Cardiac development in relation to clinical supraventricular arrhythmias : focus on structure-function relations

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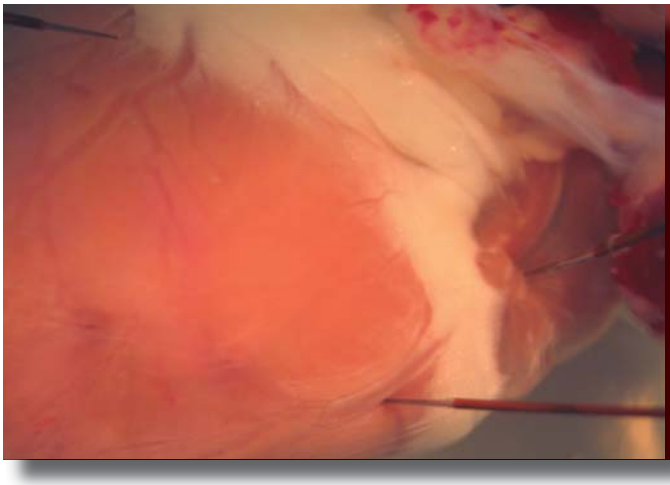
Chapter



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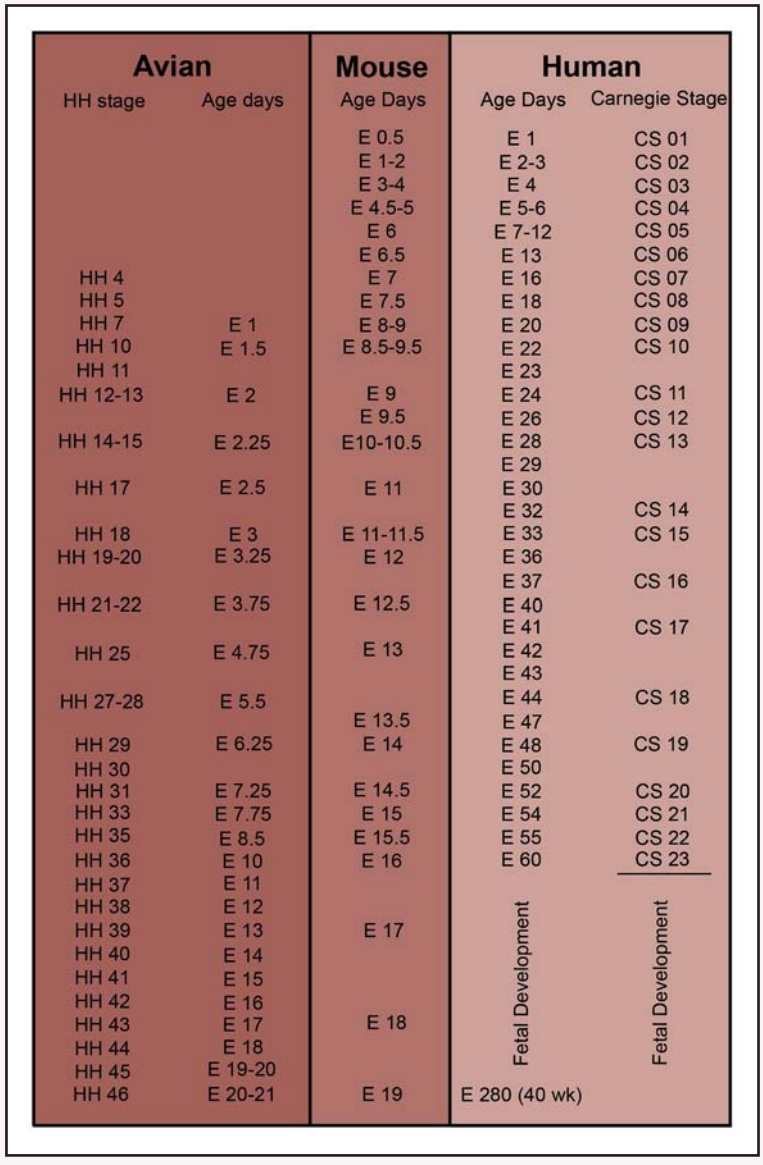
General Introduction & Outline of the Thesis

Outline General Introduction

Supraventricular tachycardias (SVTs) are amongst the most commonly encountered cardiac arrhythmias in clinical practice in both children and adults.[1, 2] The causative mechanisms underlying the appearance of most of these SVTs have however still remained as intriguing as they are unexplained. In this thesis, cardiac development is analyzed in relation to the etiology of clinical supraventricular arrhythmias with a special focus on structure-function relations.

Firstly, in **PART I** of this thesis, both the (patho) physiological development of the annulus fibrosus cordis and the etiological origin of clinical accessory AV pathway (AP) mediated AVRT in children and adults is analyzed in experimental animal models and human sections. Secondly, in **PART II** of this thesis a review of the different ontogenic theories on the embryonic development of the AV Node (AVN) in literature is followed by an experimental study postulating a new concept on the developmental origin of the AVN in relation to the etiology of AV Nodal Reentrant Tachycardia (AVNRT).

As a general introduction to both these 'basic research' (**I** & **II**) and the 'clinical' (**III**) parts of this thesis, structural cardiac development in avians (with references to equivalent mouse and human developmental timelines) (**Figure 1**) will first be described since the development of the cardiac conduction system (CCS) and structural cardiogenesis are intimately related. Next, the developmental transitions in impulse propagation and the construction of the individual components of the specialized CCS and the AVN in particular will be shortly outlined. Following a description of the changes in electrocardiograms (ECGs) during cardiogenesis, current concepts on the transitions in ventricular activation sequences during embryogenesis will be discussed. Thereafter, contemporary knowledge on the development of the isolating annulus fibrosis, the key structure involved in AP persistence, in relation to general CCS development will be reviewed. Subsequently, relevant general characteristics of the different animal models and the immunohistochemical markers used in this thesis are briefly discussed. Following the description of the structural basics of cardiogenesis, attention will be focused on current knowledge of clinical SVTs in neonates and children and the treatment of these arrhythmias. These therapeutic clinical issues will be further outlined in **PART III** of this thesis.



Avian		Mouse	Human	
HH stage	Age days	Age Days	Age Days	Carnegie Stage
		E 0.5	E 1	CS 01
		E 1-2	E 2-3	CS 02
		E 3-4	E 4	CS 03
		E 4.5-5	E 5-6	CS 04
		E 6	E 7-12	CS 05
		E 6.5	E 13	CS 06
HH 4		E 7	E 16	CS 07
HH 5		E 7.5	E 18	CS 08
HH 7	E 1	E 8-9	E 20	CS 09
HH 10	E 1.5	E 8.5-9.5	E 22	CS 10
HH 11			E 23	
HH 12-13	E 2	E 9	E 24	CS 11
		E 9.5	E 26	CS 12
HH 14-15	E 2.25	E10-10.5	E 28	CS 13
			E 29	
HH 17	E 2.5	E 11	E 30	
			E 32	CS 14
HH 18	E 3	E 11-11.5	E 33	CS 15
HH 19-20	E 3.25	E 12	E 36	
			E 37	CS 16
HH 21-22	E 3.75	E 12.5	E 40	
			E 41	CS 17
HH 25	E 4.75	E 13	E 42	
			E 43	
HH 27-28	E 5.5		E 44	CS 18
		E 13.5	E 47	
HH 29	E 6.25	E 14	E 48	CS 19
HH 30			E 50	
HH 31	E 7.25	E 14.5	E 52	CS 20
HH 33	E 7.75	E 15	E 54	CS 21
HH 35	E 8.5	E 15.5	E 55	CS 22
HH 36	E 10	E 16	E 60	CS 23
HH 37	E 11			
HH 38	E 12			
HH 39	E 13	E 17	Fetal Development	Fetal Development
HH 40	E 14			
HH 41	E 15			
HH 42	E 16			
HH 43	E 17	E 18		
HH 44	E 18			
HH 45	E 19-20			
HH 46	E 20-21	E 19	E 280 (40 wk)	

Figure 1. Schematic overview of the major staging systems of embryonic development in the avian, mouse and human embryonic developmental timeline. *Sources: Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. J Morphol. 1951;88:49-92, Fishman MC, et al. Development.1997;124:2099-2117, O'Rahilly R. Early human development and the chief sources of information on staged human embryos. Eur J Obstet Gynecol Reprod Biol. 1979;9:273-80, Edinburgh Human Developmental Anatomy (EHDA) Human versus Mouse Developmental Stage Comparison, University of New South Wales (UNSW) Carnegie Stage Comparison, University of New South Wales (UNSW) Chicken Developmental Stages.*

