ESC
Working Group
Development, Anatomy
& Pathology

Malaga Cardiovascular Development Meeting 2019

The Official Meeting of the ESC Working Group on Development, Anatomy and Pathology (DAP)

14 - 16 October 2019

INDEX

WELCOME LETTER

Dear colleagues,

It is a great pleasure for me to welcome you to **the 2019 Annual Meeting of the ESC Working Group on Development, Anatomy and Pathology (DAP).** In my double capacity as the current ESC WG DAP Chairperson and Organizer of this event, I really hope you enjoy it both scientifically and socially. We have made our best to put together an exciting series of talks, which showcase some of the hottest topics in the cardiovascular development, anatomy and pathology fields.

Málaga, the city that will host us along these three days, is the sixth largest one in Spain. Malaga's historical origins date back to 1,100-800 b.C., when Phoenicians first settled down close to the Guadalhorce river. Many civilizations have left their mark on the city, which remains a major touristic, trading and cultural center on the Mediterranean coast.

The venue of the meeting is the Rectorado (The University President's House), a central administrative building of the University of Málaga that was the city's central post office from 1923 to 1986. In the central hall of the Rectorado the old stone reservoirs used to prepare and store *garum*, a favourite Roman sauce made of fermented fish, have been preserved. This is a reminder of the unique, historical position that the Rectorado holds in the context of the city structure. Indeed, the Rectorado itself is just a few steps away from some of the most representative buildings of Málaga, like the massive Old Customs Palace (now the City Museum), the Arab Fortress (Alcazaba), or the Cathedral. Leaving the Rectorado and walking towards the narrow streets of the downtown area will discover you an endless number of restaurants and terraces serving drinks and a great variety of food, from informal *tapas* to elaborated cuisine.

I want to finish this welcome letter showing my appreciation to all those who made this meeting possible. First to the Scientific and Local Organizing Committees, which have been key in the shaping the of the scientific program and social side of the meeting. Second, to the ESC WG DAP Nucleus members and most specially to Léa Bergamaschi, our ESC Working Groups Coordinator, who was always ready to guide us through the complex task of putting a scientific meeting together. Thanks are also given to Paula Bernad and Myrsini A. Villar (BCO Congresos), whose work has been instrumental to orchestrate the complex logistics of the meeting, and to all our sponsors. Finally, thanks to our guest and keynote speakers as well as all the participants to this meeting: all this would not make any sense without your contribution.

José M. Pérez-Pomares
ESC WG DAP Chairperson
Meeting Organizer

THE ESC WORKING GROUP ON DEVELOPMENT, ANATOMY AND PATHOLOGY

Nucleus composition (2018-2020)

Chairperson	Prof. José María Pérez-Pomares, Spain
Vice-Chairperson	Assoc. Prof. Maurice van den Hoff, Netherlands
Past-Chairperson	Prof. Deborah Henderson, United Kingdom
Treasurer	Prof. Antonio Baldini, Italy
Secretary	Prof. Melanie Philipp, Germany
Web Editor	Dr. Bill Chaudhry, United Kingdom
Ordinary nucleus member	Dr. Lucile Houyel, France
Ordinary nucleus member	Dr. Monique Jongbloed, Netherlands
Ordinary nucleus member	Prof. Lars Allan Larsen, Denmark
Ordinary nucleus member	Dr. Jose Luis De La Pompa, Spain
Ordinary nucleus member	Dr. Stefania Rizzo, Italy
Ex-Officio	Prof. Thomas Brand, United Kingdom

Interim Nucleolus of the Young Community (2019-2020)

Coordinator	Dr. Claudio Cortes, France
Nucleolus Member	Dr. Nikita Ved, United Kingdom
Nucleolus Member	Dr. Guillermo Luxan, Germany

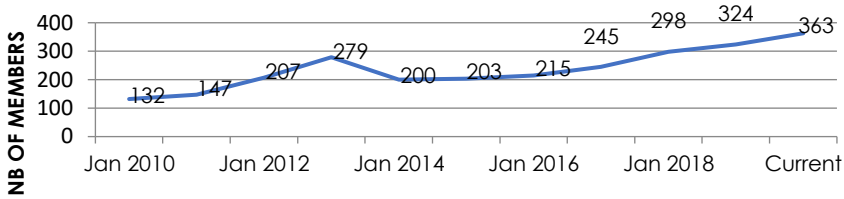
Local Organizing Committee

President	Prof. José M. Pérez-Pomares, Spain
Secretary	Dr. Juan Antonio Guadix, Spain
Vocal 1	Prof. Ramón Muñoz-Chápuli, Spain
Vocal 2	Prof. Ana Carmen Durán, Spain
Vocal 3	Dr. Borja Fernández, Spain

Scientific Committee

Prof. Deborah Henderson, United Kingdom
Dr. Robert Kelly, France
Prof. José María Pérez-Pomares, Spain
Prof. Dr. Didier Stainier, France
Assoc. Prof. Maurice van den Hoff, Netherlands
Dr. Stéphane Zaffran, France

Membership evolution



Mission

Our Working Group traditionally brings together basic scientists and clinicians with very different backgrounds, including molecular and developmental biologists, pathologists, morphologists, geneticists and cardiologists. The multidisciplinary structure represents the ideal way to approach cardiovascular diseases, either congenital or acquired.

Our goal is to attract young people to the research in the fields of cardiovascular development, anatomy and pathology, with particular attention on molecular and genetic developmental and pathological mechanisms.

At the same time, we would promote the irreplaceable value of the clinic-pathologic correlation method, which is becoming even more important in the era of advanced clinical imaging and interventional procedures.

In this perspective, joint ventures for research projects, position papers and scientific meetings or symposia with other ESC working groups will be pursued. The well attended Cardiac Anatomy and Pathology Live Sessions at every annual congress of the ESC as well as at our WG meetings are the best attestation of the educational value of our activities.

This year, we have promoted the development of a young scientist community (YC) in our Working Group and the first Nucleolus for that YC has been recently composed. Discussion has been opened with the YC representatives to design and implement specific educative actions in the fields of cardiac development, anatomy and pathology.

Our main mission will be to continue promoting the basic cardiovascular research and education with a translational approach inside the ESC.

More information

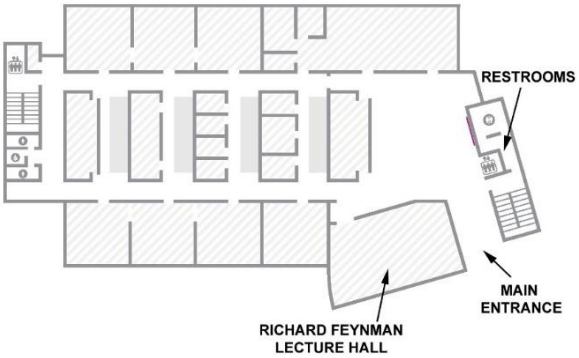
Online: www.escardio.org/anatomy-pathology

During the General Assembly: Tuesday 15 October 16:45 - 17:00h

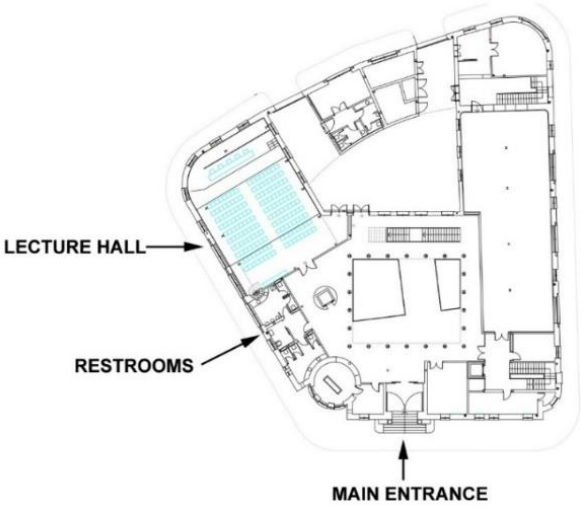
PRACTICAL INFORMATION

Conference venues & floor plans

Hands-on session
Andalusian Center for Nanomedicine and Biotechnology (BIONAND)
Parque Tecnológico de Andalucía, c/Severo Ochoa, 35
29590 Campanillas, Málaga, Spain



Conference venue
University of Malaga's Rectorado
Avenida Cervantes, 2
29016 Málaga, Spain



Wi-fi

There is WI-Fi available at the University of Malaga. Passwords will be provided.

Speakers

Faculty and presenting authors should prepare their presentation on 4:3 PowerPoint slides (non-panoramic) and arrive at the meeting room 15 minutes before the beginning of the session, introduce themselves to the chairpersons and upload their presentations. The time allotted to each oral presentation is indicated in the scientific programme section and includes discussion. Please, note that for a smooth development of the congress presentation times should be respected.

Posters

There will be 1 poster session for interaction and discussion with the rest of the attendees: Tuesday, 15 October (17.00-18.30h).

Each poster will be displayed the day of the poster session only. Posters should be installed in the morning (9:00 am) and removed before 19.00 in a hall on the first floor of the Rectorado (please, follow the indications). Left over posters will be thrown away.

Prizes

First and second best oral and poster presenters will receive a prize. They will be announced and delivered on Wednesday 16 October 2019 between 12.00-12.30h.

Sponsors

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Public transportation in Malaga

By train

This is the best option to move from the airport to the city center (single trip, around 11 minutes, 1.80€). The Renfe C1 train Line (Fuengirola-Málaga Centro Alameda) connects the airport with the city. The underground train station is located under terminal T3 and it is accessed from the terminal T3 gate through the transport interchange. Your stop is the end of the line (Málaga Centro-Alameda).

By taxi

Airport-Málaga: From 7 to 20€ depending on the time. At the airport follow the taxi signals at T2/3. Taxis can be reached everywhere around the Rectorado.

By bus

Malaga's transport consortium (EMT) runs all of Malaga's city buses. There are about 50 lines that run all across Malaga. It is also very easy to check timetables and routes in English or Spanish on the EMT website.

A single bus ticket within the Malaga urban area costs 1.30€. You can also buy a 10-journey ticket for 8.30€. The busiest central city bus routes operate every few minutes, from early morning until about midnight. After midnight, there are night buses, recognised by the letter 'N' in front of the bus line number.

The A Express line circulates between the city centre and the airport. The stop is at Terminal T3 level 0, arrivals sidewalk (single trip, around 15-20 minutes, 3€ paid on the bus) the closest stop to the Rectorado is "Paseo del Parque-Plaza de la Marina".

Line 25 can be reached at Paseo del Parque-Plaza de la Marina and is the best option to get to BIONAND for the hands-on session if you cannot catch the shuttle leaving the Rectorado at 8.15 on 14/10/2019 (see the Scientific Programme). BIONAND is inside the Technological Park (PTA). The closest stop to BIONAND 35 is 22524 Severo Ochoa 39.

Other useful bus lines are:

- Bus 3: El Palo– Malaga Centre – Puerta Blanca, runs between 06.15 h and 22.55 h, every 5-20 minutes.
- Bus N-1 Night Bus: El Palo– Malaga Centre – Puerta Blanca, runs from 23.05 h to 05.45 h every 15-30 minutes.

By metro

Occasional Ticket

This ticket allows for the charging of 1 – 9 trips. It is rechargeable and can be used by multiple users, meaning it can be used by various users on the same trip, on the proviso they all travel together. Price: 1.35 €. There is no metro connection between the Airport and Málaga city and only two lines (L1-2) are active (L3 remains under construction).

Metro de Málaga Travel Card

This travel card is a monetary card rechargeable by cash or bank card. The maximum permitted for recharging this card is 25 € and the minimum 5 €. It is not a named card and allows for multiple users, which means it can be used by up to a maximum of 9 passengers per trip, providing they all travel together. Price: 0.82 € / way.

KEYNOTE LECTURES

Pura Muñoz-Cànoves (Barcelona, Spain)

Prof. Pura Muñoz-Cánoves studied Pharmacology at the University of Valencia. She obtained her PhD in Biology at the Madrid Autonomous University and did postdoctoral work at the University of California-San Diego and The Scripps Research Institute. In 1995 she joined the Cancer Research Institute in Barcelona as a postdoc fellow, becoming an independent group leader in 1997. In 2002 her group moved to the Center for Genomic Regulation in Barcelona, and she was appointed as senior group leader in 2007 at that same institution. In 2009 she moved to the Pompeu Fabra University (UPF), as coordinator of the Cell Biology Unit. She currently is Professor of Cell Biology in the Department of Experimental and Health Sciences at the UPF. Prof. Muñoz-Cànoves research on the biology of skeletal muscle stem (satellite) cells has been published in prestigious journals including *Nature*, *Cell*, Development, *Cell Stem Cell*, *Developmental Cell*, and *Cell Metabolism* among many others. Pura Muñoz-Cánoves is an EMBO Member and recipient of several prestigious prizes (City of Barcelona, Lilly Foundation, Jaume I Biomedical Awards). In 2016, Pura Muñoz-Cánoves started her laboratory at the Spanish National Center for Cardiovascular Research (CNIC, Madrid) in the Vascular Pathophysiology Area while keeping her appointment at the UPF in Barcelona.

Jim Martin (Houston, USA)

Prof. James F. Martin got his Bachelor in Science at Fordham University (NY, USA). He completed his MD and PhD at the University of Texas Medical School at Houston (1986 and 1990 respectively). Then Prof. Martin became post-doctoral fellow at the University of Texas MD Anderson Cancer Center to continue an outstanding research career including important investigation on cranial and craniofacial stem cell biology, cell and developmental biology, molecular biology and genetics, human disease and cardiovascular sciences. He has authored more than 135 peer-reviewed papers in top journals such as *Nature*, *Science*, *Cell*, *Developmental Cell*, *PLOS Genetics*, *Development*, and *PNAS*. Current research in Prof. Martin's laboratory focuses in understanding how developmental pathways are connected to adult tissue regeneration through the analysis of the roles played by the Hippo, Wnt, and Bmp-signaling pathways in triggering the regenerative capabilities of cardiac and non-cardiac tissues.

MEETING-AT-A-GLANCE

Monday, October 14, 2019

09.00 - 12.15	Hands-on satellite session (BIONAND, Richard Feynman Lecture Hall)
13:00 – 14:00	Registration
14:00 - 14:15	Welcome address
14:15 - 15:30	Session I. Cardiac progenitor cells and the patterning of the embryonic myocardium
15:30 - 16:45	Session II. Insights in vascular biology
16:45 - 17:30	Coffee break
17:30 - 18:30	Keynote talk I
20:30 - 23:00	Faculty and Nucleus Dinner

Tuesday, October 15, 2019

9:00 - 10:15	Session III. Connective tissues in cardiac development and disease
10:15 - 10:45	Coffee break
10:45 - 11:45	Session IV. Single cell heart transcriptomics: from embryo to adult
11:45 - 12:45	Lunch
12:45 - 13:45	Keynote talk II
13:45 - 15:30	Session V. Cilia, laterality & CHD (together with the AEPC)
15:30 - 16:00	Coffee break
16:00 - 16:45	Session VI. Cellular mechanisms in cardiac disease
16:45 - 17:00	WG General Assembly
17:00 - 18:30	Poster session + guided discussion (WG Young Community)
20:30 - 23:30	General dinner

Wednesday, October 16, 2019

9:00 - 10:15	Session VII. Remodeling of the AV canal: from valves to conduction system
10:15 - 10:30	Coffee break
10:15 - 12:00	Session VIII. Animal models in cardiac development and regeneration
12:00 - 12:30	Prizes and closing remarks

SCIENTIFIC PROGRAMME

Monday 14 October 2019

08.15: bus departing from University of Malaga's Rectorado to BIONAND

Hour	Hands on session (BIONAND)	Speaker
09.00-09.05	Introduction	J.M. Pérez Pomares (Malaga, SP)
09.05-10.00	From embryology to anatomy: Di George syndrome and cardiac neural crest/second heart field outflow tract defects	Lucile Houyel & Deborah Henderson
10.00-11.00	Video demonstration of CNC/SHF outflow tract malformations: common arterial trunk, tetralogy of Fallot vs outlet VSD with malaligned outlet septum, aortic arch anomalies	Lucile Houyel (movies), Monique Jongbloed (specimens)
11.00-11.45	Hands-on session	Israel Valverde, Monique Jongbloed & Lucile Houyel
11:45-12.15	Quiz	

12:30: bus departing from BIONAND to University of Malaga's Rectorado

Hour	Welcome address	Speaker
14.00-14.15	General information	J.M. Pérez Pomares (Malaga, SP)

Hour	Session I. Cardiac progenitor cells and the patterning of the embryonic myocardium	Speaker
	Chairs: D. Henderson & S. Zaffran	
14.15-14.45	Patterning and cell fate choices in the second heart field	Robert Kelly (Marseille, FR)
14.45-15.00	Role of the cAMP signaling pathway in the regulation of cardiac progenitor cell fate	Fabien Hubert (Marseille, FR)
15.00-15.15	Transient Nodal signalling in left precursors coordinates opposite asymmetries to shape the heart loop	Audrey Desgrange (Paris, FR)
15.15-15.30	Wnt11 regulates L-Type Calcium Channel by AKAP2-PKA compartmentalization in patterning the developing myocardium	Mai Phan (Berlin, GE)

Hour	Session II. Insights in vascular biology	Speaker
	Chairs: B. Chaudhry & D. Stainier	
15.30-16.00	Using multispectral and genetic barcoding to study cardiovascular biology	Rui Benedito (Madrid, SP)
16.00-16.15	Tissue-resident macrophages pattern the developing cardiac lymphatic system	Joaquim Vieira (Oxford, UK)
16.15-16.30	Pax9 interacts with Tbx1 in the pharyngeal endoderm to control pharyngeal arch artery development	Catherine Stothard (Newcastle, UK)
16.30-16.45	NADPH oxidases promote developmental programming of pulmonary arterial hypertension by transient fetal hypoxia	Katharina Hochkogler (München, GE)

16.45-17.30 Coffee break

Hour	Keynote talk I	Speaker
Chairs: J.L. de la Pompa & J.M. Pérez-Pomares		
17.30-18.30	Biology of skeletal muscle stem cells	Pura Muñoz-Cànoves (Barcelona, SP)

Free evening for participants

20.30-23.00 Faculty and Nucleus dinner

Tuesday 15 October 2019

Hour	Session III. Connective tissues in cardiac development and disease	Speaker
	Chairs: S. Rizzo & A. Wessels	
09.00-09.30	Fibrosis in cardiac disease	Cristina Basso (Padova, IT)
09.30-09.45	Single-cell RNA-seq analysis reveals the crucial role of Collagen Triple Helix Repeat Containing 1 (CTHRC1) cardiac fibroblasts for ventricular remodeling after myocardial infarction	Adrián Ruiz-Villalba (Pamplona, SP)
09.45-10.00	Postnatal cardiac fibroblast characterization and regulation of ECM remodeling and cardiomyocyte proliferation	Luis Hortells-Garcia (Cincinnati, USA)

