Scope

The developmental complexity of cardiac morphogenesis is reflected by the high incidence of congenital heart defects that occur in up to 1% of all live births\(^1\),\(^2\). Our understanding of the aetiology and ontogeny of congenital heart defects would undoubtedly benefit from comprehensive insight into the genetic and molecular networks that orchestrate cardiac development. In the past two decades, advances in molecular biology, e.g. genetic manipulation, have supported the emergence of stem cell research and the generation of animal models of disease. Transgenic mouse models of congenital heart disease have revealed that early cardiac growth is contingent on the differentiation of a cardiac progenitor pool termed the second heart field\(^3\). Impaired development of the second heart field particularly impacts on the arterial pole, accounting for approximately one third of all congenital heart disease cases\(^4\),\(^5\). The studies described in this thesis provide clues on the developmental and genetic basis of congenital defects of the arterial pole of the heart.

In Part 1, we summarize contemporary insights into heart development and review the most recent data on the developing arterial pole and second heart field progenitor pool (Chapter 1).

Part 2 focuses on the developmental remodelling of the cardiac outflow tract and pharyngeal arch arteries into the great vessels, aortic arch and its tributaries. In 2001, the myocardial cells of the outflow tract and right ventricle in mouse were shown to be second heart field derivatives\(^6\). In contrast, precursors of the right ventricle in the chicken heart were thought to be present in the linear heart tube and not in progenitors outside the heart. Cell tracing experiments (described in Chapter 2) demonstrate that the avian trabeculated free wall of the right ventricle is derived from SHF myocardium that initially formed the myocardial wall of the outflow tract, indicating that the developmental origin of the right ventricle is evolutionarily conserved in birds and mammals.

The development of the human aortic arch system is often incorrectly interpreted and mixed up with the configuration commonly found in lower vertebrates. In our aim to resolve this, we generated interactive three-dimensional reconstructions of the developing human aortic arch system, supplemented with the distribution of developmental markers for patterning and growth to facilitate unbiased interpretations (Chapter 3). Our findings highlight that little is known about the developmental origin of the different components of the adult aortic arch and pulmonary system and that future lineage and cell-labelling experiments will be pivotal. Importantly, our findings indicate that the mechanisms underlying human and mouse aortic arch system development are largely conserved and, as such, support future genetic and molecular analyses using animal models of congenital heart disease.
In Part 3, we elaborate on the genetic underpinnings of SHF development and deployment. The gene encoding the T-box transcription factor Tbx1 is an important regulator of SHF and outflow tract development. In human, TBX1 has been implicated in 22q11.2 Deletion Syndrome (22q11.2DS), caused by an interstitial microdeletion of up to 3 Mb in chromosome 22. In Chapter 4, we studied the mechanisms underlying loss of subpulmonary myocardial precursors resulting in hypoplasia of the distal OFT in Tbx1-deficient mice. Interestingly, subpulmonary myocardium shares a lineage relationship with venous pole precursors. Using gene expression profiling, genetic and Dil tracing experiments and three-dimensional reconstruction tools, we demonstrate that Tbx1 controls venous as well as arterial pole development by regulating the segregation of a common cardiac progenitor pool into different sublineages, providing new insights into the etiology of CHD and 22q11.2DS phenotypes and how arterial and venous pole defects can coexist. In addition to Tbx1, family members Tbx2 and Tbx3 are also known to be required during OFT development. We show, using mouse genetics and gene expression analyses, that Tbx1, Tbx2, and Tbx3 constitute a T-box regulatory network that controls OFT and pharyngeal development (Chapter 5). Our findings highlight the central roles of Tbx1/Tbx2/Tbx3 in conotruncal morphogenesis and identify Tbx2 and Tbx3 as candidate modifier genes of the cardiopharyngeal phenotypes in TBX1 haploinsufficient 22q11.2DS patients.

In Part 4, we set out to identify novel candidate genes underlying tetralogy of Fallot (ToF), the most common cyanotic congenital arterial pole defect (Chapter 6). The multigenic etiology of this common anomaly was investigated by deploying a microfluidic PCR-based amplicon-tagging and enrichment strategy followed by next-generation sequencing in 480 unrelated patients with isolated non-syndromic ToF. Mutational screening of the ToF candidate genes CRKL, FRS2 or GATA6 unveiled that the majority of the sequence variants were present in non-coding sequences, including intronic and intergenic regions and the 3′-UTR. Our results are in agreement with the notion that synonymous coding variants are relatively rare in congenital heart defect patients. We propose that non-coding regions, known to be pivotal in the regulation of gene expression and mRNA translation, should be more frequently interrogated for variants and compared with accumulating databases on regulatory elements in order to fully unveil the multigenic etiology of ToF.
References