STRUCTURAL CARDIOGENESIS AND TRANSITIONS IN ELECTRICAL WIRING OF THE DEVELOPING HEART

1.1. Structural Heart Development

During cardiogenesis intriguing processes of cell recruitment, fusion, looping and septation, ultimately facilitate the formation of the four-chambered heart. The first cardiac progenitor cells can already be identified even before gastrulation in the epiblast layer as it is separating from the hypoblast.[3, 4] These heart precursor cells will invaginate through the rostral half of the primitive streak and are amongst the first embryonic cells to gastrulate.[5-9] In avians, the gastrulation process sets off at Hamburger-Hamilton (HH) stage 4 to 5 (human embryonic day (E) ~16-18, mouse ~E 7-7.5), with the recruitment of the cardiac progenitor cells from the primitive streak.[10-13] These cells will subsequently migrate to the bilateral splanchic mesodermal crescent-like primary heart fields, that express cardiac-specific genes like Nkx2.5 and GATA 4-6,[11, 14, 15] already indicating their potential to terminally differentiate into myocardial cells.[16]

At about HH stage 8 to 9 (human ~E 20-21, mouse ~E 8-9), the bilateral heart fields will fuse in the ventral midline in cephalocaudal direction to ultimately give rise to the primitive linear heart tube.[12, 17] The process of fusion of the bilateral heart fields is a sequential process, since fusion of these endocardial primordia spatiotemporally highly depends on definitive closure of the floor of the developing foregut. As a consequence, the heart tube is formed in a cephalocaudal sequence, first forming the truncoventricular portion, then the atrium and last of all the sinus venosus.[18-20] The ultimate straight heart tube contains an outer myocardium and an inner endocardium (derived from the remaining endothelial cells of the embryo that are recruited for vascular development) separated by an extracellular matrix (ECM) known as the cardiac jelly. The dorsal mesocardium, which will later on be separated to form the arterial and venous pole connections, links the primary heart tube to the dorsal body wall. Cranially the heart tube is connected to the pharyngeal arches and caudally to the omphalomesenteric veins.[12, 13]

The myocardium of the tubular and later looped heart forms a single or double cell layer at the circular periphery and is not yet covered by epicardium. However already at these early stages, anisotropic arrangement of the cardiomyocytes is clearly evident; the inner cell layer is more differentiated[21] and along the length of the heart tube preferential circular alignment of the myofibrils is seen in the AV canal and outflow tract region.[22]

The primitive heart tube will begin it's rightward folding process at about HH stage 10 (human ~E 22, mouse ~E 8.5-9.5), first transforming in a C-shaped and than in a more S-shaped structure in order to facilitate adequate mature positioning of the cardiac chambers (e.g. positioning of the future right atrium above the future right ventricle).[17, 23] This looping program is regulated by a cascade of genes of which the exact interactions are still largely unclear, but that are also critical for the left and right programming of the embryo itself.[14, 24]

Whilst the heart tube undergoes its dextral looping phase (HH stage 9-34), the cardiac jelly lining the inside of the myocardium is unevenly remodeled over the full length of the heart tube into endocardial cushions in the AV canal and the outflow tract, which are subsequently invaded by mesenchymal cells derived from the endocardium by Epithelial-Mesenchymal-Transformation (EMT).[25]

During looping, the heart tube consists of several cardiac segments: the left and right sinus venosus horns, the primitive atrium, the ventricular inlet segment and the ventricular outlet segment. These segments are divided by so-called transitional zones, brought together in the inner curvature of the heart by the looping process.[25] With continued looping, the cardiac chambers will further differentiate, a process controlled by a different subset of genes and transcription factors,[26, 27] which subsequently results in positioning of the ventricles and outflow tract of the heart in an anterior/ventral position and of the atria in a dorsal/posterior position. **Figure 2** schematically demonstrates the location of the transitional zones during the major developmental stages in cardiogenesis.

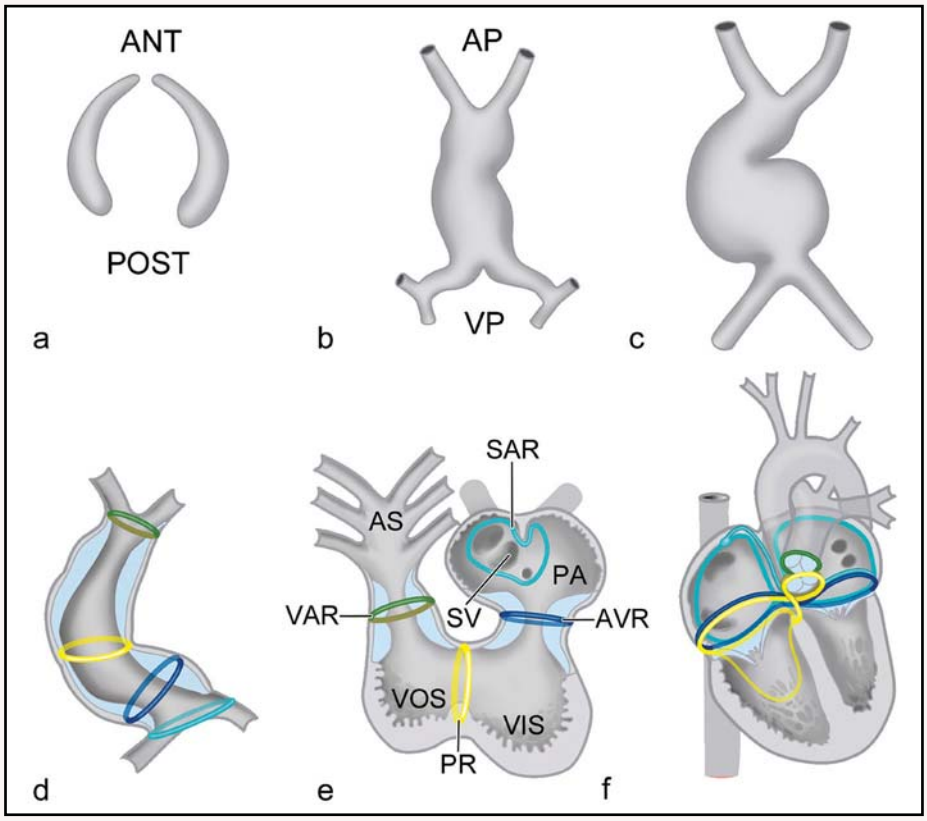


Figure 2. Schematic representation of the spatiotemporal relation of the transitional zones in cardiogenesis. The bilateral cardiogenic plates are derived from the splanchic mesoderm (a). These bilateral plates fuse in the ventral midline in cephalo-caudal direction to form the primitive linear heart tube (b). Subsequently, the linear heart tube undergoes dextral looping, that transforms the heart in a C-shape and later in a S-shape (c). After looping, the transitional zones or rings dividing the different putative chambers of the heart can be recognized, being the sinu-atrial transition (SAR), the atrioventricular ring (AVR), the primary ring (PR) and the ventriculo-arterial transition (VAR) (e-f). AP=arterial pole, VP=venous pole, PA=primitive atrium, AS=arterial segment, VOS= ventricular outflow segment, VIS= ventricular inflow segment. *Adapted from: Gittenberger-de Groot AC, et al. Pediatr Res. 2005;57:169-176.*

The next stage in ventricular morphogenesis involves the development of trabeculation, needed to increase the surface area to increase diffusion potential for nourishing the still avascular myocardium, allow the myocardial mass to increase, coordinate intraventricular conduction, enhance contractility and effectively route blood flow.[28-30] The bulk of compact myocardium is subsequently formed by trabecular compaction, which coincides with the onset of ventricular septation and the now compulsory development of coronary circulation.[29, 31]

At this time, in order to construct a mature four-chambered heart, septation is initiated at the level of the atrium, the ventricle and the arterial pole. Moreover, at the venous pole the sinus venosus becomes incorporated in the dorsal wall of the right and left atrium and receives the venous inflow of the left and right superior cardinal veins as well as the pulmonary veins.[23, 32] In the human heart, as development proceeds, the left cardinal vein regresses becoming the ligament of Marshall and oblique vein, while the remaining proximal portion (with part of the left sinus horn) becomes the coronary sinus (CS), which will open via the sinoatrial foramen into the right atrium.[10, 32-36]

As a result of subsequent endocardial cushion remodeling, the AV cushions take part in formation of the AV septal structures and AV valves (mitral and tricuspid valve), while the cushion tissues in the outflow tract are essential for the formation of the semilunar valves of the aorta and pulmonary artery and contribute to outflow tract septation.[35, 37] Due to the resultant formation of the cardiac septa and of the mitral and tricuspid valve and aortic and pulmonary valve, a functional four-chambered heart can now direct the future separate systemic and pulmonary circulation. **Figure 3** summarizes the transitions in alignment of the cardiac segments during cardiogenesis.