10.00-10.30 Coffee break

Hour	Session IV. Single cell heart transcriptomics: from embryo to adult	Speaker
	Chairs: T. Brand & T. Firulli	
10.30-11.00	Transcriptomics of cardiac progenitor cells	Thomas Braun (Bad Nauheim, DE)
11.00-11.15	Hoxb1 establishes the fate of cardiac progenitor cells	Sonia Stefanovic (Marseille, FR)
11.15-11.30	Anatomical localization of progenitors in the cardiac crescent delineated by single-cell approaches	Richard Tyser(Oxford, UK)
11.30-11.45	Conserved epigenetic regulatory logic establishes a repressive index for inferring genes governing cell identity	Nathan Palpant (St Lucia Qld, AU)

11.45-12.45 Lunch

Hour	Keynote talk II	Speaker
	Chairs: R. Kelly & J.M. Pérez-Pomares	
12.45-13.45	Hippo signaling in heart homeostasis and regeneration	Jim Martin (Houston, US)
Hour	**Session V. Cilia, laterality & CHD (together with the AEPC)**	**Speaker**
	Chairs: T. Braun & L.A. Larsen	
13.45-14.15	Regulators of cilia function in laterality development	Melanie Philipp (Ulm, DE)
14.15-14.45	Laterality in Congenital Heart Disease	Monique Jongbloed (Leiden, NL)
14.45-15.15	Fetal heterotaxy: should we still categorize?	Lucile Houyel (Paris, FR)
15.15-15.30	The Controversial Anatomy of Holes between Ventricles	Laura Edgar (Birmingham, UK)

15.30-16.00 Coffee break

Hour	Session VI. Cellular mechanisms in cardiac disease	Speaker
	Chairs: L. Field & M. van den Hoff	
16.00-16.15	Disruption of sodium-dependent vitamin transport: a potential novel cause of cardiomyopathy	Lauren Phillips (Newcastle, UK)

16.15-16.30	Low incidence of left ventricular noncompaction in a pathology archive	Bjarke Jensen (Amsterdam, NL)
16.30-16.45	Modelling Mybpc3-related HCM and LVNC	Alejandro Salguero (Madrid, SP)

Hour	WG General Assembly
16.45-17.00	Discussion

Hour	Poster session (all posters)
17.00-18.30	Poster + guided discussion (WG Young Community)

20.30-23.30 General dinner (all registered attendees)

Wednesday 16 October 2019

Hour	Session VII. Remodeling of the AV canal: from valves to conduction system	Speaker
	Chairs: D. Sedmera & W. Shou	
09.00-09.30	SOX9 and the Pathogenesis of AV Valvuloseptal Abnormalities	Andy Wessels (Charleston,USA)
09.30-09.45	Gpr126 (ADGRG6) contributes to valve development by regulating EMT of endocardial cells	Gentian Musa (Nürnberg, GE)
09.45-10.00	Mutation found in Mitral Valve Prolapse patients alters Wnt signaling through interactions with a β-catenin antagonist	Russell Norris (Charleston, SC, USA)
10.00-10.15	Temporal requirement of Nkx2-5 defines distinct scaffold and lineage restriction phases of ventricular conduction system development	Caroline Choquet (Marseille, FR)

10.15-10.30 Coffee break

Hour	Session VIII. Animal models in cardiac development and regeneration	Speaker
	Chairs: R. Muñoz-Chápuli & D. Panáková	
10.30-11.00	Coronary revascularization is regulated by epicardial and endocardial cues and forms a scaffold for cardiomyocyte repopulation	Didier Stainier (Bad Nauheim, DE)
11.00-11.15	A novel approach to understanding how pre-gestational diabetes induces embryonic birth defects	Nikita Ved (Oxford, UK)
11.15-11.30	In vivo regeneration of cardiac valves – lessons from zebrafish	Anabela Bensimon-Brito (Bad-Nauheim, GE)

11.30-11.45	Ephrin-B1 links the morphology of the adult cardiomyocyte to its inability to proliferate	Céline Galés (Toulouse, FR)
11.45-12.00	SHROOM3 is a novel component of the planar cell polarity pathway whose disruption causes congenital heart disease	Matthew Durbin (Indianapolis, IN, USA)

Hour	Prizes and closing remarks
12.00-12.30	

FACULTY ABSTRACTS

F-001 | Patterning and cell fate choices in the second heart field

Dr. Robert Kelly[1]
[1]*Aix Marseille University, Developmental Biology Institute of Marseille, Marseille, France*

Morphogenesis of the vertebrate heart occurs through progressive deployment of second heart field (SHF) progenitor cells to the cardiac poles. Defects in SHF addition to the arterial and venous poles results in a spectrum of common forms of congenital heart defects including conotruncal anomalies and atrial and atrioventricular septal defects. SHF cells are located in epithelial cardiopharyngeal mesoderm (CPM) of the dorsal pericardial wall and are patterned along the embryonic anterior posterior axis into arterial and venous pole progenitor populations through the activity of T-box transcription factors and retinoic acid signaling. CPM also gives rise to skeletal muscles of the head and neck. Results will be presented concerning the genetic and cellular properties of cardiac progenitor cells as distinct progenitor cell subpopulations emerge within the SHF as well as mechanisms regulating cell fate choice in CPM.

F-007 | Regulators of cilia function in laterality development

Melanie Philipp

Once a cell exits the cell cycle, a vesicle attaches to the mother centriole of the centrosome. Together they migrate towards the plasma membrane, where microtubule doublets of the centriole extend and form the scaffold for a unique organelle, the cilium. Cilia function as signaling platforms and propel as well as sense fluid flow. They regulate a plethora of physiological processes, among them the breaking into a left and right side of our bodies, which is a prerequisite of oriented heart development. When cilia and hence symmetry breaking fail, complex congenital heart defects may develop. Better knowledge of the control mechanism governing faithful cilium formation and function can thus help to widen our understanding of vertebrate heart development. Recently, we have identified a crosstalk with our cellular organelles to be important for cilium development and laterality development.

F-009 | SOX9 and the Pathogenesis of AV Valvuloseptal Abnormalities

Andy Wessels
[1]*Medical University of South Carolina, Charleston, USA*

Proper formation of the atrioventricular mesenchymal complex is of crucial importance for the development of valves and septa in the 4-chambered heart. In this process, a variety of mesenchymal cell populations with different developmental origins are involved. In this presentation we revisit the role of the transcription factor Sox9 in AV valvuloseptal development. The importance of Sox9 in the endocardially-derived mesenchyme of the developing AV cushions is well-described. Germline and endocardial-lineage-specific deletion of Sox9, results in abnormal valves. Here we report on the role of Sox9 in the epicardial cell lineage and Second Heart Field (SHF). We demonstrate that (1) Sox9 expression in the epicardially-derived cells at the AV junction (AV-EPDCs) is critically important for the migration of AV-EPDCs into the leaflets of the AV valves and, hence, their subsequent development, and (2) that the presence of Sox9 in the posterior SHF is essential for atrioventricular septation. We conclude that Sox9 plays a more extensive role in heart development than was previously appreciated.

F-010 | Coronary revascularization is regulated by epicardial and endocardial cues and forms a scaffold for cardiomyocyte repopulation

Didier Y.R. Stainier[1,3], Rubén Marín-Juez[1,3], Hadil El-Sammak[1], Christian S.M. Helker[1], Aosa Kamezaki[1], Sri Teja Mullapudi[1], Sofia-Iris Bibli[2,3], Matthew J. Foglia[4], Ingrid Fleming[2,3], Kenneth D. Poss[4]
[1]*Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, 61231 Bad Nauheim, Germany* , [2]*Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, 60590 Frankfurt am Main, Germany,* [3]*German Center of Cardiovascular Research (DZHK), Partner site RheinMain, 60590 Frankfurt am Main, Germany,* [4]*Regeneration Next, Duke University, Department of Cell Biology, Duke University Medical Center, Durham, USA*

Defective coronary network function and insufficient blood supply are both cause and consequence of myocardial infarction. Efficient revascularization after infarction is essential to support tissue repair and function. Zebrafish hearts exhibit a remarkable ability to regenerate, and coronary revascularization initiates within hours of injury, but how this process is regulated remains unknown. Here we show that revascularization requires a coordinated multi-tissue response culminating with the formation of a complex vascular network available as a scaffold for cardiomyocyte repopulation. During a process we term "coronary-endocardial anchoring", new coronaries respond by sprouting 1) superficially within the regenerating epicardium, and 2) intra-ventricularly towards the activated endocardium. Mechanistically, superficial revascularization is guided by epicardial Cxcl12-Cxcr4 signaling, and intra-ventricular sprouting by endocardial Vegfa signaling. Our findings indicate that the injury-activated endocardium and epicardium support cardiomyocyte replenishment initially through the guidance of coronary sprouting. Simulating this process in the injured mammalian heart should help its healing.

ORAL PRESENTATIONS

O-001 | Role of the cAMP signaling pathway in the regulation of cardiac progenitor cell fate

Fabien Hubert[1], Corentin Porada[1], Francesca Rochais[1]
[1]*Marseille Medical Genetic, Marseille, France*

During the early phase of embryonic cardiac development, second heart field (SHF) progenitors allow the rapid elongation of the embryonic heart. The precise control of SHF proliferation/differentiation balance is a prerequisite for the correct heart tube elongation and any alteration results in severe congenital heart defects. We previously identified the transcriptional repressor Hes1 as a critical regulator of SHF cell proliferation and preliminary results showed that cAMP signals may control Hes1 expression in the SHF. We thus investigated the role of the cAMP-dependent signaling in the regulation of cardiac progenitor cell fate.
Using candidate approach, the expression profile of cAMP pathway members, including the regulatory (R) subunits of the cAMP-dependent protein kinase (PKA), was analyzed at different early embryonic stages.
We first revealed regionalized expression pattern of cAMP pathway components. The R-PKA subunits are preferentially expressed in SHF progenitors and we determined that the RIa subunit (Prkar1a) is the main regulatory subunit expressed in the SHF. Interestingly, Prkar1a deficiency in mouse leads to cardiac growth arrest resulting in early (E10.5) embryonic lethality. In order to evaluate the role of Prkar1a in early cardiac progenitors, we performed conditional deletion of Prkar1a using Mesp1Cre- and AHF-Mef2cCre-Prkar1aflox/flox transgenic mouse lines. Our results revealing early developmental lethality (E11.5) and dramatic impaired SHF progenitor cell proliferation strongly support a key role for Prkar1a in the control of SHF cell fate.
Altogether, these results suggest the involvement of diverse members of the cAMP-dependent signaling pathway, including Prkar1a, in the control of early cardiac progenitor cell deployment.

O-002 | Transient Nodal signalling in left precurors coordinates opposite asymmetries to shape the heart loop

Audrey Desgrange[1], Jean-François Le Garrec[1], Ségolène Bernheim[1], Sigolène Meilhac[1]
[1]*Imagine - Pasteur Institute, INSERM UMR1163, Universite´ Paris Descartes, Laboratory of Heart Morphogenesis, Paris, France*

Establishment of left-right patterning has been well characterized in the node, with Nodal signaling as a major left determinant. However, how this molecular asymmetry is transposed into asymmetric organogenesis, such as the rightward looping of the heart, has remained poorly understood. Previous analyses of impaired left-right signaling in animal models have been limited to the direction of heart looping, with no insight into the fine shaping of the heart loop. Following our quantification and modeling of mouse heart looping dynamics, we now investigate the role of Nodal in shaping the heart loop.
In embryo culture, we have identified a transient time window during which Nodal is required for the asymmetric patterning of heart precursors. Taking advantage of a transgenic line, we mapped the contribution of cells that have expressed Nodal to the looping heart tube. We have generated a novel mouse model with mesoderm specific deletion of Nodal in which we quantified the 3D shape of the heart tube. Nodal inactivation not only randomizes heart looping direction but also generates 4 classes of abnormal heart shapes that can be reproduced by computer modeling. Our work demonstrates that Nodal is required to amplify and coordinate asymmetries at the two heart poles further supporting the existence of a random generator of asymmetry (Brown and Wolpert, 1990). With a transcriptomic approach, we show that nodal regulates the balance between cell proliferation and differentiation.
Our work provides novel insight into the asymmetric morphogenesis of the heart, which is relevant to congenital heart defects.

O-003 | Wnt11 regulates L-Type Calcium Channel by AKAP2-PKA compartmentalization in patterning the developing myocardium

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Background
During development, the L-Type Calcium channel (LTCC) is attenuated by Wnt11, a non-canonical ligand of the Wnt family, to establish the intercellular coupling gradient in the developing myocardium. Here, we seek to further characterize this regulatory axis of LTCC to identify key players working downstream of Wnt11.

Methods & Results
By means of biochemistry, quantitative fluorescent microscopy, and confocal calcium imaging, we first showed that Wnt11 via binding to its receptor Frizzled 7, regulates the LTCC. Loss of Wnt11, mirroring the effects of beta-adrenergic stimulation, increases the PKA-dependent proteolytic processing of the channel C-terminal tail in vitro.
Secondly, we utilized Gene Ontology analysis and SILAC-based quantitative proteomics to screen for potential AKAPs involved in mediating PKA activity. Systematic analysis of selected candidates showed AKAP2 compartmentalizing PKA activities as the central mechanism of Wnt11-dependent regulation of the LTCC. We found AKAP2 directly binds to the channel C-terminus, and acts downstream of Wnt11.
Finally, using high-speed optical mapping and complementary genetic methods in the in vivo zebrafish model, we demonstrated AKAP2's key roles in patterning the development of the embryonic heart, to a certain degree, in a Wnt11-dependent manner.

Conclusion
Here we have described the complete signaling cascade: Wnt11-ligand, Fzd7-receptor, PKA-effector, AKAP2-transducer, and target-LTCC. More specifically, we showed AKAP2-PKA compartmentalization is a conserved mechanism crucial for channel regulation and proper organogenesis. Taken together, our work provided a conceptual advancement not only in regulation of a key channel in cardiac physiology, the LTCC, but also in our understanding of Wnt signaling itself.

O-004 | Hoxb1 establishes the fate of cardiac progenitor cells

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Background
During early cardiogenesis, progressive addition of distinct cardiac progenitor cells originating from the second heart field (SHF) contributes to elongation of the forming heart. Whereas the subdivision of the SHF into an anterior and posterior domain is essential for morphogenesis of the definitive heart, the transcriptional programs and upstream regulatory events operating in the different progenitor cell populations remain unclear. Here, our goal is to better understand how the gene expression programs of specific sub-populations of the SHF are established.

Material and methods
We characterized mouse cardiac progenitor cells within anterior (Mef2c-AHF-Cre;RosatdT) and posterior SHF (Hoxb1-GFP) populations using RNA sequencing and transposase-accessible chromatin profiling (ATAC-seq). In order to investigate the role of Hoxb1 during heart development we used gain and loss of-function approaches.

Results and Conclusion
We identified accessible chromatin within anterior (Mef2c-AHF-Cre;RosatdT) and posterior SHF (Hoxb1-GFP) populations. Furthermore, we found that conditional activation of Hoxb1 in the anterior SHF induces the activation of a number of pSHF marker including Bmp4 a novel identified enriched posterior marker. We also observed a reduced expression of Bmp4 in Hoxb1-deficient mice and identified a conserved pSHF enhancer candidate in the Bmp4 locus. Together these findings identity a transcriptional subprogram controlling cardiac progenitor differentiation during heart morphogenesis and provide new insights into the molecular etiology of congenital heart defects (CHDs).

Funding sources
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O-005 | Anatomical localization of progenitors in the cardiac crescent delineated by single-cell approaches

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Background
The cardiac crescent is the first morphologically recognizable heart structure in the developing embryo, arising from subsets of mesoderm-derived cardiovascular progenitors. The aim of this study was to characterise the progenitor cell types that constitute the forming cardiac crescent at both an anatomical and transcriptional level.

Material and methods
SMART-Seq2 single cell sequencing was performed on isolated cells which were collected in an unbiased manner from 5 different stages of cardiac crescent to linear heart tube development, creating a high-depth dataset of 4500 cells. Whole mount immunofluorescence and multiplexed in-situ hybridization HCR was used to anatomically identify the different cell types within the anterior cardiac crescent region. In order to investigate the behavior and fate of specific cell types, time-lapse imaging using lightsheet fluorescence microscopy as well as genetic lineage tracing studies were performed.

Results and Conclusion
By combining multiple experimental approaches we have been able to define in detail the transcriptional and anatomical profile of progenitor cell types within the cardiac crescent. This analysis has allowed us to identify a novel anatomically distinct subtype of progenitor which can contribute to the forming heart. Together these data have allowed us to gain novel insight into the heterogeneity of cardiac progenitors, highlighting a greater complexity within the cell populations contributing to the developing cardiac crescent.

O-006 | Conserved epigenetic regulatory logic establishes a repressive index for inferring genes governing cell identity

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Understanding genetic control of cell diversification is essential for understanding mechanisms controlling biological complexity.

Methods
We analyzed 111 NIH epigenome roadmap data sets to identify features of genome regulation associated with cell-type specification.

Results
We show that the a priori deposition of genome-wide H3K27me3 enriches for genes governing fundamental mechanisms underlying biological complexity in cell differentiation, organ morphogenesis and disease. We tested the ability to infer regulatory genes controlling theoretically any somatic cell by interfacing genome-wide H3K27me3 values with cell-specific genome-wide sequencing data. Using more than 1 million genome-wide data sets including RNA-seq, CAGE-seq, ChIP-seq and quantitative proteomics, we identify cell-type specific regulatory mechanisms underlying diverse cell-states, organ systems and disease pathologies from species across the animal kingdom including chordates and arthropods. We used this computational inference approach for novel gene discovery. Analysis of single cell RNA-seq data from in vitro human iPSC cardiac differentiation predicted SIX3 as a novel transcription factor controlling derivation of definitive endoderm, which we confirmed by SIX3 genetic loss of function using CRISPRi hPSCs. Moreover, analysis of transcriptional data from heart development of the invertebrate chordate *Ciona robusta*, predicted RNF220 to underlie tunicate heart field formation. This was confirmed with CRISPR knockout in vivo showing that RNF220 loss of function results in pharyngeal muscle morphogenesis defects.

Conclusion
This study demonstrates that the conservation of epigenetic regulatory logic provides an effective strategy for utilizing large, diverse genome-wide data to establish quantitative basic principles of cell-states to infer cell-type specific mechanisms underpinning the complexity of biological systems.

