physiological tissue hypoxia. We found differences in myocardial architecture in hypoxic avian embryos. There was also a difference in the extent and distribution of hypoxic regions with increased HIF1alpha.
Now we used the microarray analysis for detection of differential gene expression under normoxic and hypoxic conditions. Chicken eggs were incubated at 38□C under normoxic (21% O_2) conditions for the first 48 hrs and subsequently under hypoxic (16% O_2) conditions. Sampling was performed at three different time points - acute hypoxia (6 and 24 hours) and chronic hypoxia (144 hours)). Controls were incubated in normoxia. RNA was extracted from isolated control and hypoxic chick embryonic hearts. Affymetrix Chicken Genome array analysis revealed group of transcripts that were differentially expressed between the normoxic and short term and long term hypoxic group. Among the up-regulated genes, some are involved in the gene ontology networks such as cell growth, cell differentiation, muscle contraction and signal transduction. We focused on hypoxia-

dependent genes that are involved in adaptation to hypoxia. We obtained a subset of genes with statistically different expression between control and hypoxic group. qPCR analysis confirmed upregulation VEGFA and HIF1A in hearts after 24 hours incubation under hypoxic condition. Increased expression of these genes was not observed in hearts, where the embryos were exposed to chronic hypoxia.
The interpretation of data is difficult, because some important hypoxia-regulated genes such as HIF1alpha are regulated at the protein, rather than the mRNA level.
Supported by Charles University PRVOUK 1105-280002 and GACR P302/11/1308.

16: Clonal analysis unravels the Second Heart Field as a source of epicardial-like cells

Lioux G., Temiño S., Torres M.
Cardiovascular Developmental Biology and Repair Department, CNIC, Melchor Fernández Almagro, 3 (28029) Madrid, Spain.

Mammalian heart function relies on a complex arrangement of cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells, endothelial cells and others. Cardiac cell diversity is the result of lineage establishment through specification of cardiac progenitors. So far early sources of heart lineages such as the cardiogenic mesoderm, proepicardium and cardiac neural crest cells, were identified. However the exact fate of heart progenitors is still unclear as conflicting reports on lineage contributions remain in the field.
In the laboratory, we address the question of heart lineage segregation using random retrospective clonal analysis. We are targeting heart precursors around Embryonic day 9 (E9), when the primary heart tube has been allocated and the second heart field precursors are contributing to complete heart development. Results are analyzed at E14.5, when the organization plan of the heart is established and the different heart lineages are advanced in differentiation. Heart progenitors are labelled using a ubiquitous low-level expression Tamoxifen-inducible Cre recombinase mouse line (RERT). Moreover a double-reporter strategy, combining simultaneously Rosa26R:LacZ and Rosa26R:EYFP reporters, allows us to assess the clonality of the lineage labels induced since clonally related cells are identically labelled.
Having isolated and stained more than seven hundred hearts for the analysis, we were able to point out the existence of a common progenitor for arterial epicardium-like mesothelium, smooth muscle and endothelial cells of the great arteries. Complementary lineage tracing experiments using tissue-specific cre mouse lines allowed us to reconstruct the origin and fate of such clones. We show that Second Heart Field (SHF) progenitors give rise to OFT epicardial-like cells which are themselves generating smooth muscle and endothelial cells in the Aorta and Pulmonary artery. We thus show for the first time that despite their different origin SHF-derived epicardial cells, like proepicardial-derived epicardium, are multipotent and consequently important in the formation of cardiac great arteries. We are now aiming at deciphering to which extent those cells contribute in forming the OFT and will be investigating if their function is altered in mouse mutants displaying a truncated OFT. As a result this study could be of importance at unraveling the cellular origin of specific congenital heart defects.

17: A large permissive regulatory domain controls *Tbx3* expression in the cardiac conduction system

Henk van Weerd[1], Ileana Badi[1], Malou van den Boogaard[1], Harmen J G van de Werken[2], Wouter de Laat[2], Phil Barnett[1], Vincent M Christoffels[1]
[1]Department of Anatomy, Embryology & Physiology, Academic Medical Center, Amsterdam, The Netherlands
[2]Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, The Netherlands

The evolutionary conserved *Tbx3*/*Tbx5* gene cluster encodes T-box transcription factors that play crucial roles in the development and function of the cardiac conduction system (CCS). *Tbx3* and *Tbx5* are expressed in overlapping patterns and function in strictly tissue-specific and dose-dependent manners. Genetic variants have been identified in the gene desert flanking the cluster that influence conduction system function in humans, illustrating the importance of understanding the complex transcriptional regulation of expression of both genes.
We have generated a high-resolution model of the three-dimensional architecture of the *Tbx3/Tbx5* locus using 4C-seq and found that its regulatory landscape is in a preformed, permissive conformation that is similar in embryonic heart, brain and limbs. *Tbx3* and its flanking non-coding region form a 1 Mbp loop that is physically separated from neighbouring

Tbx5 and *Med13l* loops and harbours multiple regulatory sequences that synergize to drive CCS-specific *Tbx3* expression. Using STARR-seq, combined with *in vitro* and *in vivo* enhancer assays, we identified novel active regulatory sequences within both the *TBX3* and *TBX5* domains, and we assessed genome-wide chromatin accessibility in embryonic hearts and HL1 cells using ATAC-seq to further characterize the transcriptional regulation of the locus. Furthermore, using TALEN-mediated genome editing, we deleted a 70 kbp distal region that strongly contacts *Tbx3* and encompasses the mouse orthologue of a region that in humans is associated with PR interval and QRS duration. Current analysis of the effect of this deletion will add to our understanding of the mechanisms underlying the complex regulation of the *Tbx3/Tbx5* locus and reveal how common variants affect the regulation and function of Tbx3 in the CCS.