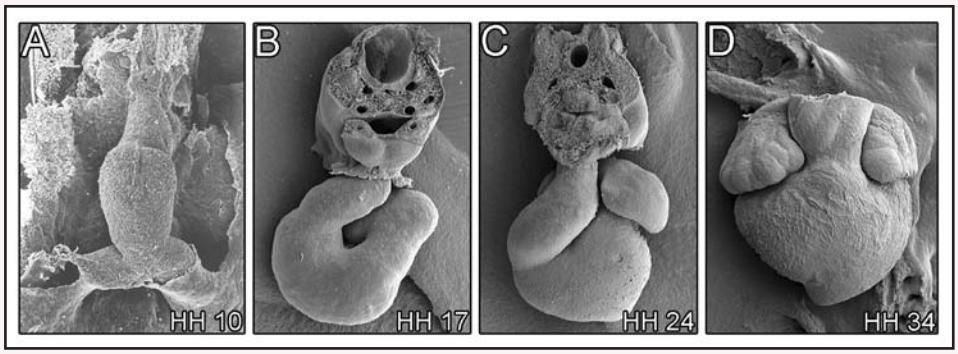


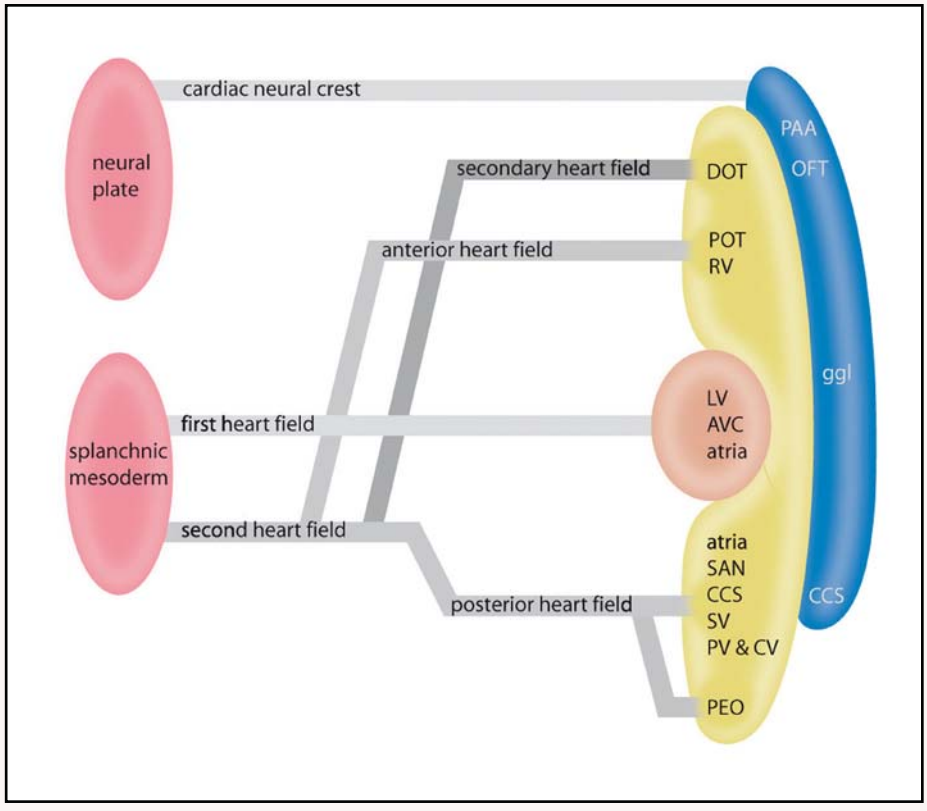
Figure 3. Transitions in alignment of the cardiac segments during cardiogenesis. **A.** At HH 10 the primitive heart tube begins it's rightward folding process. **B.** Around HH 17 the C-shaped heart tube is still in the midst of its looping phase. **C.** At HH 24, the right atrium becomes positioned above the right ventricle, whilst the left atrium is positioned above the left ventricle. **D.** Around HH 34 a four-chambered heart has been formed, which can now separate the future systemic and pulmonary circulation.

Figure 4. (right) Schematic representation of the primary linear heart tube (in brown) and the secondary added myocardium derived from the second heart field (in yellow).The second heart field is subdivided in the anterior heart field (arterial pole), the secondary heart field (arterial pole) and the posterior heart field (venous pole).The pro-epicardial organ (PEO), the source of Epicardium-Derived-Cells (EPDCs) is also derived from the posterior heart field at the venous pole of the heart. Cardiac neural crest cells (blue) enter the heart at both the arterial and venous pole. AVC=atrioventricular canal, CV=cardinal veins, CCS=cardiac conduction system, DOT=distal outflow tract, LV=left ventricle, OFT=outflow tract, PAA=pharyngeal arch arteries, ggl=ganglions, POT=proximal outflow tract, PV=pulmonary veins, RV=right ventricle, SAN=sinoatrial node, SV=sinus venosus. *Adapted from: Jongbloed MRM, et al. Development of the cardiac conduction system and the possible relation to predilection sites of arrhythmogenesis. The Scientific World Journal. 2008;8:239-269.*

1.2. Secondary & Extracardiac Contributions to the Heart

1.2.1. Secondary Contributions to the Heart

The major cardiac segments of the linear primitive heart tube - the left ventricle (LV), the AV canal (AVC) and part of the atria - are derived from the bilateral splanchic mesodermal primary heart fields (first heart field), as described above (**Figure 4**). The pharyngeal mesoderm provides the heart with a second cardiac progenitor pool (second heart field) that enters the heart at both the venous and arterial pole (**Figure 5**).[38-40] The second heart field can be subdivided in the anterior heart field (AHF) and the secondary heart field (SHF) at the arterial pole[41-43] and the posterior heart field (PHF) at the venous pole (**Figure 4**).[20, 32, 44-51]



The cardiac outflow tract (OFT) myocardium and a large part of the right ventricle (RV) are one of the last segments of the heart to form and will be added to the arterial pole of the primitive heart tube. These cardiac structures arise from a cellular population of the pharyngeal mesoderm, which initially starts migrating to the conotruncal area between HH stage 7 (human ~E 20, mouse ~E 8.0) and HH stage 13-14 (human ~E 26, mouse ~E 9.5), prior to neural crest cell invasion.[38-40, 52]

Cardiac progenitor cells derived from the posterior heart field added to the heart at the venous pole, have been shown to contribute to the formation of the atria, interatrial septum (IAS), pulmonary veins (PV), cardinal veins (CV), sinus venosus (SV) and the components of the CCS (**Figure 4,5**).[20, 32, 40-53]

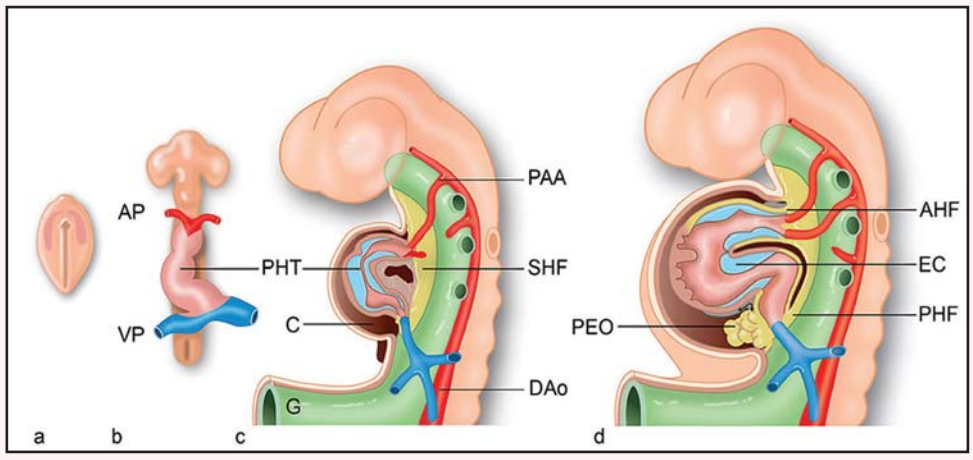


Figure 5. Schematic figure depicting the contribution of the primary (pink & blue) and secondary (yellow) heart-forming fields. The second heart field (SHF) can be divided into the anterior heart field (AHF) at the arterial pole of the heart and the posterior heart field (PHF) at the venous pole of the heart. The Pro-Epicardial-Organ (PEO) develops as part of the PHF (yellow). AP=arterial pole, VP=venous pole, PHT=primary heart tube, PAA=pharyngeal arch arteries, DAo=dorsal aorta, C=coelomic cavity, EC=endocardial cushions, G=gut. *Adapted from: Gittenberger-de Groot AC, et al. Cardiac morphogenesis. In Fetal Cardiology. 2nd ed. Yagel S, Silverman NH and Gembruch U, Eds. Taylor and Francis. London, 2008, in press.*

1.2.2. Epicardium-Derived-Cells (EPDCs)

Classically, Epicardium-Derived-Cells (EPDCs), derived from the Pro-Epicardial-Organ (PEO), have been considered as one of the extracardiac contributors to the developing heart.[54] In view of recent new concepts on the spatiotemporal addition of cells from the different heart forming fields, the true extracardiac origin of EPDCs can be debated.[47, 54] The posterior heart field (derived from the splanchic mesoderm) is located at the site where the sinus venosus enters the pericardial cavity, which is also the site where de PEO originates.[47, 55] In **Figure 6** the spatial relation of the primary and secondary (anterior and posterior) heart fields, the neural crest cells and the PEO is depicted.

