ESC Development meeting Newcastle Nov 15-17, 2016

Overview and feedback from participants:

As organisers, we felt the meeting went well and very much enjoyed the presentations and meeting the delegates. In the feedback you echoed this, with more than 94% reporting the quality, content and duration of presentations was good/excellent. The introductory lecture to each session was well received. The traffic light timer was a success and many people commented that it kept us to time. Most respondents (80%) felt we had enough time for discussion, but perhaps in future we may factor in a few more minutes. Feedback suggested we had provided a program that everyone enjoyed. There were many comments that the connection between clinical congenital heart disease, cardiac morphology and basic research is one that they would like to see more of.

We had increased the length of the poster session from previous meetings to ensure adequate opportunity to discuss, but 25% of respondents feel we need to give this more time. Overall more than 95% of respondents found Newcastle to be a great venue, easy to get to and travel around. We all found the Institute of Genetic Medicine venue a bit squashed and the microphones were poor. The Hancock (Great North) Museum was much better received. However, we completely agree with two comments made by several delegates. Shorter food queues and more coffee and alternative drinks! It is always difficult to know what delegates want to do in the evening. This time we tried to give a free evening and advance information so delegates could plan their evening. Two thirds of the responses felt this worked well, although 1/3 would have liked a reception on both nights. We had a few comments that everyone disappeared on the free night - maybe this was a good thing?!

We hope to host the meeting in Newcastle again, but at that point it will be in a new conference centre that is to be built in the university. In the meantime, we plan to hold the meeting in several different locations.

The next meeting will be in Padua in the north of Italy and will be hosted by Marina Campione, Christina Basso, and Gaetano Thiene. Padua is one of the oldest Universities in the world and was founded in 1222. The botanical gardens are a UNESCO World Heritage site and the Giotto frescos in Scrovegni Chapel are something very special indeed. This, with the hospitality and style we expect from Italy, makes it a meeting not to be missed.

Newcastle Meeting Report:

Session 1. Development, Anatomy and Pathology of the cardiac outflow tracts. (Chairs Lucile Houyel and Robert Anderson)

The opening session of the meeting was designed to demonstrate the importance of the knowledge of anatomy in underscoring the interpretation of findings from molecular biology and cardiac development. It opened with a review of the origins of the arterial pole, presented by Robert Kelly, from Marseilles. Robert emphasised the importance of understanding development as a pre-requisite for analysing the abnormalities producing congenital lesions. He outlined the steps in appreciating the temporal contributions to not only the heart, but also craniofacial muscles, from the so-called second heart field. Having begun with a consideration of the developing outflow tract, attention moved to the formation of the ventricular loop. This was approached from the stance of its formation in the zebrafish, presented by Tessadori and colleagues from Utrecht, and a mechanistic approach using a Liebau-effect pump, the latter presented by Jorg Manner, from Gottingen. The next presentation revealed the advances now being made to increase the ability to assess the cardiac components without using destructive methodology. Garcia-Canadilla and their colleagues from Barcelona showed the results of interrogation of fetal hearts using synchroton-based microcomputed tomography. The technique was shown to reveal not only the arrangement of the aggregated working cardiomyocytes within the chamber walls, but also the location of the atrioventricular conduction axis. To complete the first part of the session, Bob Anderson, from the host institute, reviewed the temporal changes occurring during development of the outflow tract, basing his account on the images provided using the technique of high resolution episcopic microscopy. He argued that, whilst recognising the importance of the investigation made by Kramer over 70 years ago, it was no longer adequate to describe the outflow tract in terms of the “truncus” and “conus”. He provided evidence showing that the outflow tract had three components, with the potential for each to be malformed so as to produce the commonly recognised congenital lesions such as common arterial trunk, bicuspid aortic valve, or double outlet right ventricle. After a break for coffee, Vi Tran, from the Institute of Child Health in London, gave a superb presentation of the lesions brought into focus by Bob Anderson, using a video camera to reveal all the intricacies of malformed human hearts. The demonstration showed elegantly how many of the changes now being seen in knock-out mice were replicating the morphology seen in the setting of human congenital disease. Jill Hikspoors from Maastricht followed with an equally impressive and convincing discussion of the development of the suprahepatic portion of the inferior caval vein, highlighting the deficiencies still appearing in current embryological textbooks. The session concluded with consideration of another well-established congenital cardiac lesion, namely hypoplasia of the left heart. Bill Chaudhry, also from the host institute, introduced the topic by presenting an analysis of a large series of archived specimens of this lesion held in Birmingham Children’s Hospital. He emphasised how attention to the morphology of the aortic and mitral valves permitted recognition of the different phenotypes making up the lesion. Anthony Firulli, from Indianopolis, then brought the session to a close by describing how he and his colleagues had hoped to produce hypoplasia of the left heart by perturbing the *Hand1* gene.