O-007 | Tissue-resident macrophages pattern the developing cardiac lymphatic system

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Background: Macrophages are components of the innate immune system with key roles in tissue inflammation, and repair. Moreover, it is now evident that macrophages support organogenesis. Given their role during lymphangiogenesis in inflammatory and tumour settings, as well as in the skin to define the calibre of lymphatic vessels, we sought to investigate a role for tissue-resident macrophages in lymphatic expansion in the developing heart.

Material and Methods: The development of cardiac lymphatics in wild-type, Pu.1-null and reporter Csf1r-CreER, Cx3cr1-CreER and Flt3-CreERT2 mouse models was characterised by immunostaining, confocal microscopy and flow cytometry. Additionally, a co-culture system was implemented to model macrophage-lymphatic endothelium interaction in vitro.

Results: Here, we demonstrate that macrophages are essential by colonizing the developing heart ahead of the initiation of cardiac lymphatic development, closely associating to, and interacting with, the leading edges and anastomosing ends to promote vessel growth and fusion, and ensure an adequate lymphatic coverage of the sub-epicardial surface. Failing to do so, downstream of macrophage-deficiency led to hyperplastic, shortened and under-branched lymphatic vessels and mis-patterning of coronary vessels. With regards to the embryological origin, extra- and intra-embryonic hematopoietic sources were found to contribute to the resident macrophage population of the developing the heart, as assessed by the use of genetic lineage tracing models.

Conclusions: These findings are novel, increasing our knowledge of lymphatic biology, and potentially of widespread interest in terms of understanding how to therapeutically modulate lymphatic growth in disease settings.

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O-008 | Pax9 interacts with Tbx1 in the pharyngeal endoderm to control pharyngeal arch artery development

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Background:
Developmental defects to the heart and aortic arch arteries are a leading cause of morbidity. Such defects arise due to the aberrant development of the pharyngeal arch arteries (PAA) and are present in 22q11 deletion syndrome (22q11DS). TBX1 has been identified as a leading causative gene, however, the wide spectrum of defects present in 22q11DS patients suggests that modifier genes may contribute to this phenotypic variation. Pax9 is expressed with Tbx1 in the pharyngeal endoderm and is downregulated in Tbx1-null mice.

Methods:
We used imaging and histological analysis to assess embryonic cardiovascular structure in multiple transgenic mouse lines. Ink injection was used to visualise PAA and immunohistochemistry was used to study neural crest cell and smooth muscle investment to these vessels.

Results/Conclusion:
We showed that Pax9-null mice have cardiovascular defects to the outflow tract and aortic arch arteries that arise from the aberrant development of the 3rd and 4th PAA. These defects stem from the lack of neural crest cell and smooth muscle investment to the PAA. Furthermore, we demonstrated a genetic interaction between Pax9 and Tbx1 as Tbx1/Pax9 double heterozygous mice presented with a significantly increased penetrance of interrupted aortic arch compared to Tbx1 heterozygotes. Using a novel Pax9Cre allele we removed Tbx1 expression from the pharyngeal endoderm and identified this tissue as the site of interaction. This data shows that a Tbx1-Pax9 signalling mechanism exists within the pharyngeal endoderm and is required for formation and remodelling of the PAA.

British Heart Foundation, Newcastle upon Tyne Hospitals NHS

O-009 | NADPH oxidases promote developmental programming of pulmonary arterial hypertension by transient fetal hypoxia

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Background: Fetal hypoxia can cause intrauterine growth restriction (IUGR) and an elevated risk of developing cardiovascular diseases in adulthood, although underlying mechanisms are largely unknown. Reactive oxygen species (ROS) generated by NADPH oxidases have been associated with cardiovascular diseases and the response to hypoxia in adulthood. However, the role of ROS in the outcome of fetal hypoxia is poorly understood. We hypothesized that NADPH oxidases contribute to fetal and adult offspring's response towards intrauterine hypoxia.

Material and Methods: Pregnant wildtype (WT) mice and mice lacking functional NADPH oxidases (nmf333) were exposed to 24 h hypoxia (10% oxygen) at E10.5. Offspring was analysed at E11.5, E17.5 and 11 weeks.

Results and Conclusion: Fetal hypoxia induced IUGR, myocardial thinning and delayed heart and lung maturation in WT embryos. In WT embryos, but not in nmf333 embryos lacking functional NADPH oxidases, ROS levels and DNA damage were increased. Importantly, nmf333 embryos developed normally without signs of IUGR, myocardial thinning and maturation delays. Following intrauterine hypoxia, adult WT mice showed enhanced levels of NADPH oxidases and DNA damage in heart and lung, and spontaneously developed pulmonary arterial hypertension (PAH) with pulmonary vascular remodelling, right ventricular hypertrophy and increased right ventricular pressure. In contrast, nmf333 mice were protected against developmental programming of PAH. Mechanistically, fetal hypoxia resulted in dysregulation of the miRNAome which was counteracted by NADPH oxidase deficiency.

Thus, transient fetal hypoxia promotes developmental programming of PAH due to increased ROS generation by NADPH oxidases leading to miRNA dysregulation and cardiac damage.
Funding: DZHK

O-010 | Single-cell RNA-seq analysis reveals the crucial role of Collagen Triple Helix Repeat Containing 1 (CTHRC1) cardiac fibroblasts for ventricular remodeling after myocardial infarction

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BACKGROUND: Ventricular remodelling is the natural process that occurs after a myocardial infarction (MI), and it is characterized by the formation of a fibrotic scar. The generation of this scar is regulated by activated cardiac fibroblasts (CF), responsible of the synthesis and maturation of collagen1a1. However, the cellular and molecular mechanisms underlying ventricular remodelling are mostly unknown.

MATERIAL AND METHODS: Collagen1a1-GFP CF has been characterized along MI by single-cell and bulk RNA-seq. ATAC-seq and functional in vivo and in vitro assays were performed to determine regulatory mechanisms. Furthermore, pig models of MI and biopsies from patients have been used to correlate cardiac function with topological RNA-seq studies.

RESULTS AND CONCLUSIONS: We describe the expression profile of 29,176 Col1a1-GFP CF along MI, and redefine the transcriptomic signature of activated CF. The in vivo absence of Cthrc1, a top marker gene for this subpopulation, showed 70% lethality within the first week after damage. Interestingly, we found a correlation between the expression of Cthrc1 and cardiac function in a pig model of MI, and topological specificity of this and other top markers in patients. In conclusion, we describe CF heterogeneity and dynamics along MI, and redefine activated CF. Our study identifies Cthrc1 as a novel target of the healing scar process with translational potential.

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O-011 | Postnatal cardiac fibroblast characterization and regulation of ECM remodeling and cardiomyocyte proliferation.

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In murine newborns, cardiomyocytes undergo cell cycle arrest and transition to hypertrophic growth, but much less is known of what happens to cardiac fibroblasts (CFs). We hypothesize that activated cardiac fibroblasts (CF), indicated by Periostin expression, remodel the extracellular matrix (ECM) and modify cardiomyocyte proliferation during the postnatal period.
CF activation was monitored by PostnMerCreMer(MCM);R26GFP lineage analysis and the resident CFs were studied using TCF21MCM;R26GFP mice. RNAseq was performed from FACS-sorted cells at Postnatal day (P)7 and P30, and both lineages were ablated by diphtheria toxin using a R26DTA allele. Cardiac ECM maturation, cell proliferation, CF identity and activation were assessed by immunofluorescence, RNAscope, and Western Blot.
Postnatal ECM remodeling includes reduced fibronectin and increased collagen1/3 expression after P7. At P7 PostnMCMR26GFP cells are highly proliferative, represent ~20% of the TCF21MCMR26GFP cells, are present adjacent to remodeling collagen and do not express alphaSMA. In contrast, TCF21MCMR26GFP cells are less proliferative at P7 and represent quiescent CF at P30. GO analysis of RNAseq data confirms increased proliferation of PostnMCM lineage and ECM remodeling of Tcf21 lineage CFs. Ablation of TCF21MCM lineage cells led to an overall reduction of collagen remodeling but PostnMCMR26GFP ablation importantly decreased CM proliferation.
Postnatal CFs include a proliferative Postn+ subpopulation that modulates cardiomyocyte proliferation, and more abundant TCF21+ CFs are required for ECM maturation. By P30, Postn+ lineage cells are not detected, but Tcf21 expression is maintained in quiescent mature CFs.

Funding:
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O-012 | FETAL HETEROTAXY: SHOULD WE STILL CATEGORIZE?

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Background:
Heterotaxy is still stratified in right and left isomerism, classically associated with abdominal, cardiac and venous abnormalities.

Material and Methods:
We analyzed 61 fetal specimens with heterotaxy divided in 5 groups: classic LI(CLI: bronchopulmonary LI, bilaterally absent pectinate muscles (PM), polysplenia, n=22), non-classic LI (NCLI: 1 discordant feature, n=15), classic RI (CRI: bronchopulmonary RI, bilateral PM extent to the crux, asplenia, n=12), non-classic RI (NCRI: 1 discordant feature, n=9), totally discordant features (n=3).

Results and conclusion:
Non-classic patterns were found in 75% RI, 40.5% LI with highly variable splenic status (NCLI: asplenia 33%, single spleen 20%; NCRI: asplenia 12%, single spleen 87%). PM extent was highly variable in NCLI (bilaterally left 40%, normal 53%, bilaterally right 6.7%), bilaterally right in 89% NCRI. Interrupted inferior caval vein was seen in LI only, total extracardiac pulmonary venous return in RI only, ipsilateral pulmonary veins in LI only. Common atrioventricular junction was constant in RI, 64% in CLI, 74% in NCLI. Left ventricle was hypoplastic in 27% CLI, 33% NCLI, 50% CRI, 44% NCRI. Right ventricle was hypoplastic in 9% CLI, 33% NCLI, 17% CRI, 22% NCRI. Ventriculo-arterial connections were always abnormal in RI, normal in 54% CLI, 60% NCLI. Pulmonary atresia/stenosis were frequent in RI (92%/67%), rare in LI (0/18% CLI, 7%/13% NCLI). Hypoplastic aortic arch was found exclusively in LI. In conclusion, heterotaxy cannot be reduced to isomerism. PM extent is not uniformly symmetrical. Each anatomic feature should be analysed individually, rather than to categorize patients in RI and LI.

O-013 | The Controversial Anatomy of Holes between Ventricles

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Background:
Isolated ventricular septal defects (VSD) can be defined as holes or channels between ventricles of hearts with normal atrioventricular and ventriculoarterial connections. Differential interpretation of VSD development has contributed to on-going controversies surrounding their classification, with the borders and geography approaches forming two rivalling schools of thought. In an attempt for a universal system, the International Society for Nomenclature of Congenital and Paediatric Heart Disease (ISNPCHD) released new nomenclature in 2018 with reference to current embryological understanding. We aimed to evaluate these terms looking specifically at interobserver agreement.

Materials and methods:
212 specimens underwent full morphological examination. Anatomical photographs were taken of both classical phenotypes and unusual findings. Using ISNPCHD definitions, the borders and geography of each VSD were described by three independent examiners and interobserver agreement was calculated using Fleiss's method.

Results:
Of 230 VSDs in the study sample, 68.7% were perimembranous, 26.5% muscular and 4.7% doubly committed. All perimembranous VSDs exhibited mitral to tricuspid fibrous continuity but only 79.7% had aortic to tricuspid continuity. The nature of this continuity was heterogeneous. The right ventricular landmarks were highly variable and specimens had varying associated lesions. Interobserver agreement revealed examiners were more likely to agree on VSD borders than geography (κ= 0.804 vs κ= 0.518).

Conclusions:
Isolated VSDs are complex and heterogeneous defects. Despite the embryological basis for the geography approach, our findings show borders terms are more objective and therefore more appropriate for classifying VSDs. Further embryological investigation may influence future iterations of the ISNPCHD nomenclature.

O-014 | Disruption of sodium-dependent vitamin transport: a potential novel cause of cardiomyopathy

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Background:
Cardiomyopathy is a heterogeneous disorder affecting adults and children and is a leading cause of death. Paediatric cardiomyopathies affect ~1 per 100,000 children and one third of these children will undergo a heart transplant or die within two years. Using whole exome sequencing we have identified a homozygous missense mutation in the Sodium Multivitamin Transporter (SMVT) gene, SLC5A6, in two sisters with paediatric cardiomyopathy. SMVT is a plasma membrane protein that transports biotin, pantothenic acid and lipoic acid throughout tissues including the brain and heart. These substrates play an essential role in energy metabolism. Therefore, reduced functionality of SMVT within the heart may lead to a decline in energy production, causing excess stress upon the heart resulting in cardiomyopathy.

Material and methods:
We have developed a Slc5a6 cardiac-specific conditional knockout mouse model. Electrocardiography (ECG) and cardiac magnetic resonance imaging (MRI) was carried out throughout early adulthood to assess cardiac functionality. Structural abnormalities were further investigated by histological staining.

Results:
Functional assessment of knockout mice revealed widening of QRS complex and T wave inversion, often associated with cardiomyopathy. Knockout mice show biatrial enlargement, ventricular dilation and an increase in myocardial fibrosis compared to littermate controls. Transmission electron microscopy revealed gross abnormalities in sarcomeric and mitochondrial structure and organisation.

Conclusion:
Cardiac specific knockout of Slc5a6 results in ventricular dilation and pathological remodelling of the heart, resulting in stiffness and rigidity throughout the myocardium. This leads to reduced cardiac function overall, suggesting that Slc5a6 plays an important novel role within the heart.

O-015 | Low incidence of left ventricular noncompaction in a pathology archive

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Left ventricular noncompaction cardiomyopathy (LVNC) is characterized by an excessive trabeculae/compact wall thickness ratio. The incidence is increasing towards 10% of the general population when the diagnoses are based on non-invasive imaging, such as echocardiography, MRI or CT. In contrast, foundational studies on autopsy collections indicated that LVNC is rare. Here, we assessed the incidence of LVNC in 728 post-natal autopsy hearts. We found 15 to have atypical trabeculation (one or more prominent trabeculae, abnormal trabeculae/compact wall ratio, or excessive number of trabeculae). Of these, 13 had a trabeculae/compact ratio less than 2 (excluding the apex), and only two cases were considered LVNC positive. In Case 1 (died in 1996), the trabecular/compact ratio was LVNC-positive (2.9), the trabeculae were not excessive, but the LV was extremely dilated and thin-walled. Case 2 was from a 74-year-old woman (died in 1991). The left ventricle had far more trabeculae than any other ventricle we inspected. Using high-resolution MRI, we compared this specimen to 6 normal hearts and found a remarkably higher trabeculae/compact ratio (6.0 vs. 1.00.1) as well as a higher percentage of LV tissue volume composed of trabeculae (40.9% vs. 15.61.7%). While Case 1 could be dilated cardiomyopathy that progressed to LVNC, Case 2 is bona fide LVNC, likely of the benign type given the long life-span. In conclusion, we report a low incidence of LVNC (1-2/728). It is not clear whether the incidence of false-negative diagnosis in post-mortem assessments is comparable to the incidence of false-positive diagnosis in non-invasive imaging.

O-016 | Modelling Mybpc3-related HCM and LVNC

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Background:
Familial cardiomyopathies are severe, incurable, genetic diseases with heterogenous phenotypes, as pedigrees may contain individuals showing features of both Hypertrophic cardiomyopathy (HCM) and Left ventricular Non-Compaction (LVNC) at the same time. This heterogeneity may be explained by the presence of more than one mutation in the affected individual, whose combination leads to the mixed phenotype. Alternatively, specific, dominant-negative mutations may lead to mixed cardiomyopathy phenotypes. Mutations in MYBPC3, encoding a cardiac sarcomeric protein, have been causally related to 15% of all HCM cases.

Material and methods:
Using Crispr-Cas9 technology, we generated three mouse models carrying three different MYBPC3 variants identified in pedigrees containing individuals with HCM or LVNC, aiming to understand how these cardiomyopathies originate and how different mutations in the same gene may produce different cardiac phenotypes.

Results and conclusions:
We analyzed the structural and functional phenotypes of our models at postnatal stages and in the adult and observed that the three different mutations result in different disease phenotypes. We want to understand the molecular changes occurring during development and postnatal stages leading to HCM, LVNC or both. We believe that establishing if HCM and LVNC have a common genetic and developmental substrate will contribute to understanding disease etiology and the design of potential therapeutic treatments.

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O-017 | Gpr126 (ADGRG6) contributes to valve development by regulating EMT of endocardial cells

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The proper formation of heart valves requires the epithelial-to-mesenchymal transition (EMT) of endocardial cells, which populate the so-called endocardial cushions. We have previously shown that Gpr126 is expressed in the ventricular endocardium during development and deletion resulted in embryonic lethality associated with hypotrabeculation. Utilizing our recently published lacZ reporter mouse and anti-GPR126 antibodies, we show here that Gpr126 is expressed also in the mesenchymal cells populating the endocardial cushions at E11.5. Deletion of Gpr126 resulted in fewer invading mesenchymal cells at E11.5. Moreover, fewer migrating cells were observed in endocardial cushion explant cultures from E9.5 Gpr126 knockout embryos compared to wildtype. In order to verify that endocardial Gpr126 is required for survival and proper heart development, we crossed Gpr126tm1b conditional knockout with Tg(Tek-cre)1Ywa/J mouse resulting in truncation of Gpr126 after exon 6 in endocardial/endothelial cells. Surprisingly, the offspring survived close to Mendelian ratio. In contrast, endocardial/endothelial-specific truncation of Gpr126 after exon 2 in Taconic Gpr126 conditional knockout mice resulted in embryonic lethality. The contrasting results suggest that the remaining fragment of Gpr126 in the Gpr126tm1b model, covering part of the extracellular domain, rescued the endothelial related phenotype. This is in concordance with previous data where the extracellular domain of Gpr126 alone rescued trabecular defect in zebrafish. Interestingly the germline deletion of Gpr126 in Gpr126tm1b using Tg(EIIa-cre)C5379Lmgd/J resulted in embryonic lethality. Taken together these data suggest that the extracellular domain of Gpr126 is sufficient for proper heart development and that Gpr126 regulates EMT of the endocardial cells during heart valve development.

O-018 | Mutation found in Mitral Valve Prolapse patients alters Wnt signaling through interactions with a b-catenin antagonist

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Background:
Non-syndromic Mitral Valve Prolapse (MVP) is a common disease with associated morbidities and mortality. The cause of the disease is poorly understood. We have recently identified mutations in the cilia gene, DZIP1 in families with MVP. To identify the function of DZIP1 in valve development, we found a unique binding partner for DZIP1—Chibby1(Cby1) which is a β-catenin antagonist. We hypothesize that DZIP1 regulates mitral valve development through Wnt signaling pathway by interacting with CBY1.