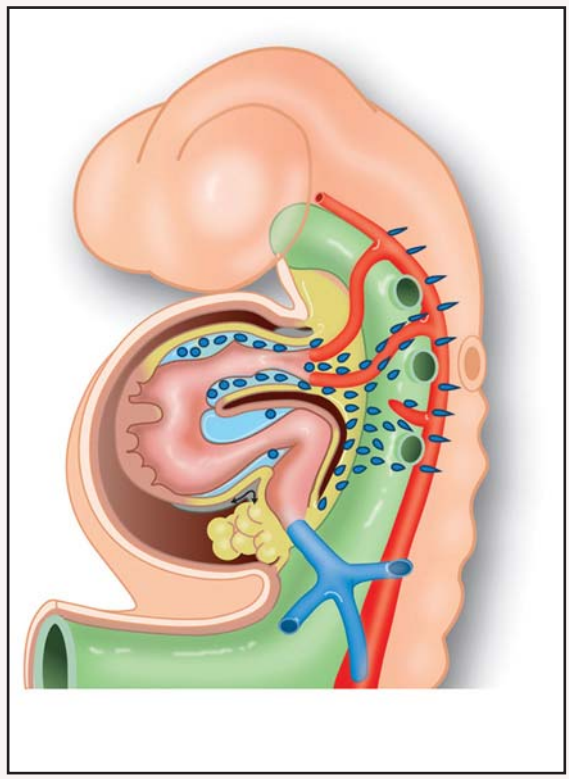


Figure 6. Spatial relation of the primary and secondary heart fields, the neural crest and PEO. Extracardiac contribution of the cardiac neural crest cells to the arterial and venous pole of the heart (blue cells). The secondary heart field is depicted in yellow. *Adapted from: Gittenberger-de Groot AC, et al. Cardiac morphogenesis. In. Fetal Cardiology. 2nd ed. Yagel S, Silverman NH and Gembruch U, Eds. Taylor and Francis. London; in press.*

During embryonic development, the epicardium is formed from the splanchnopleural mesoderm of the PHF by formation of a cauliflower like villous structure known as the pericardial serosa, proepicardium or Pro-Epicardial-Organ (or PEO) on the pericardial wall covering the SV and venous pole of the heart. The PEO protrudes from the pericardial mesothelium into the pericardial cavity in the direction of the looped heart.[56-61]

Both in mammalian and avian embryos, the PEO is initially formed as paired bilateral symmetrical structures on the transverse septum (mouse) and sinus venosus (avian). In chicken, the left PEO Anlage does however not persist, whereas the right PEO will develop into the cauliflower like protrusion (PEO).[62, 63]In avians, around HH stage 16, EPDCs will migrate to the naked heart tube by means of a tissue bridge which is formed between the SV and the dorsal wall of the AV canal of the looped heart and initially forms a mesothelial outside covering of the myocardium. After attachment to the myocardial surface, the cells start to migrate radially and start to circumvent the AV region, the inner curvature and the dorsal side of the outflow tract.[61, 64, 65] After covering the last parts of the heart – the left atrium and parts of the distal outflow tract - the heart will be completely covered by mesothelium by HH stage 26.[61] **Figure 7** schematically demonstrates the temporal relations in cardiogenesis and EPDC formation.

From HH stage 19 onwards, immediately after the onset of spreading of EPDCs over the myocardial surface, the epicardial mesothelial sheet will undergo Epithelium-to-Mesenchymal Transformation (EMT).[64, 66, 67] Initially, the resultant mesenchymal EPDCs reside in the subepicardial matrix. In chicken embryos, the subepicardum is relatively thin (one to three cell layers) at the atrial and ventricular myocardium, while it is very thick in the AV sulcus where abundant EMT is needed to provide EPDCs for coronary formation.[66]

Mesenchymal EPDCs will subsequently invade the myocardium in a spatiotemporally regulated fashion.[64, 66, 67] While the precise temporal regulation of EPDC migration has remained unknown, two distinct influxes into the myocardium of the chicken heart have been described. The first influx directly follows the process of EMT and formation of the subepicardium and takes place between HH stage 19 and HH stage 31, while the second influx takes place between HH stage 31 and HH stage 43. First influx EPDCs will take up subendocardial positions in the atrium and ventricle and will migrate into the myocardial interstitial spaces, whereas EPDCs from the second influx will mainly migrate into the AV cushions (**Figure 7**).[64, 68]

From previous studies in epicardial quail-chicken chimeras, we know that at HH stage 35, most EPDCs will have taken up their final position: around the coronary arteries as smooth muscle cells (SMCs) and fibroblasts,[66, 69-71] in the ventricular myocardium as interstitial fibroblasts,[64, 68, 70] in the AV cushions[68, 70] and in the subendocardium of the ventricular trabeculae and atria.[68]

Numerous studies in experimental models in which epicardial development has been disturbed mechanically or genetically, have proven the functional significance of EPDCs in cardiac development.[67, 72-83] In this respect, EPDCs have been inferred to play crucial roles in the development of the coronary vasculature, the AV valves, the myocardial architecture, the peripheral conduction system (Purkinje fibers) and in the formation of the isolating AV annulus fibrosis (**Figure 8**)(see also **Chapter 3**, *this thesis*).

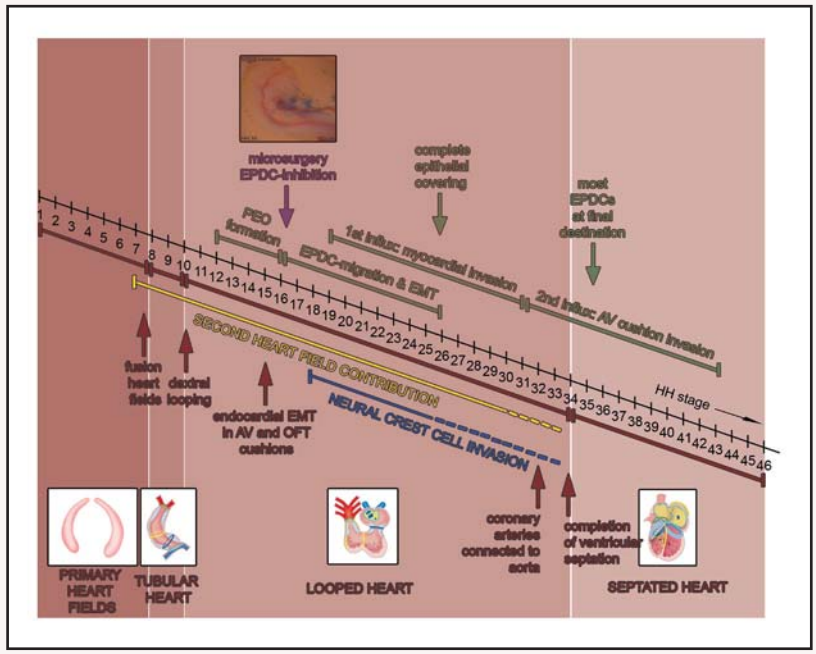


Figure 7. Temporal timeline in avian cardiogenesis and formation of Epicardium-Derived-Cells (EPDCs). The different stages of cardiogenesis are indicated by red blocks (primary heart fields, tubular heart, looped heart and septated heart) and schematic figures outlining the overall structure of the heart. At the bottom of the timeline, major events in cardiogenesis are indicated (red). Additionally, ingrowth of the second heart field population of cells and the extracardiac NCCs is indicated in yellow and blue, respectively. Contemporary processes of PEO-formation, EPDCs migration and myocardial invasion are indicated at the top of the timeline (green).