Session 2. Cardiac progenitors and Cardiogenesis. (Chairs Antonio Baldini and Peter Scambler).

The session included 5 talks, one from an invited speaker (Dr. Baldini) and 4 selected from abstract submission. Dr. Baldini introduced the topic and reviewed briefly the current knowledge about transcription factors and chromatin remodeling factors thought to play a role in cardiac progenitor cell maintenance and differentiation. He then reported the latest data from his laboratory concerning the role of Tbx1 modifying the chromatin state of target genes. Next, Dr. Sonia Stefanovic (Marseille) presented novel information about the role of retinoic acid in the development of the venous pole of the heart, and their efforts in the identification of retinoic acid receptor targets

relevant to heart development. Dr. Simon D. Bamforth (Newcastle) presented his latest data concerning the interactions between genes *Pax9* and *Tbx1* in the control of pharyngeal arch artery development, and noted a significant increase of interrupted aortic arch type B in double heterozygous mutants. Dr. Srinivasan Sakthivel (Copenhagan)) presented the identification, by whole-exome sequencing, of two missense mutations within the gene *PLEKHA6*, associated with congenital heart disease (CHD) in a family with recurrent CHD. He also validated the importance of this gene in heart development using the zebrafish model. Dr. Juan Gaudix (Leiden) reported on a novel approach to differentiate human embryonic stem cells in vitro and direct them towards embryonic epicardial progenitor cell fate and appropriate response to BMP and retinoic acid signaling. Overall, the session provided exciting new information on some of the fundamental mechanisms and signaling systems critical in cardiac progenitor cell biology.

Session 3. Endothelium, endocardium and flow. (Chairs: Shane Herbert and Jose-Luis de la Pompa)
In this session on the endothelium, endocardium and flow, we were introduced to some key recent insights into the development and function of the vascular endothelium. Shane Herbert (University of Manchester) discussed new work revealing an unexpected role for asymmetric cell divisions in the coordination of endothelial cell behaviour during new blood vessel formation. Moreover, Nicoletta Bobola (University of Manchester) shed new light on the transcriptional mechanisms regulating embryonic artery formation, implicating Gata6 in the control of branchial arch remodelling. Stanislao Igor Travisano (Madrid) provided key insights into the Jag1-Notch-dependent mechanisms driving coronary vessel morphogenesis and the impact this pathway has on subsequent myocardial development. Finally, Salim Abdelilah-Seyfried (Hannover) highlighted the role of the cerebral cavernous malformations (CCM) complex in endocardial mechanosensitive signaling and development of the cardiac valve leaflets.

Session 4 Coronary vessels and endocardium (Chairs Sarah Ivins and Jose Maria Perez-Pomares)
Does it really matter where cells come from? This was the provocative question posed by a conference delegate after the opening talk of the Coronary Vessels and Epicardium session; the first speaker (Sarah Ivins, London) guided the audience through the maze of sometimes contradictory studies relating to the origins of coronary endothelial cells. A good question, and one which was at least partly addressed by some of the talks in this wide-ranging and enjoyable session. The work presented by Sophie Payne (Oxford) showed how endothelial-specific enhancers can be used not only to trace developmental origin of vessels but also, intriguingly, to highlight which embryonic regulatory pathways are preferentially re-activated following myocardial infarction. Ghislaine Lioux (Madrid) explored the distinctive origin of the outflow tract 'epicardium' (arterial epicardial-like cells, aELCs). Her work offered the fascinating prospect of discovering how the differing origins of the outflow and ventricular epicardial cell layers actually play out in terms of 'behaviour' i.e. signalling to underlying tissues. In other talks, Catherine Roberts (London) emphasised the importance of retinoic acid regulation in differing aspects of cardiac development, with her detailed analysis of the effects of knocking out the RA-metabolising enzyme Cyp26b1 in mice. Finally, Paul Palmquist-Gomes (Malaga) outlined genetic and physical methods of inducing coronary artery fistulae in different model systems, offering diverse ways of exploring this coronary anomaly, which can be associated with severe cardiac complications in human cases.