18: Research of genetic variants underlying Bicuspid Aortic Valve by whole exome sequencing.

Amélie Pinard, Alexis Théron, Gaëlle Odelin, Emilie Faure, Jean-Pierre Desvignes, Anissa Touil, Christophe Béroud, Jean-François Avierinos, Gwenaëlle Collod-Béroud, Stéphane Zaffran
Aix Marseille Université, Inserm, GMGF UMR_S 910, 13385, Marseille, France

Bicuspid aortic valve (BAV) is the most common cardiovascular malformation (0.6-2% of the population) and is associated with premature aortic valve stenosis or insufficiency, aortic aneurysm and other congenital cardiac anomalies. Several evidences support a genetic basis for BAV, whose pathogenesis involves a complex interplay between specific gene mutations, environmental influences and stochastic factors. Although highly heritable, few causal mutations have been identified in BAV patients.
The purpose of this study was to identify new variants responsible for BAV using next generation sequencing. We recruited a cohort of 200 subjects with echocardiogram- and subsequent surgery-identified BAV. Subjects were identified as having isolated BAV or BAV associated with aortic dystrophy. Twenty DNA samples were evaluated by whole exome sequencing with the Illumina HiSeq 2000 system. Among them, 11 belong to individuals from 3 families (8 patients and 3 controls) and 9 were from isolated proband with isolated BAV. Multistep bioinformatics processing was used including an in-house pipeline for alignment and variants identification. The selection of candidate causal mutations was performed thanks to predictions from UMD-Predictor (http://umd-predictor.eu) and Human Splicing Finder v3.0 (http://www.umd.be/HSF3/). The putative disease-causing variants were then confirmed using Sanger sequencing. A first set of candidate genes was analyzed in the replication cohort to identify additional pathogenic variants. In parallel, the spatiotemporal expression of these genes was studied by RNA-sequencing of human aortic valve at different developmental stages. Further investigations using molecular and cellular experiments are ongoing to validate the implication of these candidate genes. These findings will be discussed.

19: A novel E3 ubiquitin ligase required for early cardiac morphogenesis

Arnaud Métais[1,2], Armelle Melet[1,2,3], Sandrine Uttenweiler-Joseph[1,2], Pierre G. Lutz[1,2] and Christel Moog-Lutz[1,2]
[1] CNRS; IPBS (Institut de Pharmacologie et de Biologie Structurale); 205 route de Narbonne BP 64182, F-31077 Toulouse, France
[2] Université de Toulouse; UPS; IPBS; F-31077 Toulouse, France
[3] Université Paris Descartes; 12 rue de l'Ecole de Médecine ; F-75270 Paris Cedex 06, France

Ubiquitin-mediated protein degradation comprises the major proteolytic pathway in Eukaryotes and ensures that specific protein functions are turned off at the right time and in the right place through the selective targeting of proteins to proteasome. In this pathway, E3 ubiquitin ligases are key players because they interact with the selected protein and provide specificity to the system. ASB proteins identified as containing a SOCS box domain (Ankyrin repeat-containing protein with a Suppressor of Cytokine Signaling box) were thought to act as substrate-recognition modules of E3 ubiquitin ligase complexes. Despite counting 18 members, the identity of the physiological targets of the ASB proteins remains largely unexplored.
Our group demonstrated that both isoforms encoded by the *ASB2* gene, ASB2α and ASB2β, are the specificity subunits of E3 ubiquitin ligase complexes and that ASB2 proteins trigger polyubiquitylation and drive proteasome-mediated degradation of the actin-binding proteins, filamins. ASB2α is mainly expressed in hematopoietic cells and ASB2β in heart and skeletal muscles of human adults. Using several cellular model systems, we demonstrated that ASB2

proteins, through induced proteasomal degradation of filamins, can regulate integrin-dependent functions such as cell spreading, cell adhesion and cell migration.
During development, *ASB2* gene is expressed first in the heart and later in the myotome both in mouse and chicken. To investigate the *in vivo* function of *ASB2* gene, we analyzed the impact of the total *ASB2* knock-out in mice. $ASB2^{+/-}$ offspring were viable, fertile and appeared normal. However, no viable $ASB2^{-/-}$ offspring were obtained in litters from $ASB2^{+/-}$ intercrosses, indicating that the mutation was embryonic lethal. We showed that this lethality results from major cardiovascular defects at 9.5 dpc. Our results indicate that the *ASB2* gene is critical for cardiac development and provide the first evidence that proteasomal degradation controls key steps of early heart morphogenesis.