Material and Methods:
Co-immunoprecipitation and immunofluorescence staining on valve tissues were performed for Dzip1 and Cby1. 3D reconstructions were performed on both Dzip1 conditional and Cby1+/- mitral valves. Staining for β-catenin and the downstream effector Lef1 were analyzed by Western and IHC in Cby1 and Dzip1 deficient mice.

Results and Conclusion:
DZIP1 interacts and co-localizes with CBY1 and this interaction is impaired in the context of DZIP1 familial mutations. Nucleus beta-catenin and lef1 expression are increased in Dzip1 mutant MEFs compared with wild type MEFs. However, rescue experiments by transfecting Dzip1 mutatnt MEFs with human Cby1 plasmid decreased Wnt signaling read out. Immunofluorescence suggests that beta-catenin is increased in Dzip1 mutant valve tissues compared to wild type. DZIP1 suppresses Wnt activity to direct mitral valve development through interacting with and stabilizing CBY1.This study reveals a molecular mechanism by which mutations in Dzip1 alters valve development leading to increased β-catenin signaling. As mutations in DZIP1 cause MVP in humans, altered β-catenin signaling may be an early initiating signal in the pathogenesis of MVP.

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O-019 | Temporal requirement of Nkx2-5 defines distinct scaffold and lineage restriction phases of ventricular conduction system development

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Background
The ventricular conduction system (VCS) coordinates heartbeats by rapidly propagating electrical activity through the Purkinje fiber (PF) network. PFs arise from common progenitor cells with contractile cardiomyocytes, however, the timing of segregation between these two lineages is unknown. Common progenitor cells have been localized to Cx40 expressing cells in ventricular trabeculae during embryonic development.

Methods
In order to establish the time window during which PF lineage restriction takes place we used genetic fate mapping and prospective clonal analysis of cardiomyocytes. We crossed inducible Cx40-CreERT2 or Sma-CreERT2 mice with unicolor or multicolor reporters in wildtype and Nkx2-5 heterozygous mutant mice, with severe PF hypoplasia.

Results
Our clonal analysis unexpectedly reveals the existence of cardiomyocytes committed to the PF lineage as early as E7.5, prior to Cx40 expression. These early conductive precursors give rise to a rudimentary PF scaffold. Subsequently, the segregation of common progenitors to a PF fate occurs progressively throughout trabecular morphogenesis, forming a complex PF network at birth. A pool of trabecular cells retains bipotency until late fetal stages and the contractile or conductive identities of trabecular cells are resolved at birth. While early commitment to the VCS occurs normally in Nkx2-5 haploinsufficient embryos, a high level of Nkx2-5 is required for trabecular cardiomyocytes to enter the conductive lineage during subsequent development, identifying the cellular mechanism for PF hypoplasia in Nkx2-5 heterozygous mutant mice.

Conclusion
These results define distinct scaffold and progressive lineage restriction phases during VCS development and reveal that the later requires maximal Nkx2-5 expression levels.

O-020 | A novel approach to understanding how pre-gestational diabetes induces embryonic birth defects

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Congenital heart disease (CHD) is the most common human birth defect, occurring in ~1% in the general population. All forms of maternal diabetes mellitus (type 1, type 2 and gestational) increase the risk of CHD by ~4-fold and the risk of stillbirth or miscarriage by 5-fold. We aim to understand the molecular mechanisms behind these effects by using the ßV59M mouse, a unique inducible and reversible model of diabetes. We observed a variety of effects on embryogenesis, depending on the timing and severity of maternal hyperglycaemia. Structural heart defects (ASD, VSD, TGA, DORV) and myocardial thinning increase in prevalence with increasing severity of maternal diabetes. Some diabetic embryos also show oedema, lymphatic dysfunction and a failure of coronary vascular formation. In addition to cardiac defects, diabetic embryos also present with craniofacial anomalies and/or neural tube defects. Finally, mice with very high hyperglycaemia had fewer successful pregnancies. When diabetes was reversed using glyburide pre-implantation at E3.5, all diabetic mice showed a dramatic reduction in blood glucose from >27.8mmol/l to an average of 8.4mmol/l. Both the number of successful pregnancies and number of "normal" embryos increased, however embryonic blood glucose did not normalise completely. Using a combination of µMRI, transcriptomics and histology we identify why tight glycaemic control before and during pregnancy by diabetic mothers reduces the prevalence of CHDs and other cardiac defects, and also increases the chances of conception and the likelihood of successful pregnancies.

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O-021 | In vivo regeneration of cardiac valves – lessons from zebrafish

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Cardiac valves are fundamental to ensure unidirectional blood flow within the heart. The majority of diseased valves must be replaced by mechanical, biological or synthetic implants. However, much remains unknown about the origin of the cells repopulating the implanted scaffolds, the mechanisms underlying new tissue formation and the molecular factors regulating these processes. Most in vivo models for cardiac valve replacement are large animals which present major technical limitations. Here we introduce zebrafish as a unique model to study cardiac valve regeneration due to the large number of tools and enhanced regenerative capacity. We show that genetic ablation of the atrio-ventricular cardiac valve cells induces a cascade of events encompassing cell cycle re-entry in the surrounding tissues, new valve cell differentiation and secretion of new ECM, which ultimately leads to the formation of new functional valve leaflets within 60 days. Cell tracing experiments determined the contribution of endothelium and kidney marrow-derived precursors to the newly differentiated valve cells. Interestingly, these cells do not invade the decellularized leaflets and instead secrete new ECM components ensuring the stiffness and elastic properties needed for the functional recovery of the atrio-ventricular valve. Furthermore, we identify Transforming Growth Factor beta pathway as a major regulator of the regenerative process by enhancing precursor cell proliferation and new valve cell differentiation.
Overall, we show that zebrafish is a valuable model to identify the cellular and molecular players regulating valve tissue regeneration which may contribute to new strategies to promote the success of valve implant maintenance and growth.

O-022 | Ephrin-B1 links the morphology of the adult cardiomyocyte to its inability to proliferate

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Background: Deciphering the innate mechanisms governing the blockade of proliferation in adult cardiomyocytes (CMs) is challenging for mammalian heart regeneration. Despite the exit of CMs from the cell cycle during the postnatal maturation period coincides with their morphological switch to a typical adult rod-shape, whether these two processes are connected is unknown. Here, we examined the role of ephrin-B1, a CM rod-shape stabilizer, in adult CM proliferation and cardiac regeneration.

Methods: CM proliferation was evaluated in vivo using CM-specific efnb1-KO mice and AAV9-Shefnb1 ii/ in vitro using primary adult CM culture.

Results: Ephrin-B1 expression at the lateral membrane of the CM correlated with the setting of the adult CM rod-polarity and the CM proliferation arrest. Transgenic- or AAV9-based ephrin-B1 repression in adult mouse heart led to substantial proliferation of resident CMs and tissue regeneration to compensate for apex resection, myocardial infarction (MI) and senescence. At resting state, CMs lacking ephrin-B1 did not constitutively proliferate but exhibited proliferation-competent signature (higher mononucleated state/decrease of miR-195 mitotic blocker). Accordingly, neuregulin-1 triggered CM proliferation. Mechanistically, the post-mitotic state of adult CM relies on ephrin-B1 sequestering of inactive phospho-Yap1 at the lateral membrane. Hence, ephrin-B1 repression leads to phospho-Yap1 release in the cytosol but CM quiescence at resting state. Upon cardiac stresses (apectomy, MI, senescence), Yap1 could be activated and translocated to the nucleus to induce proliferation-gene expression and CM proliferation.
Conclusion: Our results identified ephrin-B1 as a new natural locker of adult CM proliferation and a promising target in cardiac regenerative medicine.

O-023 | SHROOM3 is a novel component of the planar cell polarity pathway whose disruption causes congenital heart disease

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In a patient with Congenital Heart Disease (CHD) we previously utilized whole exome sequencing to identify a novel CHD candidate gene, SHROOM3. SHROOM3 is implicated in human neural tube and kidney defects but mostly unexplored in CHD. SHROOM3 protein induces cytoskeletal changes, including ACTOMYOSIN constriction. In addition, SHROOM3 binds DISHEVELED2 and ROCK1, key components of the non-canonical Wnt/planar cell polarity signaling pathway (PCP). PCP influences numerous developmental processes through regulating ACTOMYOSIN constriction. We hypothesize SHROOM3 serves as a link between PCP and ACTOMYOSIN constriction and that disruption causes CHD. To test this hypothesis we analyzed the cardiac phenotype of Shroom3 gene trap knockout mice (Shroom3gt/gt mice). X-Gal staining demonstrates Shroom3 expression in cardiomyocytes and cardiac neural crest cells (cNCC) of the ventricles and outflow tract (OFT) from embryonic day 9.5 onward. In addition to previously reported neural tube defects, Shroom3gt/gt mice have incompletely penetrant heart defects, including ventricular septal defects, double outlet right ventricle and Shroom3gt/gt mice have dimished cNCC staining in the OFT; this CHD spectrum phenocopies PCP disruption. To further demonstrate genetic interaction between Shroom3 and PCP, we identified increased frequency of heart defects in compound Shroom3gt/+;Dvl2+/- embryos compared to Shroom3gt/+ embryos and Dvl2+/- embryos. In addition, Shroom3gt/gt mice have disrupted PCP components by immunohistochemistry, immunoblot and gene expression analysis. Finally, we utilized bioinformatic analysis to demonstrate that patients with PCP-related CHD phenotypes have rare, potentially damaging SHROOM3 variants. These data help strengthen SHROOM3 as a novel CHD candidate gene and a component of the PCP signaling pathway.

POSTER PRESENTATIONS

P001 | Linking anatomy, development and imaging: a review of aortic arch anomalies

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Background: Aortic arch anomalies form via spatio-temporal developmental disruptions potentially leading to vascular ring formation. An update of the historical Edwards classification was recently proposed. This study reviewed anatomical specimens and assessed in vivo aortic arch imaging. This illustrated common anatomical features, evaluating their role in symptom development.

Materials/Methods: Five specimens from the BCH cardiac archive were evaluated. We also assessed antenatal echocardiograms and 170 paediatric CT/MRI of arch anomalies patients. In double aortic arches (DAA), patency and dominance were assessed. Presence of Kommerell diverticulum (KD) was noted in aberrant subclavian cases. The laterality of the arterial ligament was observed in those with a right aortic arch/mirrored branching. The manifestation of compressive ring-related symptoms was noted.

Results: All specimens have their embryological bases explained in accordance with both classifications. 55% of DAA patients had dual arch patency and 77.8% had right arch dominance. Neither of these influenced symptom development. In aberrant subclavian patients, those with KD were more likely to have symptoms ($P < 0.05$). A left arterial ligament was more common in right arch/mirrored branching patients, but there was no difference symptoms-wise between groups ($P = 0.692$).

Conclusion: Aortic arch anomalies remain rare in cardiac archives but are increasingly diagnosed in practice. New classifications may require further validation on larger samples and recent developmental findings. When present KD implies a high chance of symptom development in aberrant subclavian cases. This study did not identify the arterial ligament insertion point, which may be a useful prognostic tool. Echocardiography and/or better visualising methods could help antenatal prediction.

P002 | Duration of cardiac competence in the avian head mesoderm

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General approaches in managing cardiovascular disease burden can reduce risk factors and control symptoms. However, when a heart attack occurs, cardiomyocytes perish and there is no feasible strategy to replace these lost cells. While progress is made to generate cardiomyocytes in vitro, they fail to beat according to the rhythm set by the existing heart. Yet in the embryo, cells that achieve this are the secondary heart field cells. These cells are added onto the primitive heart over a prolonged time and beat accordingly. The challenge is to characterise these cells such that the recruitment and integration process can be recapitulated in a patient. Previous studies showed that initially, the entire head mesoderm is cardiac competent, but cells from the head mesoderm also form non-cardiac tissues such as skeletal muscle. We thus determined the time window in which the head mesoderm is heart-competent as stepping stone towards further analyses, using the chicken embryo as a model for human cardiogenesis. Bone morphogenetic proteins as known cardiac inducers were loaded on beads and implanted into the paraxial aspect of the head mesoderm that is fated to produce non-cardiogenic cell types; embryos were analysed by in situ hybridisation for the expression of various cardiac and non-cardiac marker genes. We found that the head mesoderm has full cardiac competence up to stages HH5/6, which is 12 hours after the head mesoderm forms at HH3/4. In the subsequent hours, competence declines and head skeletal muscle precursor markers become activated, suggesting a shift to myogenic competence.

P003 | Analysis of Congenital Heart Defects In Down Syndrome

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Background:
Down syndrome (DS) is caused by trisomy of human chromosome 21 (Hsa21) and leads to a large spectrum of phenotypes including congenital heart defects (CHD). These phenotypes arise from an extra copy of one or more of the ~230 genes on Hsa21, however the gene(s) needed in three copies to cause cardiac defects and the underlying mechanisms remain elusive. We generated mouse strains with duplications of regions of mouse chromosome 16 that are orthologous to Hsa21. The largest of these duplication strains (Dp1Tyb) models the types of CHD seen in babies with DS such as VSD and AVSD. Septation of the primitive heart is complex and requires the growth and fusion of several tissues. Dp1Tyb mouse hearts fail to septate properly. The overall aim is to discover a mechanistic explanation for the origin of cardiac defects in DS and answer the following question: What are the developmental processes that are impaired in Dp1Tyb mice?

Methods:
Using a combination of genetic reporters with imaging on fixed embryonic hearts, I studied the cellular behaviours and the signalling pathways that are required for heart septation and may be impaired in Dp1Tyb embryos.
Results: Preliminary data using E11.5 embryonic hearts showed that the cellular density of the atrioventricular cushions (AVC) is reduced in Dp1Tyb. Moreover, the amount of NFATC1 is decreased in Dp1Tyb AVC, indicating that NFAT signalling may be decreased.

Conclusions:
The decreased endocardial NFAT signalling might lead to decreased mesenchymal proliferation and cause the reduced AVC density in Dp1Tyb mice.

P004 | Innovative biotechnology for generation of cardiac tissue using animal model

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Background:
Cardiac tissue bio-engineering represents an important research direction for the patient-specific myocardial reconstruction therapies. The study aims to obtain cardiac tissue on a rat animal model, starting with a decellularized heart used as a matrix for grafting human cells- adult mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs).

Material and methods:
The heart of Sprague-Dawley rats was attached to decellularization devices - Langendorff-Radnoti and simplified Langendorff in alternative electric field and perfused with SDS/EDTA solutions. The decellularization efficiency was evaluated by optic and electron microscopy, measurement of DNA and protein level. iPSCs were stained for specific marker expression.
Adult MSCs and iPSCs (obtained through T cells reprogramming) were differentiated in 2D and 3D model towards cardiomyocytes using 5-azacytidine.

Results and conclusion:
Simplified Langendorff system in alternative electric field was more effective in decellularization of rat heart. Optic and electron microscopy revealed maintenance of collagen fibers architecture, the absence of cells and vascular permeability. T cells were expanded in specific media (Dynabeads© T-activator CD3/CD28) and reprogramming towards iPSCs. Immunofluorescence revealed positive marker expression on 80% of iPSC - Oct-4, Nanog, Sox-2, SSEA-4 and Tra-1-81. MSCs induced towards cardiomyocytes with 5-azacytidine in vitro 2D had a differentiation rate of 50%, while 3D differentiation, in continuous perfusion for 24 hours increased the rate.

Adult MSCs can recellularize the matrix with reduce efficiency, while the iPSCs could have a better grafting and cardiac differentiation rate. Simultaneous intraventricular injection and coronary perfusion with recellularization cells is more efficient compared with only perfusion.

P005 | CCBE1 in cardiogenesis: from developmental biology to regenerative medicine approaches

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Given that cardiovascular disease is a major cause of death worldwide, there is an important need to fully understand heart development and regeneration. We show that CCBE1 is necessary for correct coronary vessel development. Ccbe1-deficient mice exhibit defects in dorsal coronary growth that sprouts from the SV endocardium. This disruption of coronary formation correlates with abnormal processing of VEGF-C pro-peptides, indicating a VEGF-C-dependent signaling alteration. Moreover, Ccbe1 loss-of-function leads to the development of defective dorsal and ventral intramyocardial vessels, suggesting a VEGF-C independent activity. In accordance with lack of coronary endothelium, Ccbe1 mutant hearts also display noncompaction phenotype with decreased cardiomyocyte proliferation.

We also found that Ccbe1 is markedly enriched in Isl1-positive cardiac progenitors isolated from differentiating ESCs and from embryonic hearts developing in vivo. Knock-down of Ccbe1 activity impaired differentiation of embryonic stem cells along the cardiac mesoderm lineage.
To address the paracrine role of Ccbe1 in the specification of endothelial progenitor cells in vitro, we used ES Cell-derived Endothelial Precursor Cells (EPC) cocultured with mouse embryonic fibroblasts from both wild type and CCBE1-KO mice in the form of spheroids. Using this approach, we could uncover the potential role of CCBE1 in VEGF-C dependent and independent endothelial cell specification and vascular network formation.

In conclusion, our results show that, during embryogenesis, CCBE1 is essential for coronary vasculature development and proper ventricular compaction, and also required for the commitment of cardiovascular precursors and consequently, for the formation of cardiac myocytes and endothelial cells in differentiating mouse and human ESCs.

P006 | Model of the systemic inflammatory response syndrome to study the effect of antimicrobial agents on myocardial infarct size

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Purpose: To investigate the effect of antimicrobial agents (AAs) on myocardial infarct size (IS) and proinflammatory cytokine levels in obese rats with chemically induced colitis (CIC).

Methods: Diet-induced obesity was produced in male Wistar rats by daily intragastric administration of plant-derived hydrogenated fat with chemically induced colitis. During 3 days, the animals were randomized to receive one the following AAs treatment: 1) ciprofloxacin(C); 2) azithromycin(A); 3) rifaximin(R); 4) tetracycline(T); 5) ampicillin +metronidazole+clarithromycin (AMC). Five days later isolated Langendorff-perfused hearts were subjected to 30-min global ischemia and 120-min reperfusion. Left ventricular pressures, heart rate, and coronary flow were monitored throughout the experiments. IS was determined histochemically. The levels of lipopolysaccharide (LPS) and cytokines were measured with ELISA.

Results: Weight of visceral fat was significantly higher in animals treated with hydrogenated oil. CIC tended to increase IS (65±6 vs. 61±2.2 %) in obese controls. IS was 68±6.2, 60±2.0, 64±5.6, 78±2.0, and 83±4.7 % in groups C (p=NS), A (p=NS), R (p=NS), T (p=0.049), and AMC (p=0.028), respectively vs.CIC. Hemodynamic data generally paralleled the results on IS. CIC was characterized by elevation of proinflammatory cytokines: TNFa, IL-8 and LPS.