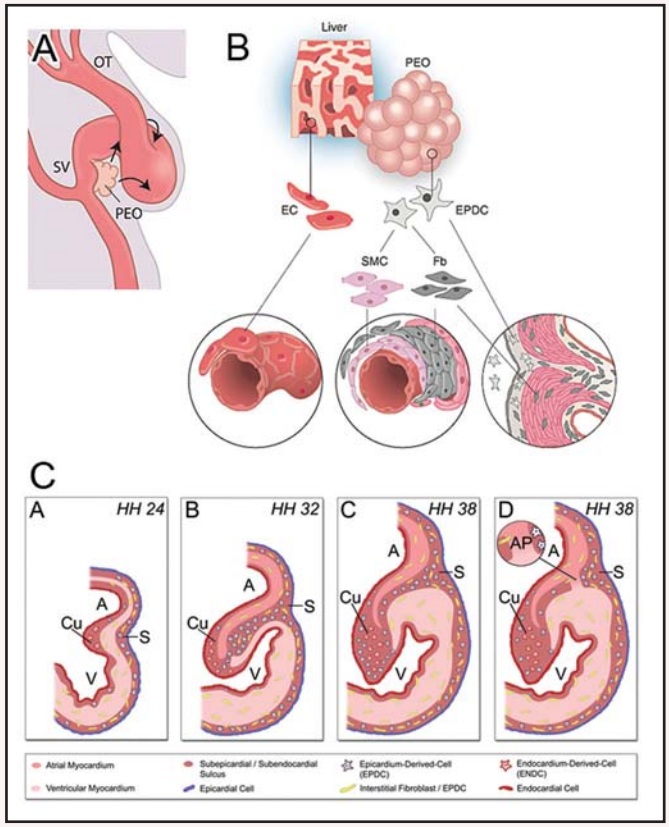


Figure 8. Schematic figure depicting EPDC fate and function. A. In avians, around HH stage 16, EPDCs will migrate to the naked heart tube from the sinus venosus region to the dorsal wall of the AV canal of the looped heart. After attachment to the myocardial surface, the cells start to migrate radially and start to circumvent the atrioventricular region, the inner curvature and the dorsal side of the outflow tract. After covering of the left atrium and parts of the distal outflow tract, the heart will be completely covered by HH stage 26. **B.** The proepicardial cells migrate from the Pro-Epicardial-Organ (PEO) to the heart tube. After migration, epithelium-mesenchymal-transformation (EMT) and formation of the subepicardium, the EPDCs start migrating into the myocardium and differentiate into smooth muscle cells (SMCs) in the media and adventitia of the coronary vessels and fibroblasts in the interstitium and the fibrous heart skeleton. **C.** From HH32 onwards, subepicardial EPDCs in the AV sulcus (S) migrate through the continuous AV junctional myocardium to ultimately populate the endocardial AV cushions (Cu). In the normal 4-chambered heart, EPDCs continue populating the AV cushions and favour 2 positions: 1) the myocardial/endocardial cushion interface and 2) the subendocardially at the luminal face of the AV cushions. *Figure A,B Adapted from: Winter EM, et al. Epicardium-derived cells in cardiogenesis and cardiac regeneration. Cell Mol Life Sci. 2007;64:692-703. Figure C adapted from Kolditz DP, et al. Epicardium-Derived-Cells (EPDCs) in Annulus Fibrosis Development and Persistence of Accessory Pathways. Circulation 2008;117:1508-1517.*

1.2.3. Extracardiac Contributions to the Heart – Neural Crest Cells (NCCs)

After looping of the single linear heart tube, the true extracardiac contributors to heart development, the pluripotent neural crest cells (NCCs), migrate from the neural crest into the arterial and venous pole of the developing heart (**Figure 6**).[84-86] Neural crest cells or ectomesenchymal cells have been traced to various parts of the embryo, including the face, thymus and the thoracic great vessels.[25] It is well established that NCCs originating from the posterior rhombencephalic segments of the neural tube (from the otic placode to the third somite) contribute to multiple aspects of cardiac development and function. However, the contribution of these cardiac NCCs has been suggested to be mostly instructive rather than constructive since the majority of NCCs are destined for apoptosis.[87] The contribution of NCCs to the vessels of the arterial pole however is substantiated, since the major part of the smooth muscle cells have a NCC origin.[88]

The mesenchymal NCCs first arrive at the outflow tract (arterial pole) and thereafter populate the inflow tract of the heart (venous pole).[25] Seminal work using NCC extirpation and analysis of quail-chick chimeras, demonstrated that NCCs entering the heart through the pharyngeal arches at the arterial pole, contribute to the neurons of the cardiac autonomic nervous system, aortopulmonary septum, the tunica media of the great arteries, the outflow tract septum and the semilunar valves.[88-97] The cardiac NCCs entering the heart at the venous pole, migrate to the dorsal mesenchymal protrusion forming the vestibular spine, from where they contribute to the base of the atrial septum and the condensed mesenchyme that is forming the membranous part of the ventricular septum.[98-100] Furthermore, NCCs entering the heart at the venous pole have been observed in vicinity of putative elements of the CCS before they undergo their fate of apoptosis. [99, 101, 102] Interestingly, neural crest ablation in the chick was recently shown to result in a lack of differentiation of the compact lamellar organization of the His bundle, which separates this essential structure from the surrounding working myocardium.[103]

1.3. Impulse Propagation During Cardiogenesis

1.3.1. Tubular Heart

In the avian embryo, when only 7 to 10 somites have yet developed (HH stage 9, equivalent age in human ~E 21-22 and mouse ~E 8.5), a small dominant pacemaking area already becomes established at the posterior inflow side of the heart (the presumptive atrium and SV region), well before formation of the tubular heart is completed and contraction is initiated.[104-106] The posterior inflow tract myocardium thus becomes electrically active, long before the primitive myocardium of the heart tube acquires the ability to contract.[107]

The very first faint and slow but rhythmic contractions (approximately 24 beats/min. in avians) will subsequently appear in the cephalic ventricular myocardium (first fused cardiac segment) around HH stage 10 (equivalent to 9-10 somites, human ~E 22, mouse ~E 8.5-9.5) even before cephalocaudal fusion of the paired primordia is complete in the atrial region.[18, 108-110] At this developmental stage, the atria and SV do not yet exist as a differentiated part of the heart, but are merely represented by endocardial primordia which are still widely separated from each other in the bilateral heart fields.[18]

These early pulsations are however still inefficient to set the blood in motion through the developing blood vessels and merely consist of non-propagating local twitchings that initially appear as fibrillar contractions along the right margin of the bulboventricular region and then coalesce to produce a concerted movement of the entire right side of the ventricle.[18, 111] Next, the left side of the ventricle becomes involved in these twitchings and subsequently the entire primitive ventricle displays synchronous contractions. These early contractions are however not yet regularly occurring, but are interrupted by rest periods, which will become progressively shortened as a slow regular rhythm gradually becomes established.[111]

The continuing Anlage of the fusing caudal cardiac segments is spatiotemporally correlated to the onset of myocardial contractions with progressively higher intrinsic pulsation rates along the anteroposterior axis, reaching its peak after final fusion of the sinus primordia at the venous pole.[105, 107, 111-113] Pacemaker dominance thus spatiotemporally spreads to the different cardiac regions in the same sequence in which they are formed by continued caudal fusion of the bilateral heart fields.[18]

In the completely fused primitive heart tube (HH stage 10), equivalent to ~23 days post conception (dpc) in humans (mouse ~E 8.5), stronger and regular

peristaltic caudal to cranial contractions, finally facilitating the first efficient propulsion of blood from the venous to the arterial pole of the heart, will be seen. At this developmental stage, when the atrial primordia have fused, spontaneous action potentials are generated in a dominant area of pacemaker cells in the left posterior inflow-site of the heart (atria and SV region).[13, 105, 111, 114-117]

Only some time after the beginning of circulation, the SV is formed (> HH stage 10) and ultimately starts to dominate the pacemaking rate. At this stage, the cardiac impulse is efficiently conducted through the heart with a constant conduction velocity from the most caudal SV inflow site of the heart (or most posterior site), through the future atrial segment, the direct myocardial AV connection between the future atria and ventricles, through the ventricles and finally to the outflow site of the heart (or most anterior site).[111]