Session 5. Valves. (Chairs Stephane Zaffran and Bill Chaudhry)
Stephane Zaffran (Marseille) gave the opening talk of the session, hightlighting that valve diseases are the most common congenital heart malformations. The cardiac valves are derived from endocardial cushions of the outflow tract (OFT) and atrioventricular (AV) regions. These cushions are populated by cells that derived from endocardium through a process of epithelial-mesenchymal transition (EMT) in response to coordinated signals emanating mostly from the adjacent myocardium. These signal include VEGF, BMP, TGFb, EGF and NOTCH. Recent studies have shown that Notch signaling is an activator of EMT, predominantly through Notch1, which is expressed in

endocardial cells. The signaling cross-talk during cardiac valve development was covered by Donal MacGrogan (Spain). MacGrogan discussed how Dll4 and Jag1 ligands activate Notch1 receptor during and after EMT. Whereas Dll4-Notch1 signaling leads to EMT for valve morphogenesis, Jag1-Notch1 signaling restricts BMP-driven valve mesenchyme proliferation by sustaining HbEGF-EGF receptor signaling. Formation of endocardial cushions requires the contribution of several cell lineages from multiple sources. However, precise source of cells that contribute to valve formation is still unclear. Stephane Zaffran (Marseille) provided evidence for the identification of novel subpopulation of neural crest cells that contribute to aortic valve formation and especially to non-coronary leaflet. Deborah Henderson (Newcastle) presented data that clearly show that endocardium and neural crest cells make a minor contribution to the intercalated cushions of the outflow tract. Her ongoing effort is now focused on discovering the origin of the ICs.
Mitral valve prolapse (MVP) affects 2-4% of the population and is characterized by an excess of tissue growth resulting in prolapse and subsequent mitral dysfunction. The genetic cause of MVP has been recently better understood through the identification of human mutations in *DCHS1* (*Dachsous 1*) locus of several MVP families and a genetic association study highlighting the role of *LMCD1* (Lim and cysteine rich domain 1) and TNS1 (Tensin 1) in the AV valves. Russel Norris (Charleston) discussed the common role of these different proteins. Interestingly, a common feature between these factors is their potential role in primary cilia. Using a two hybrids system Russel Norris showed how they identified novel ciliary interacting proteins and major signaling factors that regulate differentiation of valve progenitor cells. He is now investigating the importance of primary cilia function in valve development. All these results will help to explain the origin of congenital cardiac valve defects.

Session 6. Genetics of human congenital heart defects (Chairs David Brook and Deborah Henderson)
The session comprised six talks, focussed around identifying, modelling and interpreting the importance of potential disease-causing mutations in human patients. The first talk started with an overview of the topic from David Brook (Nottingham), where he discussed the similarities and differences in approach between recent published studies that have begun to unravel the complexities of studying syndromic and isolated cases of CHD, highlighting the different patterns of inheritance observed between the two groups. The next five presentations were selected from submitted abstracts. The first of these, from Duncan Sparrow (Oxford), discussed how environmental factors, particularly hypoxia, might influence cell signalling and gene expression in the developing embryo. Lars Allen Larsen (Copenhagan) then described the patterns of re-occurrence of CHD in families, and the different types of CHD that were found in such families. The opportunities arising from obtaining genomic DNA for genetic studies from formaldehyde-fixed archival hearts was discussed by Ahlam Alqatani (Newcastle); this was put in the context of the damage caused to the DNA by formaldehyde fixation and the caution needed when interpreting data from such studies. Amina Kamar (Beirut) and Melanie Phillipp (Ulm) described studies in which the relevance of human mutations was investigated in the context of CHD, using cell lines and zebrafish respectively. Together, these presenations gave an excellent oversight of the complexities of studying CHD in humans and provided exciting new data to this important area of cardiovascular research.