Conclusion: R and T generally reduced the levels of proinflammatory cytokines in obese animals with CIC, while other AAs had minimal effect on systemic inflammation. Treatment of obese animals with CIC with T and combination of AMC resulted in significantly larger infarct size. The model can be used to study the effect of drugs on myocardial resistance to ischemia-reperfusion.

P007 | A severe prolonged decrease in left heart flow results in left heart hypoplasia in mid-gestation fetal lambs

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Background
The aetiology of hypoplastic left heart syndrome (HLHS) is unclear. We hypothesized that low flow in the fetal left heart results in hypoplasia

Methods
LV inflow obstruction created in 75day fetal lambs by percutaneous implantation of LA coils and harvest at 125 days.

Results
19 coiled fetuses and 1 control died prior to harvest. 15 coiled fetuses reached 125 days, 1 with severe mitral regurgitation had anasarca (10.6kg). Effective coiling led to severe acquired mitral stenosis and a slit-like LV. LV inflow and aortic valve (AoV) outflow was so low in 9 that there was retrograde flow in the ascending aorta (AAo). 4 of 9 had no AoV outflow, with the brain and coronaries wholly perfused from the duct. These low-flow LV had the most severe left heart hypoplasia and the LV was no longer apex forming. Due to variation in fetal size (1.74-4.45kg), each fetus's left heart dimension was normalized to its corresponding right-heart structure. These were all smaller in the low-flow LV hearts as compared to controls:

LV/RV free-wall weight (lowflow 0.63±0.19, control 0.99±0.17; p=0.008)
AoV/Pulmonary valve diameter (lowflow 0.48±0.03, control 0.82±0.15; p= 0.0004)
AAo/Pulmonary artery diameter (lowflow 0.56±0.09, control 0.83±0.11; p=0.0004)
LV/RV end-diastolic diameter (lowflow 0.48±0.23, control 1.0±0.12; p=0.002)
LV/RV end-diastolic length (lowflow 0.67±0.21, control 1.13±0.17; p=0.0036).

Conclusion
Long after cardiogenesis, a severe, prolonged (1/3 gestation) decrease in LV flow is sufficient to produce hypoplasia with retrograde perfusion of the brain and coronaries, similar to human HLHS. This model will allow investigation of HLHS development and therapies

P008 | Genetic Lineage Tracing of Sca-1+ Cells Reveals Their Robust Myogenic Contribution to the Adult Heart

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Background:
The mammalian heart contains a pool of resident cardiac stem/progenitor cells (CSCs) initially characterized by c-kit expression as one surface marker. However, the lone c-kit expression does not equate to CSC identification. A variety of overlapping markers have been used to isolate CSCs. Nevertheless, the only population of cells in the heart which possesses in vitro/in vivo stemness property resides within the CD45negative/CD31negative/c-Kit positive/low cell fraction. Half of this cell fraction express Sca-1. Thus, we used Sca-1 as a genetic fate map marker to investigate the role of Sca-1-expressing CSCs in the adult heart.

Material and methods:
We used Sca-1Cre transgenic mice, expressing a constitutive Cre recombinase driven by the entire regulatory elements of the Sca-1 locus, to track the fate of Sca-1-expressing CSCs in vivo. We crossbred Sca-1Cre transgenic mice to R26mT/mG Cre-reporter mice to permanently label all the Sca-1-expressing cells and map their fate in the heart from embryo to adulthood and after injury.

Results and Conclusion:
In double-mutant Sca-1Cre::R26mT/mG mice, Sca-1 expression is activated in late fetal life when the heart is already structurally formed. From neonatal to adult life and until old age, the heart is progressively and robustly replenished of new CMs derived from Sca-1-labeled progenitors. New CM formation after injury is mainly the product of Sca-1-labeled progenitor differentiation in vivo. Thus, while the embryonic/fetal heart and its CM population originate from Sca-1negative progenitors, the adult heart robustly replenishes CMs lost by wear and tear and after injury mainly by endogenous CSC activation and differentiation.

P009 | Generation and validation of a novel model for mitral valve prolapse based on human familial mutations.

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Background:
Mitral valve prolapse is a common valve disease affecting 1 in 40 individuals with poorly understood etiology. Current effective treatment options for MVP are restricted to surgical interventions. Even though surgical repair/replacement is corrective for the valve defects, roughly 20% of patients still develop significant fibrosis and associated arrythmia's and heart failure. This underscores the importance for developing genetically accurate murine models to study the natural history of MVP and decipher the molecular origins of the left ventricular fibrosis.

Methods:
Using a combination of linkage and exome sequencing, we identified a mutation in the cilia gene, DZIP1 in multiple families with inherited non-syndromic MVP. CRISPR-Cas9 genome editing was used to generate a mouse model with the same human variant. Histology and immunohistochemistry (IHC) was performed to validate phenotype as well as determine whether fibrosis is evident in the left ventricle as observed in some patients.

Results and Discussion:
Histology and IHC confirmed myxomatous degeneration of the mitral valves and MVP was observed by echocardiography in 100% of adult animals analyzed. Pronounced papillary fibrosis coincident with MVP was observed in all affected animals and excess collagen deposition was also detected in the inferobasal myocardium. These data demonstrate the first generation of a genetically accurate murine model for non-syndromic MVP and can be used to identify pathways that contribute to the left ventricular fibrosis.

P010 | Myocardial healing in Leopard Gecko (*Eublepharis macularius*): a new model organism of heart regeneration

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Background:
Ischemic heart disease is the leading cause of death worlwide and leads to myocardial necrosis. Myocardial necrosis can be compensated for by regeneration, but most studies on regeneration are done on homeotherms that exhibit very limited regenerative capacity. Fish exhibit a pronounced capacity for regeneration, but they are evolutionarily and physiologically distinct from mammals. Therefore, we look for poikilothermic model species, which could provide us useful comparison to mammals. Leopard Gecko is evolutionarily closer to mammals, there is a lung circulation and also there is a more developed compact ventricular wall, higher blood pressure, and a known pronounced regenerative potential of the tail.

Materials and Methods:
In this pilot study we performed ventricular apex cryoinjury and apex amputation in two subadult males and two subadult females Leopard Geckos (*Eublepharis macularius*). Cryoinjury was performed on the apical region of the heart by liquid nitrogen frozen wire (diameter 3 mm). The other method was apex amputation (20% of the ventricle at the apex). During one month after intervention we collected hearts and used immunohistochemistry (Pentachrome, Myosin Heavy Chain Antibody) to determine healing process in injured area.

Results and Conclusion:
Cryoinjury to the apex was well tolerated, while apex amputation was not. The cryo-injured ventricles showed necrosis, ballooning of the apex, but also activation of the epicardium (thickening), vascularization of the area, and formation of islands of new myocardium. Our preliminary analysis suggests that cryoinjury in Leopard Geckos is promising model system to study the myocardial regeneration.
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P011 | Evaluation of analgesic treatment after cryoinjury in zebrafish heart

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While humans are not able to regenerate their heart after myocardial infarction, zebrafish heart can fully recover after an injury, making it an excellent model organism to study how to overcome limited regenerative response in humans. To mimic human infraction, cryoinjury method has been broadly used in order to induce an 'ischemia-like' injury in the ventricle of adult zebrafish. This procedure has been shown to be well tolerated by animals, which recover fast after the injury. Recently it has been reported, however, that fish can perceive pain, indicating that current protocols need to be revised, assuring that any possible pain and discomfort can be reduced to the minimum.
In this study, we investigated the effect of two different analgesic treatments, lidocaine and morphine, after cryoinjury. We addressed their effects on the alleviation of possible pain as well as on the heart regenerative process. Our data show that lidocaine treatment does not have any impact on zebrafish behaviour and pain alleviation, while it negatively affects the regeneration by slowing down the process. On the contrary, morphine treatment has significantly improved behaviour of fish until 6H after surgery and no histological differences in wound healing and collagen deposition were detected. Importantly, we have confirmed that the gene expression does not differ between injured untreated fish and injured fish treated with morphine by using single cell RNA-sequencing technology.
Taken together, we recommend refining the cryoinjury procedure by using the 6H-morphine treatment after the injury improving animal health without impacting the regeneration process.

P012 | Using avian models to study the developmental basis of cardiac repair and regeneration

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Tissue regeneration is a common phenomenon in Animal phylogeny that is far less frequent in Vertebrates. Some anamniote vertebrates like the zebrafish or the axolotl can regenerate multiple tissues/organs after an experimental damage, including the cardiac ventricle. Amniote vertebrates, however, seem to have lost the ability to regenerate heart tissues postnatally. This striking difference in the regenerative potential of vertebrate cardiac tissues is biomedically relevant, as the high mortality rate of cardiac ischemic disease-myocardial infarction directly relates with the lack of effective adult human myocardial regeneration. Instead, human ventricular response to injury is the generation of a fibrotic, primarily reparative wound. The absence of cardiac regeneration in mammals is frequently associated with the low proliferative capacity of adult cardiomyocytes. We herein suggest that studying cardiac tissue responses to damage in the high cardiomyocyte proliferation environment of the embryo is an alternative approach to study the cellular and molecular basis of cardiac repair and regeneraration. In this context, the avian embryo becomes a suitable model for this type of research because no experimental studies on the regenerative potential of avian and/or embryonic cardiac tissues are available in the literature. In this work, we use a cardiac cryoinjury protocol to study reparative/regenerative responses to damage in the embryonic ventricle of different avian species (chick, quail and duck). Our results show clear differences in the response to damage in these three embryonic species, suggesting that cardiac tissue regeneration mechanisms can significantly differ in closely related animals.

P013 | A mouse model for different types of atrioventricular septal defect

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Background
Atrioventricular septation requires the co-ordination of development and eventual fusion of four mesenchymal tissues, plus the proper development of the muscular atrial and ventricular septae. The fusion of these mesenchymal components creates a 'key-stone' within the heart, crucial for the septation of the four-chambered mammalian heart.

Materials and Methods
Optical projection tomography and High Resolution Episcopic Microscopy identified atrioventricular septal defects (AVSDs) in mouse embryonic hearts lacking the retinoic acid-metabolizing enzyme CYP26B1.

Results
AVSDs were found in all embryos examined, in the form of common atrioventricular junction (cAVJ) with either common or partitioned AV valves. Many AVSDs had an extremely small atrial and larger ventricular component, thus modelling an extremely rare form of AVSD found in humans. The mesenchymal cap of the primary atrial septum was frequently either missing or small. The AV valve cushions were found to be bulky and hyperplastic and have not remodelled into leaflets with normal delaminated excavated morphology. In contrast, the vestibular spine appeared normal in most hearts.

Conclusion
Retinoic acid dysregulation affects three of the four mesenchymal AVS components. We hypothesize that epithelial-mesenchymal transition defects may play a role in the aetiology of these AVSDs. Further investigation of these defects will identify the contribution of each defective AVS component to the overall pathogenic phenotype. Mouse models, provide a valuable resource for the investigation of the anatomy and causes of complex cardiac anomalies also seen in humans.

P014 | Role of Gpr126 signaling during heart development

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Trabeculae formation relies on a tightly regulated endocardium–myocardium cross-talk, in which the NOTCH signaling plays a crucial role. Gene profiling analysis of Notch mutant hearts identified the adhesion G protein-coupled receptor 126 (Gpr126) as a candidate molecule guiding trabeculation downstream of NOTCH. Previous studies reported that Gpr126 is required for chamber development in mice and zebrafish, but less is known about the cellular processes and signaling mechanisms involved. We have generated various loss-of function alleles in mouse using the CRISPR/Cas9 technology. Standard deletion of exon 6 (Gpr126 Δ6) results in the near absence of Gpr126 mRNA and causes embryonic lethality at mid-gestation. Mutants die between E11.5 and E13.5 and exhibit various cardiac defects, including thinning of the ventricular wall, ventricular septal defects and blood accumulation in the chambers. However, cellular proliferation and expression of various chamber-patterning and metabolic markers appears unaffected in Gpr126 standard mutants. Cardiac-specific conditional disruption of Gpr126 (Gpr126 flox) does not cause any heart phenotype and mutants reach adulthood, suggesting that impaired heart development observed in standard knockout embryos may be secondary to developmental defects in other organ(s). We are currently carrying experiments to ascertain what is the reason of demise of standard Gpr126 mutant mice and have also generated Gpr126 mutants in zebrafish to obtain a complementary view of the role of gpr126 in the heart.

Funding sources
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P015 | Pre-capillary Pulmonary Hypertension Associated with Signs of Peripheral Vascular Remodeling and Cardiovascular Coupling

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Background
Pulmonary hypertension (PH) is characterized by pressure increase in the pulmonary artery and can be a result of pathological remodeling of the vessel wall. Peripheral vasculopathy in the large systemic arteries is less well studied in patients with PH. Our main aim was to assess the degree of vasculopathy in the Common Carotid Artery (CCA) in patients with pre-capillary PH and compare to healthy subjects.

Material and methods
In 23 patients with pre-capillary PH (PH WHO groups 1, 3, 4, 5) and 30 healthy subjects, the intima and media layers of the CCA were measured with non-invasive high-frequency (22 MHz) ultrasound. The intima/media (I/M) ratio was calculated. The PH diagnosis was confirmed with right heart catheterization.

Results
Patients with pre-capillary PH (and all sub-groups), had a significantly thicker CCA intima layer; median difference 0.05 mm [95% CI 0.03, 0.06], $p < .0001$; and a higher CCA I/M ratio, median difference 0.23 [95% CI 0.12, 0.25], $p < .0001$, compared to healthy subjects. Adjustment for age revealed similar significance levels. All vascular parameters were associated with a number of cardiac hemodynamic markers in patients, with strongest CCA intima correlation to right ventricular systolic pressure ($rs = .716$ [95% CI -.032, .973] $p = .013$).

Conclusions
Patients with pre-capillary PH also have negatively affected peripheral arteries with signs of peripheral vascular remodeling (thicker CCA intima layer and higher CCA I/M ratio), compared to healthy subjects, even after age adjustment. Our results indicates presence of cardiovascular coupling in patients with pre-capillary PH.

P017 | Characterization of Neurotrophins Expression in Coronary Vessels

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Described for the first time in the nervous system, neurotrophins perform essential functions in this tissue contributing to axonal growth, neuronal survival or synaptic plasticity among others. However, neutrophins are also expressed in non-nervous tissues as bone, immune system or the cardiovascular system. The available information on the role of neurotrophins during heart development is scarce, although several studies have highlighted the importance of these molecules in early cardiomyocyte proliferation, sympathetic nerve growth and coronary vessel maturation. In particular, neurotrophins seem to be implicated in the development of the characteristic neurovascular bands of the adult heart. Since neurovascular interactions are vital to the homeostasis of multiple organs we suggest they play a similar role in the adult heart. In this study, immunohistochemical (laser confocal microscopy) and FACS techniques have been applied to wild type and NT3-LacZ+/- cardiac tissues to characterize neurotrophin signalling elements in adult homeostatic hearts, paying special attention to coronary vessels and cardiac nerves interaction. Our results show that neurotrophin receptor TrkB in sympathetic nerves as well as in coronary endothelium and smooth muscle during the latest stages of development. In the adult, nerves TrkB expression is reduced, but it remains conspicuous in coronary vessels, most especially in the adventitial layer. We also show that NT3 is expressed in the embryonic early myocardium but then this expression reduced in the adult, which retains NT3 coronary smooth muscle expression.

P018 | Myocardial fibrosis and regenerative processes in cardiac diseases

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Myocardial i fibrosis is a part of the pathological remodeling in cardiac diseases, leads to stiffness of the myocardial wall and cardiac dysfunction. Myocardial fibrosis may be due to pressure or volume overload, ischemic heart disease (IHD), and is characterized by an impaired collagen turnover and intensive accumulation. This dysregulation of collagen turnover occurs mainly in phenotypically transformed fibroblasts-myofibroblasts. There is a significant loss of cardiomyocytes and development of myocardial fibrosis in IHD and heart failure (HF). We suggest that increased proliferation of myofibroblasts prevents proliferation of dedifferentiated cardiomyocytes involved in the regenerative processes in the myocardium in HF.

The aim to assess fibrotic changes in the myocardium in IHD and HF, identify the dedifferentiated cardiomyocytes in the same samples.

Surgical samples of the atrial appendages of 20 patients were examined. Histological sections were stained with hematoxylin-eosin, Van Gieson and Masson. Electron microscopy was used. In almost all samples, a high degree of fibrosis was detected. Dedifferentiated cardiomyocytes were revealed.
Significant severity of myocardial fibrosis is associated with higher mortality in HF; therefore, detection, prevention and regression of fibrosis is one of the most important goals in the treatment of HF, which can lead to activation of regenerative processes, proliferation and differentiation of cardiomyocytes. The search for targets of myocardial fibrosis and then the translation of these mechanisms for personalized medicine is important for pharmacological approaches, and most importantly, for regenerative therapy for HF

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P019 | Characterization of Epicardial-Derived Exosomes

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Exosomes are small cellular vesicles (30-150nm) functioning as cell-to-cell message (miRNAs, mRNAs and divers proteins) carriers that play a key role in many biological processes. Conditions as ischemic cardiomyopathy-myocardial infarction (MI) are usually diagnosed at advanced stages of the disease, and early subclinical diagnostic markers have not been identified yet. Cardiac fibroblasts involved in post-MI ventricular remodeling are derived from the embryonic epicardium. Cardiac fibroblasts are specifically activated (proliferation, migration, collagen synthesis) upon ischemic heart damage to compensate for cardiomyocyte loss. Our work focuses in the isolation and characterization of exosomes from a continuous epicardial cell line (EPIC). Our aim is to characterize epicardial-derived exosomes and define the molecular composition of these nanovesicles in different physiological contexts. In order to tackle this scientific objective, EPIC cells are cultured under normoxic and hypoxic conditions and exosomes isolated from culture supernatant by ultracentrifugation and probed for confocal and TIRF imaging. Our data indicates that EPIC cells secrete a considerable amount of exosome, and that the amount of these vesicles increases in the culture medium exosome when EPIC cells are incubated in hypoxia (5% oxygen). Moreover, when EPIC-derived exosomes are co-cultured with EPIC cells, the former are fastly internalized by some of the cells of this continuous line. TEM and proteomic analysis are being performed to assess EPIC exosomes' structucture, size and cargo in normoxic and hypoxic states. Our short term objective is to evaluate the effect of EPIC-derived exosomes on the phenotype, function and transcriptomic profile of cultured cardiomyocytes.

P020 | FGF10: a target for heart repair and regeneration

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Background:
The stimulation of terminally differentiated cardiomyocyte proliferation represents one of the main therapeutic approach for heart regeneration. We uncovered a role for the Fibroblast Growth Factor 10 (FGF10) signalling in regulating both fetal and adult cardiomyocyte proliferation.