In short, in the primary myocardium of the embryonic tubular heart, each myocardial cell inherently possesses intrinsic pacemaker activity, which on the cellular level is reflected by action potentials displaying slow depolarizations typical of slow voltage-gated calcium ion channels (reminiscent of pacemaker action potentials). The regional differences in intrinsic beat rates, reflected in their characteristic action potential shapes and underlying action currents, most likely result from the differential expression of largely unknown gene products in different regions of the heart that cause the individual segments to have diverse types and numbers of channels and pumps.[118-120] In general, impulse propagation through the primary myocardium of the tubular heart is relatively slow, due to poor intercellular coupling in the embryonic myocardium at this developmental stage.[121-123]

1.3.2. Looped Heart

As the developing heart transforms from a tubular to a looped morphology, the pattern and speed of ventricular activation also undergo their first changes. The pattern of universally slow propagation along the primitive tubular heart develops heterogeneities in conduction properties in the different cardiac segments.[124-126] In avians, by 42 hours of development (equivalent to HH stage 11, human ~E 23, mouse ~E 8.5) as looping proceeds, a slowly conducting AV canal is forming separating the synchronous activation of the atrial and ventricular segments.[127-129] Concordantly emerging are action potentials in the atrial and ventricular working myocardium with a fast rising phase and high amplitude, characteristic of fast voltage-gated sodium channels.[122, 130]

As a consequence, the emerging atrium and ventricle in the looped heart start displaying fast conduction, while the myocardium of the AV junction is characterized by slow conduction, which is thought to result from a lack of fast sodium channels and a relative lack of the gap junctional protein connexin-43.[18, 111, 131] In the looped heart, the cardiac impulse is thus propagated with alternating conduction velocities form base-to-apex through the different segments of the looped heart resulting in a sequential contraction pattern still following the direction of the blood flow (from inflow to outflow tract).[124]

Interestingly, the cellular electrophysiology of the embryonic AV junctional tissue is already quite similar to adult nodal tissues,[132] e.g. it responds to adenosine with a reduction in action potential amplitude and dV/dt_{max}.[133] Histologically, like the Sino Atrial Node (SAN) and AVN and unlike the working myocardium, the AV junctional myocardium is relatively devoid of connexin-43.[134] Myocytes at the AV junction preferentially however express connexin 45,[135] a low conductance gap junction channel that is also expressed in the SAN as well as in the AVN of the mature heart.[136]

Coincident with the emergence of ventricular trabeculation and the formation of the primordia of the interventricular septum (IVS),[28, 137] preferential temporal anterior and posterior myocardial AV activation pathways can be identified between HH stages 16 and 24 of avian embryogenesis (human ~E 30-42, mouse ~E 11-13).[138] As development proceeds, these pathways are masked by the appearance of more trabeculae and will finally be superseded by functioning of the mature His-Purkinje system.[138] The anterior activation pathway (or anterior septal branch), is not unique to the chick heart but has also been functionally demonstrated in the embryonic rat E 11.5 heart and the embryonic mouse heart.[138, 139]

Functionally, in the looped heart the dominant pacemaking area remains localized in the left sinus primordium up to 5-6 days of incubation (HH stage 27-29, human ~E 44-48, mouse ~E 13-14), with resultant left atrial depolarization preceding right atrial depolarization.[105, 106, 140-142] Morphologically, nodal cells are also found in the right sinushorn around the 4th day of incubation (HH stage 24, human ~E 41, mouse ~E12.5) temporarily remaining functionally quiescent.[142, 143] With the outgrowth of the right atrium, between 6 and 7 days of avian development (HH stage 29-31, human ~E 48-52, mouse ~E 14-15), the SV completes its shift to the right and becomes submerged in the right atrium. Around this developmental stage, the myocardium in the dorsal mesocardium has completely developed excluding the large veins from the atria only leaving contact with the non-cardiac dorsal mesoderm at the arterial and venous pole and impulse generation switches to the adult right position.[140]

The AVN and His bundle, of mainly unestablished origin, also start to develop around this developmental stage.[144] Additionally, the atria and ventricles become subjected to chamber differentiation and trabeculation and start expressing the inward-rectifier potassium current (I_{K1}) stabilizing a strongly negative resting potential suppressing excitability, which ultimately renders the atria and ventricles electrically quiescent while the rate and rhythm will exclusively be controlled by the compact nodes.[145] Concomitantly (HH 28-29), the IVS and AV cushions have started to fuse,[146, 147] completing ventricular septation around HH stage 34.[127]

1.3.3. Septated Heart

By the stage at which the ventricles have septated (> HH stage 34), the AVN and His bundle will have formed and attained their definitive positions close to the inferior edge of the atrial septum, while the annulus fibrosis still has to undergo extensive developmental changes (see also **Chapters 2-5**, *this thesis*). To facilitate propulsion of blood into the arterial trunks of the four-chambered heart, the initial base-to-apex direction of impulse propagation is reversed to a more mature apex-to-base oriented conduction and myocardial contraction (which will be further outlined in paragraph **1.6. "Transitions in Ventricular Activation During Cardiogenesis"**).

1.4. Development of the Specialized Cardiac Conduction System (CCS)

The specialized Cardiac Conduction System (CCS) is comprised of separate subcomponents with distinct functions and has mainly been studied in avian embryos.[141, 142] Firstly, the SAN generates the cardiac impulse and sets the leading pacemaker rate. The electrical impulse will subsequently be conducted via the internodal pathways to the AVN. After a short AV delay, the cardiac impulse is then rapidly transmitted to the His bundle, bundle branches and Purkinje fiber network.

1.4.1. The Origin of the CCS

The origin of the CCS has been a subject of debate for many years now. In the debate of the 19th century, both "*myogenic*" and "*neurogenic*" origins of the CCS were suggested. Temporarily, with the discovery of the existence of intraventricular neurons in the early 19th century, the balance was tipped in the neurogenic direction.[148, 149] Again, a quite similar debate arose at the end of the 20th century with the demonstration of neural cell-type gene expression in the cells of the CCS, now proposing the neural crest cell (NCC) as a candidate parental population for the developing CCS.[99, 150-153]

An elegant series of 20th century retroviral lineage studies has however unambiguously demonstrated that cardiomyocytes are the true and sole progenitors of the CCS cells.[99, 154] Indeed, cardiomyocytes of the CCS share with the cardiomyocytes of the ordinary working myocardium four basic elements: 1) contraction, 2) autorhythmicity, 3) intercellular conduction and 4) electromechanical coupling.[120] The still unresolved question however remains, if the CCS cardiomyocytes are derived from the division of differentiated (pre-specified) conduction cells (*the"specification"-model*) or are recruited from a pool of multipotent undifferentiated cardiomyogenic cells (*the "recruitment"-model*).[155]

1.4.2. CCS Development and The 4-Ring Theory

In an attempt to distinguish the working myocardium from the myocardium of the specialized CCS, the observation was made that after looping of the heart tube, 4 rings of 'special' tissue could be distinguished from the working myocardium, as was described by Wenink and others.[156-158] These rings or transitional zones consist of: 1) the sinoatrial ring in between the SV segment and the primitive atrium, 2) the AV ring in between the primitive atrium and primitive left ventricle, 3) the primary ring or fold separating the primitive left ventricle from

the primitive right ventricle and 4) the ventriculo-arterial ring positioned at the junction of the primitive right ventricle with the truncus or putative outflow tract of the heart (**Figure 2**).

This 'ring-theory', hypothesized that these 4 rings of 'special' tissue are the precursors of the CCS. During development these rings will come together in the inner curvature of the heart and partly loose their specialized character, while the remaining parts are identified as putative parts of the mature CCS.[158] Classically, in this theory, the SA ring was thought to contribute to formation of the SAN, the SA ring and the AV ring to contribute to the AVN and the primary ring to give rise to the His bundle and bundle branches.[23, 25, 37, 48, 52, 158] This theory has however been the subject of discussion and controversy for many years. Later on, contemporary marker studies could again confirm the important contribution of the SA ring to the developing SAN and AVN by HNK-1 expression patterns in the developing human embryo and analysis of CCS-LacZ and MinK-LacZ expression in the mouse embryo identified the SA ring, AV ring and primary ring as important contributors to CCS development.[23,25,37,48,52]

1.4.3. Molecular Markers for CCS Development

In the early embryonic heart, the individual cells of the CCS can hardly be distinguished from the surrounding myocardium by unique histological features, while their separate arrangement and topography can in some cases be helpful.[159-162] Histologically, in the adult heart nodal cardiomyocytes of the CCS display some characteristics comparable to embryonic working cardiomyocytes: they are small compared to the cardiomyocytes of the surrounding adult working myocardium and have poorly organized actin and myosin filaments and a scantily developed sarcoplasmatic reticulum.[159]

By applying the criteria established by Monckeberg and Aschoff in 1910, using the AV conduction axis as the paradigm, discrete specialized conduction tracts in the postnatal heart: 1) are histologically distinct, 2) can be followed from section to section and 3) are insulated from the adjacent working myocardium by fibrous tissue.[163] While these criteria permit adequate recognition of the specialized components of the CCS in the postnatal human heart, identification of the embryologic conduction tissues in the developing heart has remained fairly challenging. A multitude of transgenes, such as minK-LacZ,[49] Engrailed2-lacZ/CCS-LacZ[48, 164] has however been consistently proposed to reflect the arrangement of the developing CCS.