20: Investigation of Myomesin2 - a potential candidate gene for congenital heart defects

Karin de Linde Troelsen Pia Rengtved Lundegaard Maren Mönnich Lars Allan Larsen
University of Copenhagen, Copenhagen, Danmark

Congenital heart defects (CHD) are the most common congenital malformations in newborns and are a major cause of infant morbidity and mortality. Despite many genetic studies, much of the genetic mechanisms behind normal and abnormal heart development remain to be elucidated.
The Myomesin 2 gene (*MYOM2*) encodes a protein located in the M-band of the sarcomere in cardiac and skeletal muscle. The assembly of the sarcomere is of tremendous importance for a fully functional skeletal and cardiac muscle. Mutations in sarcomeric proteins *ACTC1*, *MYH6* and *MYH7* have previously been associated with CHD and rare *MYOM2* variants have been reported in CHD patients. This study focuses on the role of MYOM2 during heart development. The zebrafish genome encodes a *myom2* gene with a 55% homology to the human MYOM2 gene. Therefore the zebrafish was used to model the expression of *myom2* during development and to explore the role of gene knockdown in live developing zebrafish hearts.
In situ hybridization showed that *myom2* is expressed specifically in heart and skeletal muscle during zebrafish embryonic development. Two approaches to knock down the gene in zebrafish were used. First, zebrafish carrying a nonsense mutation in the *myom2* gene were obtained from the Zebrafish Mutation Project (Sanger Institute, UK). No obvious cardiac or muscle phenotype was present in homozygous mutants, suggesting that mutants compensate for the loss of Myom2 function. Whereas the development of zebrafish embryos injected with morpholinos targeting *myom2* was delayed and a range of developmental defects were observed. The preliminary data indicates a possible role of Myom2 during heart development, but further experiments are needed.

21: Comparison of different tissue clearing methods and 3D imaging techniques in connexin40:GFP-expressing mouse embryonic hearts and whole embryos

Hana Kolesová [a], Martin Čapek [b], Jiří Janáček [b], David Sedmera [a, b]
[a] Institute of Anatomy, First Faculty of Medicine, Charles University in Prague, Czech Republic
[b] Institute of Physiology, The Czech Academy of Sciences, Prague, Czech Republic

Aims. To find a suitable tissue clearing protocol for whole mount imaging of embryonic and adult hearts and whole embryos of transgenic mice preserving GFP fluorescence and to compare different imaging modalities.
Methods. We tested various published organic solvent- or water-based clearing protocols claiming to preserve GFP fluorescence in central nervous system: tetrahydrofurane dehydration and dibenzyl ether protocol (DBE), SCALE, CLARITY, and CUBIC and evaluated their ability to render transparent hearts and whole embryo. Tissue transparency and preservation of GFP fluorescence was evaluated in hearts of Cx40:GFP knock in mice at stages: ED10.5 – 18.5 ED and adults. Imaging was performed on a dissecting microscope equipped with epifluorescence and an upright single photon confocal microscope (Olympus) with 4x, 10x, and 25x objectives after mounting the specimens into cavity slides or custom imaging chambers. For comparison of different imaging modalities we used optical projection tomography (OPT). 3D renderings were performed and evaluated using ImageJ.
Results. Despite careful control of all critical parameters, DBE clearing protocol did not preserve GFP fluorescence; in addition, it caused considerable tissue shrinking and worse deformation than the golden standard BABB protocol used for whole mount immunohistochemistry. The CLARITY method considerably improved tissue transparency at later stages, but also decreased GFP fluorescence intensity. The SCALE clearing resulted in good tissue transparency up to ED12.5; at later stages and in the adults the useful depth of

imaging was limited by tissue light scattering. The best method for the hearth proved tissue clearing using the CUBIC protocol, which very well preserved GFP fluorescence, and furthermore cleared nicely the specimens even at the adult stages. In addition, it decolorized the blood and myocardium by removing iron from the tissues. High-resolution images were thus obtained even from deep tissue layers with ScaleView immersion 25x objective. Good 3D renderings of whole fetal hearts and embryos were obtained with OPT, although at resolution lower than with a confocal microscope.
Conclusions. From the tested methods, the CUBIC protocol turned out to be the best for whole mount GFP cardiac samples. Scale technique is also suitable for younger embryos. Single photon confocal microscopy is complementary with the OPT. These protocols will be used for three dimensional reconstructions of normal and abnormal specimens to visualize the developing cardiac conduction system and coronary vasculature. **Acknowledgements.** Supported by 13-12412S from the Czech Science Foundation, Ministry of Education PRVOUK P35/LF1/5, institutional support RVO:67985823, and Charles University UNCE.

22: RNA-tomography provides an unbiased way to identify the spatial regulation of genes during heart regeneration

Fabian Kruse[1]; Jan Philipp Junker[1]; Emily Noël[1]; Alexander van Oudenaarden[1]; Jeroen Bakkers[1]
[1] Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, The Netherlands

In contrast to adult mammals, zebrafish can completely regenerate heart injuries by reactivating developmental gene programs and inducing proliferation in differentiated cardiomyocytes (CMs). However, the signaling pathways and molecular mechanisms underlying these processes remain largely unknown. We recently developed RNA tomography, a technique to obtain whole-genome transcriptomics with spatial information (Junker et al., 2014, Cell), and applied this to the regenerating heart.
Here, we show that we are able to obtain genome-wide transcriptome information, combined with high spatial resolution of the transition from injured area to border zone to healthy myocardium of the injured zebrafish heart. We identify over 1000 genes that show a specific expression peak in one of the three zones, 10% of which are still uncharacterized. Moreover, we characterize a novel zone in the regenerating zebrafish heart, which is located between the borderzone of the regenerating myocardium and the injury area. We are in the process of further defining this zone and the cell types that it is comprised of. We hypothesize that this novel zone is an important signaling source for the dedifferentiating and proliferating cardiomyocytes in the borderzone.
Altogether, we show that RNA tomography can be used to study whole-transcriptome changes with spatial resolution in the regenerating zebrafish heart. This dataset provides a valuable resource to identify novel molecules and pathways involved in the regenerative process and we show the characterization of a previously unknown zone during heart regeneration.
This work was supported by the Netherlands Heart Foundation grant CVON2011-12.