Session 7. Conduction System (Chairs Thomas Brand and and Marina Campione)
The session on the cardiac conduction system (CCS) was opened by a general introduction into the development of the conduction system by Thomas Brand (London). He went on to talk about the Popeye domain containing (POPDC) gene family encoding transmembrane proteins, which functions as cAMP effector proteins controlling membrane trafficking of ion channels and anchor proteins. POPDC proteins are highly expressed in the cardiac conduction system but also in working myocardium. After a general review on POPDC gene function, he talked on disease association of POPDC genes. A recently discovered missense mutation of POPDC1 (S201F), which was found in a family suffering from limb girdle muscular dystrophy (LGMDX) affects the ability of POPDC1 to bind

cAMP. The mutant protein displays impaired membrane trafficking of itself and of interacting proteins. The POPDC1 mutant caused impaired channel transport and function and also structrally affected skeletal muscle causing aberrant formation of the myotendinous junction. Silja Burkhard (Utrecht), used tomo seq in order to gain novel insight into pathways involved in pacemaker development in the zebrafish. Interestingly, the pacemaker in the zebrafish is not a node but instead forms a ring at the sinuatrial junction and can be identified by islet-1 or the recently described enhancer trap line ET33-mi28. The tomo-seq approach revealed that canonical Wnt signalling is important for cardiac pacemaker formation downstream of Isl1. Impairing canonical Wnt signaling affected cardiac pacemaker function. Since Isl1 is also expressed in the sinus node of higher vertebrates, it will be interesting to find out whether canonical Wnt signalling is also required in mammals. The ventricular conduction system (VCS) originates from precursors present in the trabecular myocardium and can be identified by Cx40 expression. An important determinant of VCS development is Nkx2.5. Caroline Cochet (Marseille) performed a lineage analysis of trabecular myocardium using *Cx40-CreERT2* mice, which were crossed with *Rosa26-Confetti* mice. As early as E9.5, the first VCS precursors were found to be present in the trabecular compartment. Additional cells are recruited to the VCS lineage as development proceeds. Nkx2.5 plays an important role during recruitment but is also required for the maintenance of CCS identity.

Session 8. Myocardium and myocardial regeneration (Chairs: Mathilda Mommersteeg and Maurice van den Hoff).

The session included 5 talks, one from an invited speaker Mommersteeg) and 4 selected from abstract submission. Mommersteeg (Oxford) gave a brief overview of the state-of-the art knowledge on cardiac regeneration in the zebrafish, mouse and human. Next she introduced a new experimental fish model, the Mexican cavefish, two variants exist, of which the cave variant is not able to regenerate its ventricle upon scaring and the surface variant is able to do so. Using a global genetics approach, she aims at unraveling the molecular pathways involved. Boudewijn Kruithof (Leiden) introduced his ex vivo closed flow system to culture intact adult mouse hearts. He showed that it is possible to mimic the induction of cardiac fibrosis and influence it by altering cell signaling. Helen Phillips (Newcastle) showed that conditional downregulation of the cell signaling molecule ROCK during cardiac development is an important novel genetic modifier that increases the chance of developing hypertrophic cardiomyopathy in adult life. PhD Student Sanches-Iranzo (Bern/Madrid) showed that in the zebrafish the ventricle is mainly derived from the first heart field but that during regeneration of the injured heart, the regenerated cardiomyocytes are mainly derived from the second heart field. Interestingly, their analysis also showed that trabecular cardiomyocytes can contribute to the outer “compact” layer of the ventricle. PhD Student Kruse (Utrecht) introduced the method of whole genome sequencing on sections (Tomo-Seq) throughout the entire embryo and individual cardiac cells on Zebrafish. The single cell sequencing results of zebrafish hearts after injury revealed that the differentiation state of the cardiomyocytes ranged from poorly to fully differentiated.