Moreover, each subcomponent of the CCS expresses a distinct set of

discriminating ion channels,[165, 166] channel-associated proteins,[167] connexins,[136, 168-172] cytoskeletal components[173, 174] and transcriptional regulators,[175, 176] useful for immunohistological recognition. Additionally, important known signaling and transcription factors implicated in the induction, maturation and patterning of the CCS including endothelin (ET),[177-182] neuregulin,[139, 183] Notch,[183] Wnt,[184] Msx,[185] Nkx,[44, 186-188] Hop,[189] Id-2,[50] podoplanin[47] and Tbx and GATA gene families[190-193] can also be of help. State-of-the-art studies focusing on the transcription factors involved in cardiogenesis have made evident that myocardial differentiation to CCS cells cannot be dependent on a single gene, but should be considered as a multifactorial process in which a multitude of different gene families must contribute.

1.4.4. The Individual Components of the CCS

1.4.4.1. The Sino Atrial Node (SAN)

In humans and other mammals, the first morphological signs of the developing SAN are present at Carnegie stage 15 (~5 weeks of human development, avians ~HH stage 18, mouse ~E 11.5)[120] in the anteromedial wall of the right common cardinal vein, which will ultimately give rise to the superior caval vein.[160, 194]

In the adult heart, the SAN is located in the crista terminalis (representing the internal fusion-line of the SV and the primitive atrium) near the superior caval entrance into the right atrium.[119, 195] During formation of the SAN, a considerable portion of the right horn of the SV becomes incorporated in the dorsal wall of the right atrium. The SAN myocardium, thus represents myocardium which was originally associated with the right sinus horn. Interestingly, as described above, in the early stages of development the sinus horns belong to the most caudal regions of the cardiac primordia harboring the highest cephalocaudal pacemaking rate.[111]

While all adult heart muscle cells retain the capacity to rhythmically beat without an external stimulus, the cells of the SAN are those with the most rapid intrinsic rate of excitation (the dominant pacemaking rate).[196] In generating the pacemaker action potential of the SAN, the hyperpolarization activated I_f (pacemaker or "funny") current plays a major role. Furthermore, the pacemaking action potential is regulated by several genes, including those for the T- and L-type calcium currents and the sustained inward current, producing a slow and diastolic depolarization.[166, 197] From genetic studies in human and mouse we know that Hyperpolarization-activated Cyclic Nucleotide gated (HCN)

channels are required to generate the I_f current or normal pacemaking current, but it is however still unclear how the complex expression of HCN channels is induced and regulated at specific regions of the developing heart.[198, 199]

1.4.4.2. The Internodal Tracts

Considerable controversy and debate, lasting for almost a century, has surrounded the mostly semantic discussion on the existence of specialized, insulated internodal tracts in the atrium between the SAN and AVN. Within the right atrium three internodal tracts for preferential interatrial conduction have been demonstrated between the SAN and AVN: 1) the anterior bundle running through the septum spurium (SS),[23,46,48] which connects to *Bachmann's bundle*[200-202] running in a retroaortic position connecting the right atrium to the left atrium, 2) the posterior bundle running through the right venous valve (RVV)[23,46,48] partly corresponding to the posterior bundle or *Thorel's bundle* localized along the crista terminalis and 3) the posterior bundle running through the left venous valve (LVV)[26] partly corresponding to the middle bundle or *Wenckebach's bundle*.[23, 46, 48, 158, 200-202]

Currently, it is well established that preferential conduction between the cardiac nodes (SAN and AVN), through the ultrastructural and electrophysiological heterogenic atrial myocardium, highly depends on the nonuniform anisotropic arrangement of the normal working myocardial fibers,[203] instead of on the existence of truly specialized insulated atrial internodal tracts. These non-specialized internodal atrial tracts are made up in part of transitional cells, which interpose between the working atrial myocardium and the unequivocally histologically specialized compact AV Node.[204] While structurally these tracts have been extensively demonstrated,[23, 46, 48, 49] their functionality has still not been shown.

1.4.4.3. The Atrioventricular Node (AVN)

In the human embryo, the developing AVN becomes gradually identifiable from Carnegie stage 16/17 (~5/6 weeks of human development) onwards.[161, 162, 205] Early in the sixth week of human development (~HH stage 25, mouse ~E 13) a compact cluster of cells makes its appearance in the posterior wall of the AV canal, towards its right side.[206] This cluster of cells is thought to represent the primitive AVN, which is in cellular continuity with the atrial muscle and AV bundle.

The architecture of the adult AV conduction axis was first described by Sunao

Tawara in 1906.[149] In the mature heart, the compact AVN is positioned in the apex of the triangle of Koch at the base of the interatrial septum, where it lies only a few millimeters anterior to the coronary sinus (CS) ostium and directly beneath the right atrial septal endocardium and the septal attachment of the tricuspid valve where it rests on the central fibrous body, which forms the anchor for the septal portion of the mural leaflet of the mitral valve. The atrial margin of the AVN is apposed to the myocardialized vestibular spine, containing the tendon of Todaro, while the ventricular margin of the AVN is continuous with the bundle of His.[207, 208]

The triangle of Koch occupies the atrial component of the muscular AV septum and is limited by three anatomical landmarks: 1) superiorly by the tendon of Todaro (the fibrous commissure of the flap guarding the openings of the inferior caval vein and the CS), 2) inferiorly by the attachment of the septal leaflet of the tricuspid valve and 3) at the base by the mouth of the CS. The apex of the triangle of Koch overlies the membranous component of the AV septum and lies at the center of the short axis of the heart.[209] The triangle of Koch not only harbors the AV nodal tissues but also the remnants of the embryologic primordium of the specialized myocardium that surrounds the primary interventricular foramen (primary ring), extending rightward and inferiorly from the compact node.[204]

The main functions of the adult AVN are: 1) gathering the incoming signals from the SAN, 2) directing the signals through the AVN to the His bundle, 3) maintaining an AV delay, 4) generating an escape rhythm when needed and 5) responding to the autonomic nervous system and humoral signals.[210, 211]

The ontogenic development of the AV specialized tissues has, since the first detailed report on the AVN by Tawara in 1906, been studied for over 100 years now. In the debate of the 20th century, competing theories based on observations in different species complicated by the use of variable terminology for identification and non-specific staining, however failed to provide a resolution on this subject. The developmental origin of the AVN will more extensively be reviewed and analyzed in **Chapter 6 & 7** of this thesis.

1.4.4.4. The His Bundle and Bundle Branches (His & BBs)

From the AVN, propagation of the electrical impulse is subsequently accelerated along the AV bundle (His bundle) and bundle branches. Around 6 weeks of human development (avians ~HH stage 25, mouse ~E 13), the AV bundle can first be found to run across the top of the thick IVS, behind and under the dorsal endocardial cushion. Subsequently, after 8 weeks of human development (avians

~HH stage 35, mouse ~E 15.5), the bundle branches arise from the terminal end of the His bundle.[212]

The His bundle in the adult heart, as first described in the mammalian heart by His in 1893, originates at the posterior right atrial wall near the atrial septum above the AV groove and than passes over the upper margin of the ventricular septal muscle, where its fibers intermingle with the cardiomyocytes. Near the aorta it subsequently bifurcates in the right and left bundle branch, the later terminating at the base of the aortic leaflet of the mitral valve.[149, 176, 213]

Controversy concerning the development of the His bundle has led to various proposals on the origin of the His bundle: 1) Viragh and Challice demonstrated in 1976 that the AVN and His bundle develop simultaneously,[205, 214] while 2) others found that the AVN develops first and the AV bundle arises later as an outgrowth of the AVN,[206, 215-217] 3) others have suggested that the AV bundle develops first and than the AVN develops as an outgrowth of its proximal portion[218-220] and 4) in the classical 'ring theory', the AVN and AV bundle have been shown to originate independently from the AV and bulboventricular ring respectively and join secondarily.[158, 221, 222]

1.4.4.5. The Purkinje Fibers

In the human embryo, Purkinje fibers do not appear until rather late, between the 10th and 15th week of development.[206] The original description of the Purkinje fiber in 1845 by Purkinje stated that these special cells can ultrastructurally be identified as cardiac fibers without transverse tubules.[223] Since the Purkinje fibers have been found to co-express both myogenic and neurogenic gene products, the origin of the Purkinje fiber system has also been a subject of longstanding controversies.