23: Fetal human epicardial-derived cells exhibit an enhanced activation state compared to their adult counterparts

Asja T. Moerkamp, Anna M. D. Végh, Fredrik Tengström, Tessa van Herwaarden, Kirsten Lodder, Sjoerd N. Duim, Marie-José T. H. Goumans & Anke M. Smits

During cardiac development, the epicardium actively contributes to the formation of several cellular components of the myocardium and valves. In the adult heart the epicardium is a quiescent layer enveloping the heart. Interestingly, the epicardium is reactivated upon cardiac injury, resulting in thickening of this layer, (re)expression of Wilms' Tumor-1 (WT1), proliferation and possibly migration of adult epicardial-derived cells (EPDCs) into the myocardium. Given their function during embryonic development, adult EPDCs may represent an interesting source for endogenous cardiac repair following injury.
One of the key processes during activation of the epicardium is epithelial-to-mesenchymal transition (EMT). Whether EMT occurs in a similar fashion in a developmental or adult setting is unknown. To investigate this, we have established an isolation and culture protocol for EPDCs derived from both human adult and fetal cardiac specimens. In culture these cells maintain their cobble-stone like morphology and exhibit an epicardial specific gene and protein expression profile. Upon TGF-beta stimulation, both fetal and adult EPDCs undergo a morphological change to a mesenchymal, spindle-like shape. The epithelial markers WT-1 and E-cadherin are downregulated while the mesenchymal genes alpha-SMA, F-actin and Vimentin are upregulated indicating EMT. Interestingly, we observed that in contrast to adult

cells, fetal EPDCs readily undergo spontaneous EMT. Additional data suggests that fetal EPDCs are in a more advanced activation state, which may reflect their more active role in development.
We are currently focusing on specific differences between fetal and adult EPDCs and are identifying targets which may enable us to change the adult EPDC response to a developmental one which may ultimately increase their response post-injury.

24: The role of trabeculae in conduction through the early embryonic mouse heart

David Sedmera [a,b], Barbora Sankova [a,b], Peter Uriel Hamor [a]
[a] Institute of Anatomy, First Faculty of Medicine, Charles University in Prague, Czech Republic
[b] Institute of Physiology, The Czech Academy of Sciences, Prague, Czech Republic

Background. Trabeculae are the first morphological marker of differentiation of the ventricular chambers from the primitive heart tube. In the chick embryo was shown that they play a role in preferential propagation of the electrical impulse in the ventricles.
Aims. To assess the role of trabeculae in the early ventricular activation sequence in the mammalian heart.
Methods. Two transgenic mouse models of deficient trabeculation (ErbB2 null: rudimentary trabeculation, Nkx2.5 null: no trabeculation) were used to study conduction of electrical impulse in the embryonic ventricles. Optical mapping using di-4-ANEPPS complemented by morphological examination (histology, immunohistochemistry, whole mount confocal microscopy) was used on ED9.5 (Nkx2.5 line) or 9.75 (ErbB2 line) wild type, heterozygous, and null (lethal by ED10) embryonic hearts.
Results. In both lines, the recovery of viable hearts was lower than the expected Mendelian ratio, indicating negative selection against homozygotes. In both cases, the ventricles with deficient trabeculation showed activation patterns reminiscent of activation sequence characteristic for the primitive tubular heart, i.e., absence of apical epicardial breakthrough or primary ring activation. The speed of propagation was notably slower and the ventricular activation times prolonged compared with the wild type or heterozygous littermates, especially in the Nkx2.5 nulls with a complete lack of trabeculae. The rudimentary trabeculae in less dysmorphic ErbB2 mutants showed a low, but detectable expression of connexin40.
Conclusions. This is the first direct evidence that the early trabeculae play an important role in electrical activation of the embryonic mammalian ventricle.
Acknowledgements. We are grateful to Drs. Karin Birchmeier and Tim Harvey for providing the ErbB2 and Nkx2.5 lines, respectively. Supported by 13-12412S from the Czech Science Foundation, Ministry of Education PRVOUK P35/LF1/5, and institutional RVO:67985823.

***25: miR-200b* plays a role modulating cardiac lineage cell specification**

José Manuel Vilches, Francisco Hernández-Torres, Estefanía Lozano-Velasco, Diego Franco, Amelia Aránega.
Cardiovascular Development Group, Department of Experimental Biology, University of Jaén, Jaén, Spain