Aim: Our study aims to determine the relevance of FGF10 as a potential target for heart regeneration.

Methods: To this end, we used an experimental mouse model of Myocardial Infarction (MI), together with Fgf10 gain and loss of function mouse models.

Results:
Adult transgenic mice with reduced Fgf10 expression subjected to MI displayed impaired cardiomyocyte proliferation and enhanced cardiac fibrosis, leading to a worsened cardiac function and remodelling post-MI. In contrast, conditional overexpression of Fgf10 post-MI revealed that by promoting cardiomyocyte proliferation and preventing cardiac fibrosis infiltration, FGF10 post-MI preserves cardiac remodelling and function. Deep RNA-sequencing analysis performed on WT and Fgf10-overexpressing mice 3 weeks post-MI suggests that FGF10 promotes cardiac regeneration by modulating diverse signalling events already identified as regenerative processes including the Hippo pathway. We then determined, using in vitro experiments, that FGF10 directly prevents cardiac myofibroblast activation and thus fibrotic infiltration. Finally, we investigated FGF10 expression in human failing explanted heart samples. Our results revealed that elevated myocardial FGF10 levels in the injured ventricle strongly correlate with enhanced cardiomyocyte proliferation and reduced fibrosis infiltration.

Conclusion:
Altogether, this study thus identifies FGF10 as a potential target to improve the limited innate regenerative capacities of the myocardium after injury, of direct clinical relevance for heart regeneration.

P021 | Specific protein degradation mechanisms underlying recessive cathecolaminergic polymorphic ventricular tachycardia

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Background:
Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inheritable cardiac disorder associated with exercise and stress-induced life-threatening arrhythmias occurring in the structurally intact heart, leading to sudden death in young individuals. The second most common type of CPVT is caused by mutations in the cardiac calsequestrin gene (CASQ2), which are responsible for the rare autosomal recessive form of the disease. CASQ2 and its interacting partner Triadin (TRDN) regulate the release of calcium ions from the sarcoplasmic reticulum via the Ryanodine Receptor (RyR2). So far, the mechanisms underlying the degradation of these proteins are not understood.

Material and methods:
Here we used a knock-in mouse carrier of a CASQ2 point mutation that causes an amino acid change at position 33 (R33Q). Protein expression and posttranslational modifications were analyzed by mass spectrometry proteomics.

Results and conclusions:
CASQ2 and TRDN levels are reduced in this model, with no changes in the mRNA levels. Mass spectrometry revealed an increase in the endoplasmic reticulum stress pathway, and in regulators of calcium release and uptake, such as the luminal sarcoplasmic reticulum histidine rich calcium binging protein.We also found a decrease in phosphorylated phospholamban. These changes may deregulate calcium homeostasis that would lead to CASQ2 and TRDN protein degradation. Experiments in neonatal cardiomyocytes demonstrated that CASQ2 R33Q is degraded through the ERAD pathway and by the proteasome. In contrast, TRDN is degraded by calpain.

Our findings may increase our understanding of the molecular mechanisms underlying CPVT and unveil new therapeutic targets to treat this disease.

P022 | Histopathology of explanted cardiovascular devices for interventional therapy of congenital heart disease

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Objectives:
The field of interventional therapy for congenital heart disease is rapidly developing. However, knowledge about biocompatibility of the metallic and/or textile implants is limited.

Methods:
We studied and compared tissue reactions of >100 cardiovascular implants after surgical removal (vascular stents, valved stent grafts, septal defect occluder, occluder of the arterial duct) with an implantation time of between few hours and 15 years. Explants were worked up using a uniform protocol with methylmethacrylate embedding after fixation in formalin. Histology and immunohistochemistry was performed after sawing and grinding of the hard resin blocs.

Results:
After initial fibrin clotting at the implant surface, ingrowth of fibromuscular cells with antigen characteristics of vascular smooth muscle cells was seen in a material-dependent time pattern in stents ("intimal hyperplasia") as well as within occlusion devices. Immunohistochemistry revealed the growth of a monolayer of endothelial cells on the intravascular surface of the implants as early as 14 to 20 days after implantation. Different types of inflammatory reactions (granulocytic, lymphocytic, macrophages/foreign body reaction) could be identified at the implant/tissue interface. Lymphocytes and foreign body giant cells were detected up to 15 years after implantation. Explants with significant chronic inflammation tended to show more calcifications.

Conclusions:
Biocompatibility screening revealed regular ingrowth and endothelialisation of most explants but persisting inflammatory reactions after implantation of interventional devices within the cardiovascular system. Calcifications seem to coincide with inflammatory reactions. Our results emphasize the importance of advances in the development of biodegradable implants.

P023 | Desert Hedgehog signaling contributes to mitral valve remodeling during development

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Background: Mitral Valve Prolapse (MVP) is a prevalent cardiac disease that can lead to serious secondary complications. Recent discoveries by our lab demonstrated that primary cilia are critical regulators of valvulogenesis and their perturbation results in myxomatous degeneration. Hedgehog (HH) signaling is associated with primary cilia, and here we sought to determine this pathway's involvement in mitral development and disease.

Materials and Methods: RT-PCR, Westerns and immunohistochemistry were utilized to investigate HH signaling component expression in vitro and in vivo. Desert hedgehog (Dhh) was conditionally ablated in valvular interstitial cells (VICs) and endocardial cells (VECs) of murine mitral valves to assess changes in morphology via 3D-reconstructions. Embryonic chicken VICs were cultured and seeded in collagen hydrogels to evaluate contraction.

Results and Conclusions: Developing mitral valves display active HH signaling through localization of GPCR, Smoothened, onto primary cilia of VICs and DHH ligand expression by VECs. Global Dhh ablation in mice results in a myxomatous phenotype similar to that of MVP patients. Endocardial-specific Dhh deletion is sufficient to cause dysmorphic valves, suggesting paracrine cross-talk between endocardium and cilia-expressing VICs. DHH treatment induced hydrogel contraction by chicken VICs, which was attenuated by HH-inhibitor, Cyclopamine. DHH signaling functions via a non-canonical (Gli-independent) mechanism to induce temporal smooth muscle actin (SMA) expression from E13.5 through neonatal timepoints. These studies describe novel mechanisms of Cilia-DHH-SMA signaling that promote mitral valve remodeling and may help to explain known associations between cilia defects and MVP.

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P024 | From mutation to malformation: molecular mechanisms associating HOXA1 mutations to bicuspid aortic valve

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Background
Bicuspid aortic valve (BAV) is the most common cardiovascular malformation (0.6-2% of the population). It is hereditary in 89% of diagnosed cases, however, only few genes, particularly NOTCH1 and GATA5, have been linked to BAV in humans.
Among a cohort of 338 patients with BAV, 33 patients were identified to display heterozygous in-frame addition or deletion of nucleotides in the HOXA1 gene. These mutations modify a short N-terminal repeat of 10 histidines (His), thereby resulting in either 7-His, 9-His or 11-His repeats in the patients (frequency in the general population being 0.000778, source GNOMAD). HOXA1 is a transcription factor of the HOX family known to play key roles in the embryonic development of mammals.
The objective of our work is two-fold: (1) characterizing the molecular processes connecting HOXA1 and heart development, and (2) investigating the role of this unusual His-repeat in the activity of HOXA1 and, thereby, in the etiology of BAV

Material and methods
Human cell line transfection, protein abundance observed by Western blot after cycloheximide treatment, co-precipitation.

Results and Conclusion
We first observed that the half-life of the mutant HOXA1 proteins is reduced by the His tract modifications. Second, we identified that the interaction of HOXA1 with the Wnt pathway regulator MDFI is modified by the mutations. Together our data support that the shortening or the extension of its His tract modify distinct molecular properties of HOXA1 which now need to be related to the onset of BAV

P025 | DCHS1 interacts with LIX1L and SEPT9 to promote mitral valve remodeling through an actin dependent mechanism

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Background:
Mitral valve prolapse (MVP) affects 1 in 40 individuals worldwide and is characterized by abnormal thickening and billowing of one or both leaflets into the left atrium. Nonsurgical treatments for MVP do not exist and development of such has been hindered by an incomplete understanding of disease inception and progression. Our group, however, has contributed significant insights as we were the first to identify Dchs1 as a causal gene for MVP. We recently performed two-hybrid screens and co-immunoprecipitation assays that define a novel complex between DCHS1, LIX1L and SEPT9. Our current studies investigate the role of this interaction in the valve interstitium early in development.

Materials and Methods:
Wild-type, DCHS1 and/or LIX1L heterozygous neonate murine heart tissue was isolated for 1) primary culture of cardiac fibroblasts (CFs) and analyzed with immunocytochemistry, collagen compaction, and fibrin pillar to post assays and 2) morphological analyses with 3D reconstructions of hematoxylin and eosin (H&E) stains. Cardiac function was measured in aged mice of each genotype.

Results and Conclusion:
We observed increased smooth muscle actin and ECM expression and decreased tissue compaction in CFs deficient of DCHS1 and/or LIX1L. In-vivo epistasis studies support these findings as mitral valve enlargement, smooth muscle actin expression and prolapse in adulthood is exacerbated in DCHS1/LIX1L compound heterozygote mice. These data suggest a mechanism by which cell-cell contact regulates the actin cytoskeleton through a DCHS1/LIX1L/SEPT9 complex during critical stages of valve morphogenesis and disease inception.

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P026 | The role of the mitral valve prolapse gene, DCHS1, in stabilizing the valve endocardium post-EMT

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Background:
Mitral valve prolapse is defined as the billowing of one or both mitral leaflets back into the left atrium. In patients, the valves become enlarged and mechanically incompetent. Our group previously identified mutations in the DCHS1 as causative to MVP in large families and recent genetic studies have indicated that as many as 24% of the MVP population may harbor damaging DCHS1 variants. Thus, understanding the role of DCHS1 in the valves can provide insight into the developmental origin of the disease and potentially lead to new non-surgical therapies.

Methods:
Two hybrid screens were conducted to identify direct protein binding partners for the intracellular domain of DCHS1. Out of 115 million clones screened from a human heart library, only one confident interactor, an RNA binding protein, was identified. Co-IP, immunohistochemistry and Western analyses revealed a unique interaction between DCHS1 and the miRNA machinery.

Results and Conclusions:
Herein we report that the MVP protein, DCHS1 is able to interact with an RNA binding protein and the miRNA machinery at the cell membrane. Disruption of this interaction results in failure to generate mature miRNAs: miR200c, Let7-e and Let7-g, known regulators of Zeb2 and EMT. Increased Zeb2 is evident in our knockdown cells as is reduced VE-cadherin. Coincident with these molecular changes, we observe poorly stabilized endocardial adherons junctions and increased EMT in the mitral valves of our Dchs1 MVP murine model. Thus, our data support Dchs1 in stabilizing the valve endocardium after EMT through a miRNA mechanism.

P027 | Histopathology of bioprosthetic pulmonary valved conduits with endocarditis

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Background:
Bioprosthetic pulmonary valves have a high incidence of endocarditis. We examined a series of explanted valved pulmonary conduits with a history of endocarditis by means of histology and immunohistochemistry.

Methods:
All valve specimen were surgically excised due to endocarditis. Macroscopic and histological analysis of the hard resin embedded specimen focused on localization and quality of endocarditic lesions.

Results:
26 valved pulmonary conduits (Melody transcatheter valve n=12, Hancock n=3, Homograft n=2, Contegra n=2, Sapien n=1) were analyzed. Average time between implantation and explantation had been 66 months (4 to 231 months). Endocarditic lesions were localized at the basis of the semilunar valves in 24 of 26 specimen, at the conduit wall in 17 of 126, and at the valve edges in 13 of 26 patients. All identified endocarditic lesions consisted of typical thrombus material and fibrin condensations containing granulocytes and lymphocytes.

Conclusions:
In our series of explanted pulmonary conduits, we demonstrate formation of typical endocarditic lesions macroscopically and histologically. Lesions at the basis of semilunar valves were an almost constant finding whereas the edges of the valves were affected much less frequent. We speculate that the high incidence of endocarditis in bioprosthetic valves may in part be explained by thrombus apposition at the valve basis as a primary nidus for development of an endocarditis. In contrast, the often supposed mechanism of endothelium injury due to shear stress at the edges of the valves may play a minor role. Findings imply that intensified anticoagulation should be discussed for bioprosthetic heart valves.

P028 | Fusion of conotruncal ridges during cardiac outflow tract septation relays on an endocardial-mesechymal transition process

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Background
Conotruncal septation (CS) divides the embryonic cardiac outflow tract (OFT) and gives rise to the semilunar valve primordia by fusion of the two opposite conotruncal ridges (CRs), composed of mesenchymal cells covered by endocardium. The aim here is to clarify the mechanism involved in the fusion of the CRs, which remains currently unknown. Two mechanisms have been proposed: endocardial apoptosis and endocardial-mesenchymal transition (EMT).

Material and methods
ED 11-12 hamster embryos were used. Immunofluorescence for active caspase 3 and TUNEL assays were performed to assess apoptosis. Immunofluorescence for endocardial (VE-CAD and CD34) and migration (a-actin) markers, as well as localization of carboxifluorescein (CFSE) after 12h ex-vivo incubation, were performed to assess EMT.

Results and Conclusion
Endocardial cells covering the CRs were VE-CAD+, CD34+ and a-actin-, except for those at the fusion area, which were positive for the three markers. Apoptotic mesenchymal cells were found at the proximal portion of the CRs, but no apoptosis was detected either in the endocardium or in the fusion area during CS. Scattered CFSE+ endocardial and mesenchymal cells, usually a-actin+, were found in the fusion area, closed to the endocardium, after ex-vivo incubation.
The results indicate that fusion of CRs during CS relays on the transformation of contacting endocardial cells from opposite CRs into mesenchymal cells. This EMT process seems uncoupled from that involved in CR formation. This finding may explain the common etiology of the distinct anatomical types of bicuspid aortic valve.

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P029 | Maternal iron-deficiency perturbs embryonic heart development

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Background
30% of congenital heart disease (CHD) cases are genetic in origin, but the causes of the remainder are not well understood. Some of these are likely to be caused by environmental factors. Previously-described examples of such factors include maternal viral infection, pre-existing diabetes and exposure to teratogenic pharmaceuticals. Here we describe a novel risk factor for CHD: maternal iron deficiency.

Materials and methods
Embryonic heart morphology was examined by HREM; changes in gene expression and signalling pathways by RNASeq and immunohistochemistry; and the contribution of progenitor cells to the embryonic heart by lineage-labelling.

Results
Maternal iron-deficiency disrupts embryonic heart development. This manifests at E15.5 by peri-membranous VSDs, OFT mal-rotation, and valve and aortic arch defects; at E12.5 by dysmorphic OFT and AVC cushions; and at E10.5 by a shortened distal OFT and hypoplastic pharyngeal arch arteries. These defects are similar to those in Tbx1 null embryos, and we show that these arise via a combination of premature SHF differentiation, and dysregulation of retinoic acid signalling. Finally, maternal iron supplementation before E8.5 completely rescues the phenotype.

Conclusion: Maternal iron-deficiency causes highly-penetrant heart defects in mouse. Iron-deficiency is the most common nutritional deficiency in both developing and developed nations, affecting 20-40% of women of child-bearing age. This previously unappreciated environmental risk factor may contribute to the high global CHD prevalence. Our results suggest that ensuring adequate maternal iron levels in early pregnancy should be a clinical priority.

Funding: BHF, Novo Nordisk, John Fell OUP Fund, OU Medical Sciences Division Research Fund

P030 | Characterization of FSP1+ cell populations in the developing and adult heart

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Fibroblast specific protein 1 (Fsp1), also known as Metastasin (Mts1) or S100A4, is a small polypeptide belonging to the calcium-binding protein family S100, which include two kinds of proteins, S100A and S100B (Moore, 1965). S100 family members are involved in a wide range of biological processes such as proliferation, migration and/or invasion, inflammation and differentiation (Leclerc and Heizmann, 2011). FSP1 is expressed in different adult cell types like fibroblasts (Strutz et al., 1995), macrophages, monocytes and polymorphonuclear leukocytes (Takenaga et al., 1994). This protein is also expressed by several cardiac embryonic cells, but it remains unclear whether it is required for the proper development of such heart tissues. In this work, we have analyzed the expression of FSP1 during heart morphogenesis aiming at identifying specific functions for this protein. In order to tackle this goal, we have used a mouse transgenic line in which a GFP cassette is under the control of the FSP1 promoter allowing us to trace the location and fate of FSP1+ cells throughout embryonic development. Our results show that FSP1 is expressed by cardiac fibroblasts, myocardial cells located at the distal portion of trabecules (prospective cardiac conduction Purkinje cells), and a discrete population of cardiac venous and lymphatic endothelium associated to coronary valves. Ongoing experiments in this project include testing the effects of FSP1 gain of function in vitro and the tissue-specific conditional deletion of this gene using the Cre/LoxP technology.

P031 | Immunohistochemical study of developing cardiac conduction system using HNK-1 and pan-neuronal markers

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Background:
Human natural killer (HNK)-1 antibody is an established marker of developing cardiac conduction system (CCS) in birds, mammals, and also reptiles. However, the HNK-1 is not a functional marker and is also present in the extracellular matrix and peripheral nervous system including cardiac nerves. Therefore, we sought to confirm HNK-1 specificity for the CCS using double immunohistochemistry with alternative markers - Protein Gene Peptide (PGP) 9.5, Contactin-2, and Beta-III Tubulin.

Material and methods:
The hearts of selected model species (chick, rat, mouse) were collected at various post-septation stages of embryonic development to map immunoreactivity in cardiac tissues. We stained alternating serial frozen or paraffin sections using double immunofluorescence staining with HNK-1 antibody, and with a selection of the pan-neuronal markers for possible collocation of staining. Sections were imaged on an Olympus FluoView confocal laser-scanning microscope using 4x, 10x, and 40x objectives.

Results:
HNK-1 positive structures included the cardiac nerve fibres and ganglia, the extracellular matrix of the cardiac cushions/valves, the myocardium of the sinus venosus, and the atrioventricular conduction axis. In all tested species, cardiac nerves and ganglia were stained by Beta-III Tubulin. The pan-neuronal markers showed species specificity.
Conclusions: According to preliminary results of double immunofluorescence staining (HNK-1/pan-neuronal marker), the PGP 9.5 antibody could be used as an alternative for labelling developing CCS in mouse and rat.