Stem cells represent a potentially attractive source of cardiac cells for the treatment of cardiovascular diseases. The use of pluripotent stem cells may enable the generation of large quantities of specialised cells that can be used as *in vitro* tools for drug development as well as for future applications in regenerative medicine. However, most of the currently used differentiation protocols yield inefficient quantities of differentiated cells and low purity of the final cell preparations. The discovery of *miRNAs* and their roles as important post-transcriptional regulators may provide a new means of manipulating stem cell fate. Here we shown that *miR-200b*, which has been previously showed to be significantly up-regulated during cardiomyocyte differentiation, modulates the contractile phenotype during *in vitro* cardiac differentiation from embryonic stem cells (ESCs). Interestingly, *miR-200b* regulates the expression of cardiac genes that define the electrophysiological properties of cardiomyocytes, such as the ion channels genes *Scn5a* and *Scn3b* (coding for alpha and beta subunits of the cardiac sodium channel Nav1.5) and *Cx45* (connexin that form low conductance channel). miR-200b also modulates the expression levels of the transcription factor *Tbx5,* a cardiac determinant gene, recently linked to atrial arrhythmias. In this sense, LNA *in situ* hybridisation analysis revealed that *miR-200b* is expressed in a subset cell population in the embryonic and fetal heart. We are currently characterizing the *miR-200b*+ cardiac cell population by co-localization with specific cell-markers. Collectively, these data showed that *miR-200b* could modify the cell fate of the cardiac lineage in ESCs. Additional experiments and further analyses could leads us to purpose this *miRNA* as a key molecule regulating cardiac cell type specification.

26: Krox20 loss-of-function leads to bicuspid aortic valve

Gaëlle Odelin[1,2], Emilie Faure[1,2], Alexis Théron[1,2,3], Fanny Coulpier[4,5], Jean-François Avierinos[1,2,3], Patrick Charnay[4,5], Piotr Topilko[4,5] and Stéphane Zaffran[1,2]
[1]Aix Marseille Université, GMGF UMR_S910, Faculté de Médecine, 27 Bd Jean Moulin 13385, Marseille, France.
[2]Inserm, GMGF U910, Faculté de Médecine, 27 Bd Jean Moulin, 13005, Marseille, France.
[3]AP-HM, Hôpital de la Timone, Département de Cardiologie, 13005, Marseille, France.
[6]Inserm, U1024, IBENS, École Normale Supérieure, 46 rue d'Ulm, 75005, Paris, France.
[4]CNRS, UMR8197, IBENS, École Normale Supérieure, 46 rue d'Ulm75005, Paris, France.
[5]Aix Marseille Université, CERIMED, Faculté de Médecine, 27 Bd Jean Moulin 13005, Marseille, France.

Bicuspid aortic valve (BAV), which occurs when the aortic valve has two cusps rather than three, represents the most common form of congenital heart defects, affecting 1-2% of the population. Despite the clinical significance of this pathology, its etiology is poorly understood. During embryonic development, the aortic valve arises from endocardial cushions that form in the outflow tract region. Genetic lineage tracing analyses have identified that several cell type contribute to the formation of the aortic valve including endothelial and neural crest cells. Recently we have shown that the zinc finger transcription factor, Krox20, is an important regulator of aortic valve development. Indeed, Krox20 deficient mice have aortic valve regurgitation associated with a disorganization of the extracellular matrix. We now report that *Krox20*-/- mice have also BAV. Targeting deletion of Krox20 suggests that its expression in neural crest cells migrating from the rhombomeres 6 toward the 3rd branchial arch is required for normal aortic valve formation. Interestingly, targeted deletion of Krox20 in the neural crest cell lineage leads to BAV with aortic dysfunction. Thus, *Krox20* appears to be a candidate gene for human aortic valve disease such as BAV.

27: The Early Role of Rho Kinase (ROCK) in the Development of the Ventricular Wall and Cardiomyopathy

Bailey KE, Blain A, Tual-Chalot S, Mohun T, Bamforth SD, Henderson DJ, Phillips HM.

Congenital heart defects are extremely common, affecting over 1% of live births, while adult heart disease is the main cause of death in the UK. Defects acquired during fetal development can have a lasting detrimental effect on adult heart function. Therefore understanding the underlying mechanisms involved in cardiac development and disease progression are of particular importance. Rho Kinase, ROCK, is a protein which is expressed in the heart during development and has many cellular functions including cell polarity, proliferation, apoptosis, migration and cytoskeletal arrangement. ROCK is required for heart development to occur normally, however, the exact function of ROCK within the cardiomyocytes, the muscle cells of the heart, remains unknown.
Transgenic mouse models using Cre-LoxP technology have been utilised to downregulate ROCK specifically in the ventricle of the heart. Downregulating ROCK in the outer layer of the heart, the epicardium, and the muscle layer of the heart, the myocardium, results in the development of a number of heart defects including an abnormally thin myocardium, as well as disruption in the arrangement of the cardiomyocytes. Interestingly these mice survive into adulthood allowing us to investigate the effect of downregulating ROCK during development has on the adult heart. Histological analysis shows that these hearts develop hypertrophy and fibrosis. Also MRI studies have shown that the function of these hearts is reduced due to increased thickening and rigidity of the wall of the heart; characteristics associated with the development of cardiovascular disease. This model will help generate cellular mechanisms underlying the development of cardiovascular disease.
Keywords: Rho Kinase, heart development, myocardium, hypertrophic cardiomyopathy

28: Criocauterization of the chick embryonic ventricular wall induces the formation of arterio-ventricular connections

Paul Palmquist-Gomes, Juan Antonio Guadix, José M. Pérez-Pomares
University of Malaga, Malaga, Spain

Tissue ablation is a classic experimental approach to study normal and abnormal embryo development and patterning. Some of these methods are an alternative to the local removal of cells and tissue by surgical excision. Accordingly, cauterization procedures using heat or ice-cooled metallic probes to 'burn' adult tissues have been extensively reported in the literature[1-2], but no data are available on their application to embryonic tissues. In this

study, we have developed a method to cauterize chick embryonic tissue *in ovo* [3]. Our results indicate this method is suitable to the study of organ-specific tissue responses to damage. Specifically, we report that cryocauterization of the embryonic ventricular wall induces the local discontinuity of the myocardium, disruption of coronary blood vessel development and the formation of characteristic transmural arterio-ventricular communications (fistulae) which are reminiscent of the homonymous congenital heart disease described in humans[4]. We suggest this is a unique method to study the pathogenesis and the cellular and molecular mechanisms underlying certain types of congenital coronary artery diseases.

1- Chablais F, Veit J, Rainer G, Jaźwińska A. The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. Dev Biol. 2011; 11:21.
2- Taylor AJ, Chan W. Post-Infarction LV Remodeling: Remote Changes Do Not Necessarily Occur Remotely From Time of Infarction. JACC Cardiovasc Imaging. 2015; 8(7):790-2.
3- Palmquist-Gomes P, Guadix JA, Pérez-Pomares JM. A chick embryo cryoinjury model for the study of cell differentiation tissue growth and organ repair. Differentiation. *In press.*
4- Mangukia CV. Coronary artery fistula. Ann Thorac Surg. 2012; 93(6):2084-92.

29: A novel role for NFATC1 in Heart, Eye, and Skin diseases

Hadla Hariri, Theresa Farhat, Akl Fahed, Mazen Kurban, Christiane Haddad, Sarin Poladian, Zakaria Kambris, Fadi Bitar
American University of Beirut, Beirut, Lebanon.

Valvulogenesis is among the most intriguing event during cardiac development. Complex signaling hierarchies of transcription and growth factors orchestrate such process. We have previously shown that two compound novel missense mutations (P66L, I701L) in NFATC1 are linked to tricuspid atresia in a Lebanese patient.
We extended our screening for mutations in NFATC1 to all familial cases of Congenital Heart Disease (CHD) at the American University of Beirut Medical Center. Out of the 20 families with two affected children and more, we found a previously documented polymorphism (rs62096875) leading to a missense mutation (V210M) in one family. The two affected children (twins) and their father were heterozygous for this variant while the healthy mother and sibling do not and the all three have an Epstein-like phenotype in the heart, and suffered from congenital Glaucoma. This variant was neither detected in any of the 200 patients with CHD registered in our center, neither in 100 healthy controls. We thus generated the V210M mutant plasmid and assessed the expression and function of the protein in vitro. Our results unravel a novel pathway implicating an interaction with Tbx5, probably responsible for the underlying phenotype both in the eye and the heart by showing a physical and functional interaction between NFATC1 and Tbx5 over the VEGF promoter. The V210M variant shows a gain of function that would account for the underlying defects in both organs. Moreover, we unravel a novel phenotype in the three affected individuals from analyzing skin biopsies, which showed a complete lack of hair follicles. We believe that this phenotype is also associated to the V210M variant based on the phenotype observed in patients treated with cyclosporine A, which inhibits NFATC1 resulting in aberrant growth of hair follicles.
Our results strongly suggest that NFATC1 plays a major role in congenital heart, eye, and skin defects. We hypothesize according to our result that the vascular endothelial growth factor (VEGF) is the main downstream target responsible for the observed phenotypes.

30: A novel dual reporter line for analysis of cardiomyocyte subtype specification

Robert Leigh, Heikki Ruskoaho, Bogac Kaynak
University of Helsinki, Helsinki, Finland

Proper formation and function of the heart requires subtype-specific gene expression in cardiomyocytes of the developing atria and ventricles. To study the specification and maturation of cardiomyocyte subtypes in vitro, we have generated a fluorescent dual reporter embryonic stem cell line which marks early ventricular and atrial cardiomyocytes. Addition of all-trans retinoic acid to differentiation cultures results in a reduced yield and purity of cardiomyocytes in a time- and dose-dependent fashion, as well as a reduction in ventricular cardiomyocytes and activation of an atrial-specific transgene. This dual reporter line will be used to study the molecular mechanisms behind specification of cardiomyocyte subtypes as well as the optimization of subtype-specific protocols with implications for regenerative medicine and drug screening.

31: Generation and purification of human stem cell-derived cardiomyocytes

Verena Schwach[1] and Robert Passier[1,2]
[1]Dept. Anatomy and Embryology, Leiden University Medical Center, Einthovenweg 20, P.O. box 9600, Leiden,and [2]Department of Applied Stem cell Technologies, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, P.O. Box 217, 7500 AE Enschede, the Netherlands.

Efficient and reproducible generation and purification of human stem cell-derived cardiomyocytes (CMs) is of fundamental importance for regenerative medicine, drug discovery and safety pharmacology, as well as for studying early cardiogenesis and disease.
In recent years, several methods to generate CMs from human pluripotent stem cells (hPSC) have been described and optimized leading to improved efficiencies and higher yields of cardiomyocytes. Widely accepted methods include spin-embryoid body (spin-EB) and monolayer-based differentiations. However, the majority of differentiation protocols result in heterogeneous populations which includes non-CMs.
For purification of CMs from mixed populations, fluorescent activated cell sorting (FACS) is a commonly used method and allows selection of fluorescent single cells, labelled via either genetic manipulation or cell-specific antibodies. However, FACS-based purification of CMs comes along with several disadvantages, such as undesired contaminations and low viability of target cells.
Here, we describe two rapid procedures for the robust enrichment of hPSC-derived CMs under sterile culture conditions, using VCAM1-coupled magnetic Dynabeads. As assessed by immunostaining, quantitative PCR or FACS, both purification methods resulted in vastly viable populations of cardiac cells with high purity. Selection of CMs will especially be important for cardiac differentiations of cell lines with poor differentiation efficiencies, though they will also substantially enhance reproducibility and standardization of cardiomyocyte assays in general.