Funding:
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P032 | Left atrial ligation affects electrophysiology and morphogenesis in chick embryonic atria

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Background:
Mechanical loading is an important factor in cardiac morphogenesis. Experimental unloading of left ventricle via left atrial ligation results in left ventricular hypoplasia and delayed electrophysiological development. Similar effects were demonstrated also at the level of whole ventricle in vitro. However, effects of mechanical loading on the atria were not studied yet. We thus set to investigate the effects of exclusion of a portion of the developing left auricle from circulation on electrophysiological and morphological development of the atria.

Methods:
Left atrial ligation was performed on embryonic day 4 (Stage 21-23). Impulse initiation and conduction through the atrial was studied by optical mapping after 48 hours at day 6. Morphology was studied by whole mount confocal imaging and micro-CT. Additional immunohistochemical investigation was performed on exclude bits of left atrial after 96 hours on day 8.

Results:
Electrophysiological examination showed that the excluded portion of the left atrium generated ectopic pacemaking activity, and in the sinus rhythm, there was a considerable slowing of the impulse propagation in the ligated portion. Morphologically, there were no or only rudimentary pectinate muscles in the ligated portion. Later on, there was also a significant decrease in myosin heavy chain expression.

Conclusions:
We conclude that atrial pectinate muscles and ventricular trabeculae share similarity in their role in impulse propagation and dependence of their development on hemodynamic loading. Thus, they could be regarded as homologous structures in chamber myocardial morphogenesis.

Funding:
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P033 | hiPSC-derived SAN-like cardiomyocytes for studying lineage specification

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Reproducing molecular and functional profiles of specialized cardiomyocyte (CM) subtypes is required to improve our understanding of developmental programs involved in lineage specification and to establish models that recapitulate in vivo cell types for drug screenings and regenerative medicine.
Using human induced pluripotent stem cells (hiPSCs) and their directed differentiation towards different cardiomyocyte subtypes including sinoatrial node (SAN)-like cells, we aim to gain better understanding of the differentiation processes. For the generation of SAN-like cells, a cocktail of growth factors and small molecules were added after mesoderm induction, as previously described. Beating cells were obtained from day 7 onwards. Cardiomyocyte identity in differentiated cells was confirmed by quantitative PCR, as well as flow cytometry using the cell surface marker SIRPa and intracellular marker, cardiac Troponin T. Gene expression profiles were analyzed by quantitative PCR on day 18 of differentiation. Functional characteristics of these cardiomyocytes were analyzed by single-cell patch clamp.
SAN-like cells showed significantly higher expression of key transcription factor genes specific for SAN development, as well as the ion channel genes HCN1 and HCN4. Furthermore, hiPSC-derived SAN-like cells showed decreased expression of NKX2.5, similar to SAN cells in vivo. Electrophysiological measurements confirmed pacemaker-like characteristics in hiPSC-derived SAN-like cells, which showed increased pacemaker current, I_f. Ongoing work is focused on in-depth molecular and functional characterization of these cells, particularly during differentiation.

Funding:
Funding from the European Research Council (ERC) and The Netherlands Organization for Health Research and Development (ZonMW) is gratefully acknowledged

P034 | THE PON1 GENE Q192R POLYMORPHISM IN POSTINFARCTION PATIENTS WITH CHRONIC HEART FAILURE

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Background/Introduction.
The PON1 gene is candidate gene of atherogenic macrovascular pathology. It is interesting to research this gene in patients with CHF and previous myocardial infarction (MI).

Methods.
The study included 157 postinfarction patients. The samples of peripheral blood were used to determine the allelic variants of the PON1 gene (polymorphism Q192R). The distribution of the genotypes of the PON1 gene corresponded to the Hardy-Weinberg equilibrium (p>0,05).

Results.
All patients included in the study had CHF. Most of the patients (67,5%) had CHF of II-III functional classes (FC) of NYHA.
Carrying the genotypes of the Q192R polymorphism of the PON1 gene was as follows: homozygote AA – 6,4% (n=10), homozygote GG – 49% (n=77), heterozygote GA - 44,6% (n=70).
Group of patients with HFpEF consisted of 119 patients, group with HFrEF consisted of 38 patients. In group of patient with HFrEF was more often observed with Q-wave MI (p=0,002, x2=9,01), STEMI (p=0,004, x2=8,53) and CHF of II-III FC (p=0,01, x2=7,39). GA genotype was in 50% of cases in group of patients with HFrEF and in 42,9% of cases in group with HFpEF.
It was found that the GA genotype of the Q192R polymorphism of the PON1 gene was associated with a more frequent CHF of II-III FC (OR=2,27; 95% CI 1,12-4,59; p=0,026, x2=5,34).

Conclusion.
Taking into account the comparability of groups in the severity of coronary atherosclerosis, we can talk about the possible contribution of the PON1 gene to the formation of CHF phenotypes in postinfarction patients.

P035 | Association of polymorphism rs662799 with development of Stroke in patients with cardiovascular pathology

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Purpose:
to study SNP rs662799 association (G> T) with development of Stroke in the patients with cardiovascular pathology and risk factors of its development who are representatives of the east Siberian population.

Material and methods.
260 patients with Stroke participated in a research (age [57.0; 51.0-62.0]) and 272 patients of control group (age [55.0; 51.0-62.0].). Among the patients who transferred Stroke, 157 men and 103 women. The control group included 170 men and 102 women. Inspection of the main group included: collecting complaints, anamnesis, clinical examination, computer tomography of a brain, electrocardiography, ultrasonic duplex scanning of brachial arteries, daily monitoring of arterial blood pressure and cardiac rhythm, analysis of a coagulant system of blood. The control group is examined within the international HAPIEE project. The molecular and genetic research was conducted by PCR method in real time.

Results.
In all analyzed groups and subgroups of patients connection between rare to alleles of G and the increased risk of Stroke is established. The genotype of GG showed significant associations with Stroke only in the main group of patients, in subgroup of men and in subgroup of patients with Hypertension

P036 | Association of polymorphism rs556621 with development of stroke in patients with cardiovascular pathology

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Purpose:
to study SNP rs556621 association (G> T) with development of Stroke in the patients with cardiovascular pathology and risk factors of its development who are representatives of the east Siberian population.

Material and methods.
260 patients with Stroke participated in a research (age [57.0; 51.0-62.0]) and 272 patients of control group (age [55.0; 51.0-62.0].). Among the patients who transferred Stroke, 157 men and 103 women. The control group included 170 men and 102 women. Inspection of the main group included: collecting complaints, anamnesis, clinical examination, computer tomography of a brain, electrocardiography, ultrasonic duplex scanning of brachial arteries, daily monitoring of arterial blood pressure and cardiac rhythm, analysis of a coagulant system of blood. The control group is examined within the international HAPIEE project. The molecular and genetic research was conducted by PCR method in real time.

Results.
Statistically significant distinctions of frequencies of genotypes and alleles of ONP rs556621 (G> T) in subgroup of patients from Stroke and the patient of control group it is not revealed. Gender distinctions when comparing frequencies of genotypes and alleles are not revealed. Statistically significant connection of a rare genotype of a TT of SNP rs556621 is established (G> T) with development of Stroke in patients with a dislipidemia and atherosclerotic defeat of coronary arteries (p =0.041; OR1.86,95%CI:1.02-3.41).

Conclusion.
SNP rs556621 TT genotype (G> T) increases risk of development of an acute disorder of cerebral circulation in patients with a dislipidemiya and atherosclerosis in comparison with carriers of genotypes of GG and GT

P037 | Association of polymorphism rs1800801 with development Stroke in patients with cardiovascular pathology

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Purpose:
to study SNP rs1800801 association (G> T) with development of Stroke in the patients with cardiovascular pathology and risk factors of its development who are representatives of the east Siberian population.

Material and methods.
260 patients with Stroke participated in a research (age [57.0; 51.0-62.0]) and 272 patients of control group (age [55.0; 51.0-62.0].). Among the patients who transferred Stroke, 157 men and 103 women. The control group included 170 men and 102 women. Inspection of the main group included: collecting complaints, anamnesis, clinical examination, computer tomography of a brain, electrocardiography, ultrasonic duplex scanning of brachial arteries, daily monitoring of arterial blood pressure and cardiac rhythm, analysis of a coagulant system of blood. The control group is examined within the international HAPIEE project. The molecular and genetic research was conducted by PCR method in real time.

Results.
As a result of the conducted research in one of the analyzed groups and subgroups statistically significant associations of genotypes and alleles of polymorphism of rs1800801 were not revealed (C> T) with Stroke.

Conclusion.
SNP rs1800801 (C> T) has no significant effect on Stroke in persons of the east Siberian population regardless of the previous cardiovascular pathology and risk factors.

P038 | Role of USP8 in cardiac development and disease

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Ubiquitylation is a reversible post-translational protein modification consisting to the covalent attachment of ubiquitin to a lysine residue within a substrate protein creating a mono- or poly-ubiquitin chain, referred to as the 'ubiquitin code'. This Ub-code determines whether a protein modulates a specific signaling cascade or becomes degraded. De-ubiquitilating enzymes (DUB) remove ubiquitin from target proteins. Therefore, DUB's modify the 'ub-code' and the subcellular distribution of specific proteins. USP8 is a DUB involved in the ubiquitin removal from developmental proteins such as Neuregulin or VEGFR2, and it is also involved in the regulation of intracellular vesicular trafficking, but little is known about its function in vivo. We detected USP8 expression in the developing mouse heart, both in the myocardium and in the endocardium, located at specific intracellular vesicles. Deletion of USP8 in specific cardiovascular tissues produces embryonic lethality associated with morphogenetic defects in ventricles, valve progenitor cells and interventricular septum. By Tandem Ubiquitin Binding Entities (TUBE) and classical biochemical approaches we will uncover what specific proteins USP8 de-ubiquitylates in vivo. Germline activating mutations of human USP8 produces Cushing Disease (CD) symptoms secondary to the formation of ACTH-secreting adenomas, including dilated cardiomyopathy with congestive heart failure. We are generating a new CD mouse model to understand the cardiac alterations described in CD patients. Our results reveal new mechanisms of regulation of intercellular signals implicated in cardiac development and disease.

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P039 | Non-coding Copy Number Variation (CNV) in Congenital Heart Disease (CHD)

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Congenital heart disease (CHD) is one of the most common congenital disabilities with a neonatal incidence of 0.8 - 1 % worldwide. Several factors have been identified to contribute to disease pathogenesis, including genetic and environmental causes. Despite recent advances in discovering genetic factors for CHD, the majority of cases cannot be explained thus far on the protein-coding level. Therefore, our study aims to evaluate the impact on the non-coding level by re-evaluating already published datasets for disease-causing pathogenic non-coding CNVs.

We have established a cohort (7958 cases vs 14082 controls) to study the impact of intergenic and intragenic regions on CHD pathogenesis. We show that affected patients have an increased burden of large and rare CNVs spanning the regulatory genome compared to controls. By targeting three classes of putative regulatory elements, we identified candidate sequences that potentially contribute to the development of CHD. We provide in silico evidence of potentially pathogenic CNVs overlapping enhancer elements that have been previously associated with CHD, including regulators for micro-RNA variant miR-570 and the gene DAD1. Our findings with this bioinformatics approach demonstrate the relevance of CNVs affecting the non-coding genome in CHD patients and emphasize the importance of studying regulatory causes of genetic disorders. In order to increase the validity of these results, in vivo experiments are currently underway to define the regional and temporal expression domains during heart development.

P040 | Confirmation of the role of pathogenic SMAD6 variants in bicuspid aortic valve-related aortopathy

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Progressive dilatation of the thoracic aorta leads to thoracic aortic aneurysm (TAA), which is often asymptomatic but predisposes to lethal aortic dissections and ruptures. TAA is a common complication in patients with bicuspid aortic valve (BAV). Recently, rare loss-of-function SMAD6 variants were shown to contribute significantly to the genetic aetiology of BAV/TAA. Intriguingly, patients with craniosynostosis have also been reported to be explained molecularly by similar loss-of-function SMAD6 variants. While significantly reduced penetrance of craniosynostosis has been reported for the SMAD6 variants as such, near-complete penetrance is reached upon co-occurrence with a common BMP2 SNP risk allele. Here, we report on the results of a SMAD6 variant analysis in 473 unrelated non-syndromic TAA patients, of which the SMAD6-variant positive individuals were also studied for the presence of the BMP2 risk allele. Although only 14% of the TAA patients also presented BAV, all novel likely pathogenic SMAD6 variants (N=7) were identified in BAV/TAA individuals, further establishing the role of SMAD6 variants to the aetiology of BAV/TAA and revealing limited contribution to TAA development in patients with tricuspid aortic valve. Familial segregation studies confirmed reduced penetrance (82%), variable clinical expressivity with coarctation of the aorta being a common comorbidity (10%). None of our six BMP2+/SMAD6+ patients presented with craniosynostosis. Hence, the proposed digenic model for craniosynostosis was not supported in the presented BAV/TAA cohort, suggesting that additional factors are at play. Finally, our data provides improved insights

into the clinical spectrum of SMAD6-related BAV/TAA and has important implications for molecular diagnostics.

P041 | Planar Cell Polarity Pathway Regulates Mechanosensitive Muscle Differentiation Program

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Background:
Throughout cardiac morphogenesis from the simple linear heart tube (LHT) to the multi-chambered organ, the heart is constantly exposed to persistent and mutable level of mechanical strain. The nuclear compartment is also subjected to organ morphogenesis, since its nucleoskeleton connects directly to the cytoskeleton. Consequently, the nucleus withstands strong biophysical forces from two active systems: the cytoskeleton and the genome.

Material and Methods:
Transgenic zebrafish lines for genes of interest were combined with immunohistochemistry and quantification of cell-specific relative gene expression levels.

Results and Conclusion:
Here, we have discovered that cardiogenesis is concomitant with changes in the nuclear morphology. The nuclei of ventricular cardiomyocytes are round at 24 hpf shortly after the myocardial contraction starts, while becoming more lobular and smaller at 30 hpf continuing until the two cardiac chambers are formed at 54 hpf. We have identified a role for Wnt non-canonical Planar Cell Polarity (PCP) signalling in modulating the tensional homeostasis of the nucleus at LHT stage as PCP-deficient hearts display abnormalities in nuclear morphology. We have shown that PCP signalling targets nuclear levels of globular actin in the nucleus of cardiomyocytes which is reflected by transcriptional changes of the mechanosensitive Myocardin-Related Transcription Factor A-Serum Response Factor (MRTFA-SRF) pathway during cardiomyocyte maturation. Thus, PCP pathway-driven remodelling of the myocardium compartmentalizes tensional tissue homeostasis by targeting nuclear actomyosin localization and links it to heart muscle differentiation mediated by mechanosensitive MRTFA-SRF signalling.

P042 | pre-mRNA processing factor 8 (Prpf8) dysfunction results in aberrant laterality establishment and cardiac development

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During development errors that occur in laterality establishment may cause congenital heart defects (CHD). Despite the prevalence of CHD, the developmental processes that ensure that heart forms correctly are still being elucidated. Using chemically induced mutagenesis, a novel homozygous lethal missense mutation in the spliceosome complex protein 'Pre-mRNA splicing factor 8 (Prpf8)' was identified. The mutant phenotype includes laterality defects with higher incidence of reversed heart looping. Previous studies have confirmed that Prpf8 homozygous mutant mouse embryos have altered expression of laterality genes and reduced cilia motility at the embryonic node, which may cause errors in the left-right specification of the heart. This study will contribute to understanding the role of Prpf8, and its links to cilia function, during embryonic heart development. Homozygous mutant Prpf8 embryos showed significantly delayed heart development compared to heterozygotes. Cardiac trabeculation was significantly altered in mutant homozygotes with reduced thickness of the myocardial compact zone. Developmental cardiac cell proliferation was not significantly altered and the percentage of cardiomyocytes and endocardial cells contributing to the developing heart also remained unchanged. Reduced cilia length was observed in Prpf8 homozygous hearts, especially in endocardial cells and epicardial cells of reversed looped hearts. Variants in Prpf8 have been detected in CHD patients; these variants did not alter Prpf8 protein expression in cultured mammalian cells. Further investigations are required to establish the link between Prpf8 dysfunction and aberrant cardiac development, revealing developmental pathways critical for heart formation, which are likely to be disrupted in human CHD patients.

P043 | microRNA-mediated modulation and gene regulatory networks of proepicardium (PE)/septum transversum (ST) differentiation

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The proepicardium (PE) is a transitory embryonic structure that provide cells to the outer layer of the embryonic heart. In vitro PE explants culture can derived beating cardiomyocyte while in vivo their plausible contribution is still controversial. We provide herein a comprehensive characterization of the developmental expression profile of multiple microRNAs during PE/epicardium development in chicken. We identified that miR-125, miR-146, miR-195 and miR-223 selectively enhance cardiomyogenesis both in the PE/ST and epicardial cultures, a Smurf1- and Foxp1-driven process. In addition we identified three novel long non-coding RNAs with enhanced expression in PE/ST, that are complementarily regulated by Bmp and Fgf administration as well as by microRNAs that selectively promote cardiomyogenesis, supporting a pivotal role of these long non coding RNAs in microRNA-mediated cardiomyogenesis. To further understand the molecular mechanisms driving PE and epicardial differentiation we have performed whole genome coding and non-coding RNAseq in mouse Wt1GFP+ E9.5 PE and E10.5 embryonic epicadium (EE) FACS-sorted cells. 1291 (752 up, 539 down) mRNAs, 448 (329 up, 119 down) lncRNAs and 18 (10 up, 8 down) microRNAs were differentially expressed in PE as compared to EE. Enrichment of Spry1, Aldh1a2, Tcf21 in PE and Hoxb1 and Prox1 in EE were identified and subsequently validated. microRNA-mRNA and mRNA-lncRNAs gene regulatory networks were built unravelling novel putative interactions such as let7b-Hoxb1 and miR-205-Spry1 that might be important for PE/EE formation. Overall, we provide a comprehensive analysis of differential gene expression during PE/EE development as well as how microRNA overexpression influence PE/EE cell fate determination.

P044 | Dissecting the role of WNT signalling target genes in cardiogenesis using human pluripotent stem cells

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Upon myocardial infarction, the heart muscle undergoes catastrophic loss of cardiomyocytes. Natural replacement of these cells is inefficient, making finding therapeutic strategies to promote heart muscle regeneration a priority. Wnt signalling plays a major role in cardiomyocyte differentiation during embryonic development. By identifying Wnt target genes in cardiac development we aim to contribute towards the innovation of novel effective therapeutic strategies to promote regeneration in damaged adult hearts.

We are using human pluripotent stem cell models in combination with RNA-seq, ChIP-seq and ATAC-seq to reveal Wnt targets that are crucial for embryonic cardiomyocyte differentiation. Following initial analysis of RNA-seq data, we identified critical genes that are responding directly to WNT inhibition following mesoderm formation. These include both positive targets with reduced expression, as well as, negative target genes that are not expressed without WNT inhibition. We are now exploring how both types of Wnt targets might be involved in promoting cardiogenesis as a consequence of Wnt inhibition. We are also further directly validating WNT targets by performing beta-catenin ChIP-seq in mesoderm cultures.