32: Fetal noncompaction cardiomyopathy is aberrant compact wall maturation rather than retention of the embryonic state

Bjarke Jensen[1], Vincent Christoffels[1], Antoon FM Moorman[1], Allard van der Wal[2]
[1]Department of Anatomy, Embryology & Physiology, Academic Medical Center, University of Amsterdam, the Netherlands.
[4]Department of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands.

Introduction: Ventricular noncompaction or hypertrabeculation is a cardiomyopathy characterized by an excessive amount of trabeculations compared to compact wall. Hypertrabeculation is commonly believed to be the retention of the spongy ventricular design of the embryo. If true, trabeculations in left ventricular hypertrabeculation should resemble those in the embryonic ventricle, being approximately 50µm wide, without coronary circulation, and molecularly distinct from the compact wall by expression of ANF and little expression of structural proteins.
Materials and methods: We performed immunohistochemistry on ventricular sections of 5 cases of fetal hypertrabeculation (two sets of twins, and a fifth unrelated fetus with Ebstein's anomaly), 1case of fetal hypertrophy, and 2 healthy fetal hearts.
Results: All cases of hypertrabeculation had an excessively thick layer of trabeculation compared to the compact wall. The trabeculations were up to orders of magnitude wider than in the embryo. There was substantial coronary supply. ANF was strongly expressed in a very small sub-endocardial part of the trabeculated myocardium. The ANF rich myocardium had less troponin I than the trabeculations generally and the compact wall.
Conclusion: The trabeculations in the setting of fetal hypertrabeculation were different from embryonic trabeculations. Embryonic trabeculations give rise to the Purkinje system which continues to express ANF and the myocardium rich in ANF in the hypertrabeculated ventricles had the localization and molecular phenotype of the Purkinje system. To the best of our knowledge, we provide the first molecular characterization of ventricular hypertrabeculation in human tissues. Our data are consistent with hypertrabeculation as the result of aberrant compact wall maturation, as has been implied by mouse models, but not as the retention of the embryonic ventricular design.

33: The evolution of cardiac conduction system in the crocodilian heart

Alena Kvasilova[1], Martina Gregorovicova[2], Bjarke Jensen[3], Vincent Christoffels[3], David Sedmera[1, 2]

[1] Institute of Anatomy, First Faculty of Medicine, Charles University, Czech Republic

[2] Institute of Physiology, The Czech Academy of Sciences

[3] Anatomy, Embryology & Physiology, AMC, The Netherlands

The crocodilians have a completely septated heart, similar to birds and mammals, thus occupying a unique position among reptiles. In avian and mammalian development, the functional and morphological emergence of the atrioventricular bundle and its branches of the cardiac conduction system correlates with ventricular septation; we have therefore tested the hypothesis that evolution of the specialized conduction system is linked to ventricular septation, rather than homoiothermy (endothermy), as postulated by Davies.

We studied a group of 11 embryos of the Siamese and Mugger Crocodile between 3 and 84 days of incubation, and serial embryonic sections with HNK-1 and Hematoxylin staining of the Nile Crocodile, using optical mapping, ultrasound biomicroscopy, histo- and immunohistochemistry, *in situ* hybridization and 3D reconstruction.

At 12 days of incubation there was no ventricular septum and the ventricular activation pattern progressed in a left-to-right/base-to-apex sweep, similar to that observed at the early stages of avian and mammalian cardiogenesis. At 45 and 84 days the ventricular septum has formed and the epicardial activation patterns showed apex-to-base activation, indicative of presence of a preferential conduction pathway within the ventricles. In two hearts (50 days of incubation) we saw an epicardial breakthrough near the right ventricular apex. Immunohistochemistry showed HNK-1 positivity in the crest of the ventricular septum (and pacemaker area) and thus resembles the expression in the chicken, rat and human embryonic hearts where it marks the developing conduction system. *In situ* hybridization for crocodilian Cx40 transcripts (marker of Purkinje fibers) showed uniform expression in the trabeculated ventricular myocardium.

We conclude late embryonic crocodilian hearts possess a preferential conduction pathway somewhat similar to one present in the hearts of homoiotherms. The emergence of this pathway during development correlates with ventricular septation and presence of some conduction system markers.

Supported by Ministry of Education PRVOUK P35/LF/5 and institutional grant RVO: 67985823 (The Czech Academy of Sciences).

34: Molecular autopsy of sudden cardiac death with structurally normal heart: the circumstances of death and ECG tracings can address the final diagnosis

Kalliopi Pilichou, Marco Cason, Federico Migliore, Stefania Rizzo, Elisa Carturan, Emanuele Bertaglia, Gaetano Thiene, Cristina Basso.

Background. Sudden cardiac death with normal heart can occur in the setting of inherited ion channel diseases. Among these, Brugada syndrome (BrS) is characterized by non-ischemic ST segment elevation associated with PR prolongation and right bundle branch block, whereas Lenégre disease is characterized by atrio-ventricular block with progressive structural changes of the conduction system. In these arrhythmic syndromes, more than 1300 nucleotide variations has been identified in SCN5A gene.

Methods & Results. A 35-years old asymptomatic male died suddenly during sleep. Post-mortem examination excluded extracardiac causes of sudden death as well as the absence of structural abnormalities in the working myocardium. Personal clinical history re-assessment revealed a 'coved-type' ST segment elevation on right precordial leads with PQ prolongation (220 msec) in a patient's ECG performed during blood donation, compatible with BrS. Detailed conduction system investigation by serial section technique showed severe fibrosis of the bifurcating His and proximal bundles with sclerotic interruption of the left bundle branch. Genetic screening of SCN5A (NM. 198056.2) gene identified a mutation in exon 22 (c.3673 G>A , E1225K), defined as "likely to be pathogenic" by in silico tools, and previously linked to BrS phenotype with atrio-ventricular block. Cascade genetic screening detected 5 additional family mutation carriers, and provoked electrical stimulation unmasked ECG abnormalities in 2 of them.

Conclusion. Lenégre disease and BrS are overlapping clinical entities, «bookends» in a continuum of sodium current deficiency, accounting for a structural cardiomyopathy of the specialized conducting tissue occurring in the setting of an otherwise normal heart.

35: EMERGE: a flexible modelling framework to predict genomic regulatory elements from genomic signatures

K. van Duijvenboden, B.A. de Boer, N. Capon, J.M. Ruijter and V.M. Christoffels
Dept. Anatomy, Embryology & Physiology, Academic Medical Centre, Amsterdam, the Netherlands

Regulatory DNA elements, short genomic segments that regulate gene expression, have been implicated in developmental disorders and human disease. Despite this clinical urgency, only a small fraction of the regulatory DNA repertoire has been confirmed through reporter gene assays. The overall success rate of functional validation of candidate regulatory elements is low. Moreover, the number and diversity of datasets from which putative regulatory elements can be identified is large and rapidly increasing. We generated a flexible and user-friendly tool to integrate the information from different types of genomic datasets, e.g. ATAC-seq, ChIP-seq, conservation, aiming to increase the ease and success rate of functional prediction. To this end, we developed the EMERGE program that merges all datasets that the user considers informative and uses a logistic regression framework, based on validated functional elements, to set optimal weights to these datasets. ROC curve analysis shows that a combination of datasets leads to improved prediction of tissue-specific enhancers in human, mouse and Drosophila genomes. Functional assays based on this prediction can be expected to have substantially higher success rates. The resulting integrated signal for prediction of functional elements can be plotted in a build-in genome browser or exported for further analysis.

36: The development of the caudal venous return to the heart in man

Jill PJM Hikspoors[1], Mathijs MJP Peeters[1], Hayelom K Mekonen[1], Greet MC Mommen[1], S Eleonore Köhler [1], Wouter H Lamers[1,2]
[1] Department of Anatomy & Embryology, Maastricht University, Maastricht, The Netherlands
[2] Tygat Institute for Liver and Intestinal research, Academic Medical Center, Amsterdam, The Netherlands

The drainage of the umbilical and vitelline veins into the sinus venosus at 4 weeks of development (CS12) is well documented, but whether these veins play a role in the development of the hepatic venous system and the proximal portion of the inferior caval vein remains to be established. We reinvestigated this issue in a closely spaced series of human embryos between 4 and 6 weeks of development. Embryological structures were visualised with Amira 3D reconstruction and Cinema 4D remodelling software.
In the 5th week (CS13), the vitelline and umbilical veins became enclosed by the expanding mesenchyme of the septum transversum, while the expanding liver primordium enclosed and interrupted the vitelline veins. Concomitant with the repositioning of the entrance of the sinus venosus into the right atrium, the most distal part of the right vitelline vein ("hepatocardiac channel") increased in diameter, whereas the corresponding left portion did not.
In early CS14 embryos (~33 days of development), the liver primordium enclosed the umbilical veins. While the most distal parts of these veins disappeared, a connection (ductus venosus) appeared between the portion of the left umbilical vein that entered the liver and the right hepatocardiac channel. The intrahepatic part of the right vitelline vein proximal to the connection with the ductus venosus persisted as a fairly large, "C"-shaped vessel along the right-sided outer edge of liver until the ductus venosus had acquired a similar size as the umbilical vein. Thereafter, the remaining part of the left and the intrahepatic portion of the right vitelline vein regressed. The right umbilical vein started to regress as soon as it was enclosed by the liver.
In late CS14 embryos (~35 days of development), vessels larger than sinusoids emerged from the right vitelline (portal) vein and ductus venosus in the central part of the liver, while a well-developed sinusoidal network was identifiable in the liver periphery. In CS15 embryos, the portal branches extended further into the liver periphery, while the sinusoidal network expanded towards the centre. In CS16 embryos (~39 days of development), hepatic veins began to develop from this sinusoidal network starting at the hepatocardiac vein.
Conclusions: The vitelline and umbilical veins contribute to the hepatic venous system, but at their entrance and exit from the liver only. The sidedness of the development of the hepatocardiac channels appears imposed by that of the sinus venosus, whereas that of the umbilical veins may relate to that of the umbilical cord. Development of the intrahepatic portal veins precedes that of the hepatic veins, indicating that the liver segments follow the branching pattern of the portal veins.