We are combining this work with an in vivo Xenopus model. We expect to uncover conserved and important mechanisms of early cardiomyocyte differentiation, which promises to improve future regeneration strategies.

P045 | Maturation of the cardiomyocyte surface crests contributes to an atypical physiological cardiac hypertrophy during the late postnatal period

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Background: We previously characterized a new architecture of the cardiomyocyte (CM) lateral membrane in mammalian adult species with periodic crests and intermittent lateral crest-crest interactions between adjacent CMs through claudin-5. Given that the lateral membrane is specific of the adult rod-shaped CM that maturates during the postnatal period (P0-P20 days), we examined the postnatal setting of the CM surface crests.
Material and Methods: Crests, sarcomeres, myofibrills were quantified by electron microscopy in rat tissues or in CM-specific efnb1 KO mice at different postnatal days (P0/P3/P5/P10/P20/P60).
Results: Western-blot analysis revealed a significant increase in claudin-5 expression from P0 to P5 in rat cardiac tissues. However, immunofluorescent staining indicated that maximal targeting of claudin-5 at the CM lateral membrane occurred between P20 and P60, while the maturation of adult CMs is supposedly complete at P20. Concomitantly, at P20, while CMs harbored their typical adult rod-shape, their surface crests were still immature and maturated between P20 and P45. Sarcomeres also significantly increased in size while the myofibrill number was unchanged, evocative of CM mechanical stretching. Consistently, after P20 (P45), we observed a significant increase of the CM cross-sectional area, reflecting a new late physiological hypertrophic process, confirmed by echocardiography. Examination of WT and efnb1 cKO mice indicated that ephrin-B1, a claudin-5 partner, was specifically involved in the P20-P60 short axis stretch of the lateral membrane and the maturation of the surface crests.
Conclusion: Our results unveil an atypical physiological cardiac hypertrophy during the late postnatal period related to the CM surface crest maturation.

P046 | Live imaging of cell division and intercalation in the murine second heart field

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Background
Growth of the embryonic heart occurs by deployment of second heart field (SHF) cells to the cardiac poles. Perturbation of this process results in a spectrum of congenital heart defects, such as those seen in 22q11.2 deletion/DiGeorge syndrome patients. SHF cells reside in epithelial splanchnic mesoderm of the dorsal pericardial wall (DPW), from which cells progressively contribute to the arterial and venous poles of the heart. However, the cell biology of the SHF remains relatively unexplored. Here we use live imaging to investigate cell behaviour and polarity in the murine SHF.

Results
Characterization of the dynamic properties of SHF cells using fluorescent reporter genes and time-lapse imaging of thick transverse slice cultures revealed that cell division in the SHF is biased towards division in the plane of the epithelium. In addition, individual mesodermal cells from underlying mesenchyme were observed to intercalate into the epithelial DPW, consistent with polyclonal growth patterns in the SHF. Further analyses revealed differential polarities of the centrosome- and Golgi-to-nucleus axis in cells in different regions of the DPW. This suggests that epithelial cells in the SHF are on divergent trajectories, consistent with differential contributions to alternate cardiac poles. Moreover, cell behaviour and polarity in the SHF are modified in embryos lacking Tbx1, the major gene implicated in 22q11.2 deletion syndrome.

Conclusion
Our results provide insight into how cell behaviour and progenitor cell patterning are integrated in the murine SHF, and further our understanding of the dynamics of cell deployment during heart tube elongation.

P047 | Epicardium-derived cells migration is mediated by miR-200b during cardiogenesis and after myocardial ischemia

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Development of the heart is a complex and dynamic process in which cells from the First and Second Heart Field contribute importantly to the distinct cardiac regions formation. Other cells populations, the neural crest and epicardium, provide the heart with a considerable amount of non-myocardial cells that are indispensable for correct heart development. During the past 2 decades, the importance of epicardium-derived cells (EPDCs) in heart formation became increasingly clear. Several fate mapping and cell lineage studies have demonstrated that coronary vascular smooth muscle cells (cVSMC) and cardiac fibroblasts develop from the EPDCs in a multi-step process involving cell proliferation, epithelial-to-mesenchymal transition (EMT) and cell migration. However, the exact molecular signalling cascades driving EPDC specification still need to be elucidated. Here we show that miR-200b is expressed at E12.5 and E15.5 during heart development. LNA in situ hybridization analysis in Wt1Cre-YFP, G2GATA4Cre-YFP embryos, as well as qPCR of sorted cells, showed that miR-200b is present in a cell subpopulation of EPDCs at these stages of heart development. In vitro experiments of gain and loss of functions, by using EPDCs and whole-organ cultures from mouse embryos, evidenced that miR-200b regulates cell motility. Additionally, analysis in ventricles from infarcted adult mice, strongly demonstrates that miR-200b is upregulated from post-myocardial infarction day 3, peaking at day 7. Collectively, our data suggest that this miRNA might be a key molecule regulating epicardial cell lineage diversification and migration during cardiac development, as well as, in EPDC activation after myocardial infarction.

P049 | Epicardium derived cells promote sympathetic ganglionic outgrowth towards myocardium in vitro via NGF and SEMA3A

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Background: A balanced cardiac sympathetic and parasympathetic innervation maintains normal cardiac function. After cardiac damage, like myocardial infarction (MI), excessive sympathetic neurite outgrowth referred to as sympathetic hyperinnervation can occur, which is associated with ventricular arrhythmias. The mechanism of this hyperinnervation remodelling is yet unresolved.

Objective: To investigate the potential role of adult mesenchymal EPDCs in stimulating sympathetic neurite outgrowth towards myocardium.

Material and methods: Murine superior cervical ganglia (SCG) were co-cultured with adult mesenchymal epicardium-derived cells (EPDCs) and/or myocardium in a 3D co-culture system. Neurite outgrowth and density of neurites sprouting directionally towards myocardium was assessed with a semi-automatic quantification method. Neurite differentiation of PC12 cells in corresponding conditioned medium was quantified to detect paracrine effects. Expression of neurotrophic factors in myocardiam was examined by RT-qPCR and western blotting.

Results: Co-culture with only mesenchymal EPDCs increased the neurite outgrowth of SCG, but neurite sprouting was non-directional. Co-culturing with myocardium induced directional (i.e. towards myocardium) neurite outgrowth, and adding mesenchymal EPDCs to the co-culture significantly increased directional neurite sprouting. Conditioned medium of myocardium co-cultured with EPDCs showed the highest promotional effect on neurite differentiation of PC12 cells. EPDCs upregulated NGF and downregulated SEMA3A expression in myocardium.

Conclusions: Adult mesenchymal EPDCs stimulate the outgrowth of cardiac sympathetic ganglia towards myocardium in a paracrine manner, via modulation of expression of NGF and SEMA3A.
Findings indicate a role for EPDCs in the occurrence of sympathetic re/hyperinnervation after cardiac damage.

P050 | The GPCR kinase 4 controls cilia without affecting the heart

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Background: GPCR kinases (GRKs) are best known for their regulation of receptor signaling. Several of the 7 GRK subtypes modulate both, heart development as well as cardiac performance. Recently, we and others have found that GRKs exert their functions through the cilium, a microtubule-based antenna required for cellular signaling and tissue morphogenesis. Here, we report that GRK4, which previously has not been investigated during embryonic development, controls cilium length and hence ciliary function.
Material and methods: GRK4 loss-of-function (LOF) was achieved using common knockdown approaches in zebrafish and human fibroblasts.
Results: Depletion of Grk4 in zebrafish embryos resulted in strong pericardiac edema and hydrocephalus. Interestingly, neither cardiac performance nor heart morphology were grossly changed. Instead we observed defects in the formation and function of the developing kidney. Grk4 LOF resulted in proteinuria, kidney cysts, massive dilatation of the pronephric tubule, patterning defects, increased numbers of cilia and cilium elongation. Experiments in fibroblasts revealed similar cilium extension effects as seen in zebrafish and aberrant centrosome numbers. RNAseq revealed downregulation of the tubulin modifier CFAP20. Reconstitution with human CFAP20 restored cilium length in zebrafish embryos.
Conclusions: We identified GRK4 as a novel protein preventing uncontrolled cilium elongation.

P051 | Analysis of specific microRNAs profiles during sinus venosus differentiation in early cardiac development

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Background: It has been demonstrated that multiple microRNAs play crucial roles in distinct and diverse biological processes, including cardiovascular development.

Material and methods: Using in situ hybridization in developing chick embryo with microRNA-specific LNA probes, we carried out a detailed study of several microRNAs: miR-15a, miR-23b, miR-130a, miR-106 and miR-100, among others.

Results: We observed their expression pattern from early cardiac looping, in particular at the level of the inflow tract/sinus venosus. Additionally, by means of TargetScan bioinformatic predictive analysis, we identified that these microRNAs putatively target several 3´UTRs of distinct genes related to inflow tract/sinus venosus development
The model here proposed establishes specific roles for microRNAs during sinus venosus differentiation, playing crucial roles in several signaling pathways involved in early cardiac development, including MAPK/ERK signaling pathway. In this sense, miR-15a (by repressing its target proteins FGFR1 and RAF1), and both miR-130a and miR-106 (by repressing their target protein ERK) could modulate the pathway as anti-proliferative factors. Moreover, miR-23b could also act as by repressing its target protein SPROUTY2 (a RAF1 modulator). On the other hand, miR-100 could act at later stages by repressing its target protein FGFR3.

Conclusion: Therefore, these microRNAs might function as multiple necessary modulators, in the FGF/MAPK/ERK signaling pathway, during sinus venosus differentiation in early cardiac development

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P052 | Cooperation of miR-133a and miR-130a as regulators of retinoic acid role during early cardiac segmentation

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Background
It is well known that microRNAs regulate gene expression during heart development. In particular, we demonstrated that miR-130a and miR-133a regulate signaling pathways during early cardiac specification, mediated by Bmp2. We have also shown that retinoic acid plays a crucial role in atrio-ventricular cardiac segmentation.
Material and methods
In this work, we studied the cooperative role of miR-133a and miR-130a as intrinsic regulatory mechanisms involved in early cardiac segmentation. Gain- and loss-of-function experiments in chick embryos were performed by means of in vitro electroporation with miR-133a and miR-130a, as well as anti-miRs, respectively.

Results
We observed that miR-133a and miR-130a suppress the expression of specific atrial markers, such as Tbx5, Tbx20 and AMHC-1, and modify the specific ventricular expression of Anf, modulating thus atrial and ventricular chamber gene expression during early cardiac looping.

Conclusion
These results support that miR-133a and miR-130a, putative microRNAs that target RARB (Retinoic Acid Receptor B) and Tbx5 3´UTR, respectively, constitute necessary links in the control of Anf, Tbx20, Tbx5, and AMHC-1, giving a key role to the retinoic acid, and Bmp2, as modulators of atrial chamber specification.

Funding sources
This work has been financed with research grants IB18123 (to CLS) and GR18185 (to VGM, CTS005) from the Junta de Extremadura, with FEDER co-financing, and CTS-446 (to DF and AA) from the Junta de Andalucía Regional Council.

P053 | Mylk3 is a novel effector of the Planar Cell Polarity pathway during cardiac morphogenesis.

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Planar Cell Polarity (PCP) signaling, a major morphogenetic pathway, drives cardiac chamber formation through regulation of actomyosin tension. This is in accordance with the downstream effectors of the PCP pathway, specifically those regulating the actomyosin complex, ultimately influencing cytoskeleton dynamics, cell adhesion and migration. The Myosin Regulatory Light Chain (MRLC) activity is regulated through the interplay of Myosin Light Chain Kinase (MLCK), Rho-associated Protein Kinase (ROCK) and Myosin Phosphatase (MYPT). The Myosin Light Chain Kinase 3, encoded by the mylk3 gene, is a cardiac-specific kinase that is highly expressed in the zebrafish heart during the time of cardiac chamber formation. Its role and signaling pathway interaction during heart development remain unclear. Here, using zebrafish as a model organism, we show that the reduced levels of mylk3 cause cardiac abnormalities, including cardiac edema and looping defects resembling PCP-deficient hearts. Loss of mylk3 decreases the MRLC phosphorylation levels in the myocardium, similarly to ROCK inhibition. The phosphorylated MRLC accumulates apically in the outflow tract (OFT) region; this organ-scale polarization of tissue tension is required for heart tube looping and is regulated by PCP signaling. In mylk3-deficient heart tube, the localization pattern of the phosphorylated MRLC is modified. We found a genetic interaction between Mylk3 and the PCP core components and propose Mylk3 as a novel effector protein of the PCP pathway. PCP signaling through regulation of Mylk3 activity and/or localization may polarize the actomyosin complex generating tissue-tension necessary for shaping of the heart.

P054 | Epicardial cell lineages and the origin of the coronary endothelium

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The contribution of epicardial-derived mesenchymal cells (EPDC) to the coronary endothelium is controversial. We have used murine cell-tracing models to compare, using flow cytometry and confocal microscopy, the developmental fate of four cellular lineages related with the EPDC. Mice bearing R26REYFP reporters were crossed with mice expressing Cre-recombinase under control of Wilms' tumor suppressor gene (Wt1), cardiac troponin gene (cTnT), and GATA5 gene drivers. We have also used the G2 enhancer of the GATA4 gene as a driver. Recombination was found in most of the epicardial cells. Unexpectedly, recombination induced by the troponin-Cre driver also labelled the epicardium.

The fate of the four lineages revealed striking differences. The G2-GATA4 cell linage contributes more than any other one to the coronary endothelium and this contribution increases along fetal and postnatal stages, probably due to the recruitment of circulating endothelial progenitors and higher proliferation rate. The contribution of the WT1 cell lineage increases along development to a lesser extent, probably due to further activation of the WT1 expression in the endothelium. Both, GATA5 and cTnT lineages contribute to about 4% of all the cardiac CD31+ cells in the embryo and 1% in the adult, a fraction that probably represents the actual EPDC contribution to the coronary endothelium. These results suggest caution when using a sole cell-tracing model to study the fate of the epicardial-derived cells.

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P055 | The impact of admission red cell distribution width on myocardial perfusion and short-term prognosis after primary percutaneous coronary intervention

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Background:
RDW is a marker of the variability in erythrocyte volume and is a routinely available component of the complete blood count.
Clinical usage of RDW has usually been restricted to differential diagnosis of anemia. However, elevated RDW levels are associated with poor prognoses in acute myocardial infarctions (MI) heart failures stable angina in patients undergoing PCI and general population even in the adjustment for multiple potential confounders, including anemia.

Methods:
A total of 72 patients were included in this prospective cohort study. The study population was divided into two groups
- Group (I): 54 patients with post-procedure ST-T resolution ≥ 50%.
- Group (II): 18 patients with post-procedure ST-T resolution < 50%.

Results and Conclusions:
Results of the current study showed that there was significant difference between the 2 groups regarding RDW (P= 0.003), LV systolic function (P= 0.040), TIMI flow after PPCI (P= 0.006) and MBG post PCI (P= 0.002).In addition, there was significant difference regarding in-hospital MACE (P= 0.023).
Cut-off value for RDW as a predictor of outcome was ≥ 14.3 % with accuracy of 68% i.e. the higher the RDW the worse outcome result should be predicted out of PCI results.
So, more focused care should be delivered to patients presented with STEMI and high RDW value.
More investigations should be perfomed regarding this topic.

P056 | Identification of new regulators of cardiomyocyte proliferation

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The discoveries of low but detectable level of adult cardiomyocyte proliferation have orientated cardiovascular research toward therapeutical strategy aiming to activate adult cardiomyocyte cell cycle reentry. Moreover, the loss of mammalian cardiomyocyte renewal potential shortly after birth causes the loss of regenerative capacities supports that a detailed understanding of fetal cardiomyocyte proliferation regulation is essential to identify targets for cardiac regeneration. We have uncovered FGF10 as a regulator of both fetal and adult cardiomyocyte proliferation and our recent unpublished results strongly suggest FGF10 as a potential target to promote heart regeneration after myocardial infarction.
This study aims to identify new regulators of cardiomyocyte proliferation downstream of FGF10 signaling.
Genome wide transcript (RNA-seq) analysis was performed on right and left ventricles from control and conditionally overexpressing Fgf10 fetal mouse hearts.
We identified 207 and 437 transcripts up-regulated and 159 and 330 transcripts downregulated, in the right and left ventricles, respectively, from Fgf10 overexpressing fetal mouse hearts. Moreover, 129 common genes were also identified suggesting that both regionalized and identical mechanisms are operating in both ventricles downstream of FGF10 signaling. Gene ontology analysis revealed main biological processes including cell proliferation and metabolism among which the most relevant candidates have been validated using qRT-PCR experiments. The role of two selected targets in modulating cardiomyocyte proliferation is under investigation and their impact in promoting cardiac regeneration will be addressed.
Altogether this study will identify novel regulators of fetal and adult cardiomyocyte proliferation including downstream targets of FGF10 signaling, representing potential targets for heart regeneration.

P057 | ROLE OF LRRFIP2 IN MOUSE HEART DEVELOPMENT

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Heart is the first functional organ in the early embryo. Cardiogenesis is controlled by several signalling pathways, like canonical and non-canonical Wnt which regulate proliferation, polarity and actin cytoskeleton.
LRRFIP2 (Leucine Rich Repeat in Flightless Interacting Protein 2)1 is known to interact with different partners like Disheveled and Flightless, involving LRRFIP2 into several signaling networks.
We show that the absence of LRRFIP2 leads to embryonic lethality around E13.5 due to severe cardiac malformations.
From E10.5LRRFIP2 mutant embryos have a reduced cardiomyocyte (CM) number. Despite the same proportion of cycling CM, there is a significant decrease of pHH3 positive CM. Moreover, we observed a defect in the proportion of CM in mitosis, associated with a defect of cytokinesis suggesting a cell cycle alteration and a possible precocious binucleation phenomenon.
We also investigated the actin cytoskeleton organization and the state of the sarcomeres in embryonic mutant CM. We found precocious sarcomerisation associated with the formation of bigger adherent junctions and a decrease of phosphorylation of some of their components. These results suggest a premature maturation of these cells and a defect in the adhesion capacity between them.
These observations could be linked with the mis-localization of Flightless, member of gelsolin family. This protein is known to be implicated in the remodeling of cytoskeleton, a defect of its gelsolin activity could be a valid explanation of this phenotype.
Thus, the lack of LRRFIP2 could lead to a lengthening of the cell cycle due to a precocious maturation of cardiomyocytes.

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Thursday 15 - Saturday 17 October 2020

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