Individual Purkinje fibers are scattered throughout the myocardium but can be distinguished from the working myocardium by their distinct electrophysiological and molecular characteristics. Functionally, these cells of the fast conduction system are electrically coupled to neighboring muscle cells via gap junctions and exhibit a faster action potential upstroke, a prolonged action potential duration, a higher membrane diastolic potential and greater electrical restitution properties in comparison to the slow conducting components of the CCS.[178, 182, 224]

While the proximal components of the Purkinje system run subendocardially regardless of species, the presence and distribution of the more distal intramyocardial branches of the fast conduction network is highly variable among species.[120, 174] Furthermore, in avian hearts in addition to the subendocardial Purkinje fibers, intramyocardial Purkinje fibers penetrate along the coronary artery branches (periarterial Purkinje fibers).[154, 176, 182, 225, 226]

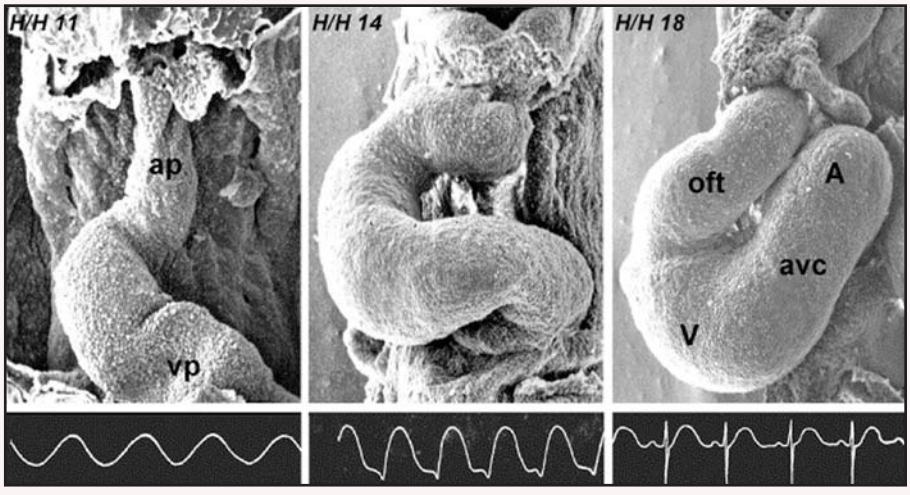
Cell tracing studies have demonstrated that Purkinje fiber recruitment from the myocardium takes place at two restricted sites: periarterially and subendocardially.[71] In this respect, recent studies have shown that Purkinje fiber differentiation is tightly regulated by hemodynamic alterations, while endothelin-1 (ET-1) and ET-converting enzyme 1 (ECE1) were identified as inductive molecules.[179, 182, 227] Concomitant retroviral expression of mature ET-1 and ECE1 was even shown to be sufficient for the ectopic conversion of adjacent cardiomyocytes into Purkinje fibers.[182] Prompted by the periarterial and interstitial arrangement of EPDCs in the developing heart, an instrumental role of EPDCs in Purkinje fiber differentiation could recently also be demonstrated.[68, 77]

Figure 9. (right) **Scanning electron microscopic photographs of the developing chicken heart with matching electrocardiograms** (*adapted from Seidl W, et al. A few remarks on the physiology of the chick embryo heart (Gallus gallus). Folia Morphol. 1981;29:237–242).* At Hamburger Hamilton (HH) stage 11, a linear peristaltic contracting heart tube has developed, from which a matching sinusoidal electrocardiogram can be derived. At HH stage 14, a sharp downward deflection approximately 80 ms ahead of the QRS-complex can be recorded (presumptive inverted P-wave). When the ventricular loop is subsequently looped backward, around HH 18, and becomes to be positioned caudal to the outflow tract, the P-wave appears above the iso-electric line and an adult type electrocardiogram can be recorded. ap = arterial pole; A = atrium; avc = atrioventricular canal; oft = outflow tract; V = ventricle; vp = venous pole. *Adapted and modified from Moorman AFM, et al. Anatomic substrates for cardiac conduction. Heart Rhythm 2005;2:875– 886.*

1.5. The Electrocardiogram (ECG) in Cardiogenesis

The youngest embryo of which a primitive ECG has been recorded, is a chick embryo of only 15 somites (33-36 hours of development, HH stage 9-10), a developmental stage at which the heart tube almost solely consists of the common ventricle. The ECG recorded at this developmental stage, shows a sinusoidal curve dropping below and above the isoelectric line, reflecting the vector of myocardial contractions in caudocephalic direction.[111, 128, 228, 229] In **Figure 9**, an example of such a sinusoidal ECG recorded in the developing tubular chick heart is shown, reflecting linear and isotropic impulse conduction with constant low velocity resulting in the typical primitive peristaltic unidirectional contraction pattern.[230, 231]

With progression in caudal fusion of the cardiac primordia, the SV is formed and becomes positioned posterior to the atrium and a sharp downward deflection approximately 80 ms ahead of the QRS-complex can be recorded (presumptive inverted P-wave). When the ventricular loop is subsequently looped backward and becomes positioned caudal to the outflow tract (day 4 or HH stage 23-24), the P-wave starts to appear above the isoelectric line (**Figure 9**).[128]



As described above, in the looped embryonic heart, the different cardiac segments will contract sequentially and conduct the electrical impulse with distinct conduction velocities - slow conduction in the AV canal and outflow myocardium and fast conduction in the future atrial and ventricular myocardium - from the ventricular base to the ventricular apex of the developing heart.[124] As a consequence, the electrocardiogram of a 3 to 4 day old chick (~HH stage 18-22) already reveals the presence of a PR interval (AV delay) in the absence of a structural AVN.[230, 232] In the looped embryonic heart, an adult type electrocardiogram including a P-wave reflecting atrial activation, an AV delay caused by slow conduction in the AV junctional myocardium and a QRS complex reflecting fast ventricular activation, can thus be recorded.[107, 230]

The first electrocardiographic tracings in the human fetal heart have been recorded in the 1930-ies with direct chest leads from fetuses removed by hysterectomy. An adult type tracing could be obtained from an embryo of between 6 and 7 weeks of gestation (avians ~HH stage 25-30, mouse ~E 13-14) and of about 16 mm Crown-Romb-Length (CRL) (**Figure 10**). At this developmental stage, the AVN and His bundle are morphologically recognizable but their differentiation is still far from complete yet. [233, 234]

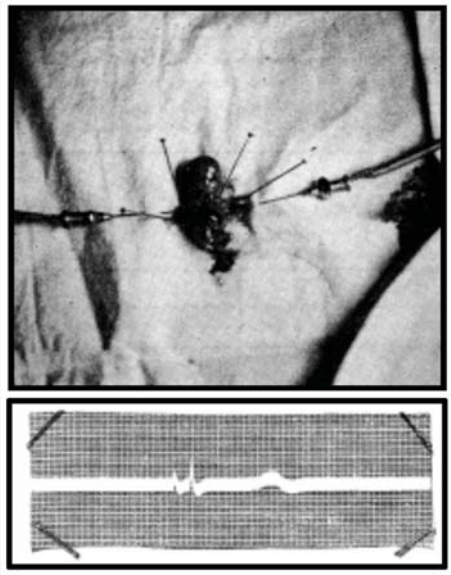


Figure 10. The first electrocardiographic tracings in the human fetal heart, recorded in the 1930-ies with direct chest leads form a foetus of 6-7 weeks of gestation (avians ~HH stage 25-29, mouse ~E 13-14) removed by hysterectomy. *Adapted from: Marcel MP, Exchaquet JP. L'electrocardiogramme du foetus human. Arch Ml Coeur. 1938;1:52.*

1.6. Transitions in Ventricular Activation During Cardiogenesis

During cardiogenesis, the ventricular activation sequence changes concomitantly with changes in ventricular geometry and microarchitecture, from a slow peristaltoid base-to-apex pattern in the tubular heart, through a sequential base-to-apex pattern in the looped trabeculated heart and ultimately to the mature apex-to-base sequention in the septated heart.[138, 235] Generally, myocardial activation proceeds from the venous inflow towards the arterial outflow end of the heart and thus consistently follows the direction of the blood flow (**Figure 11**).

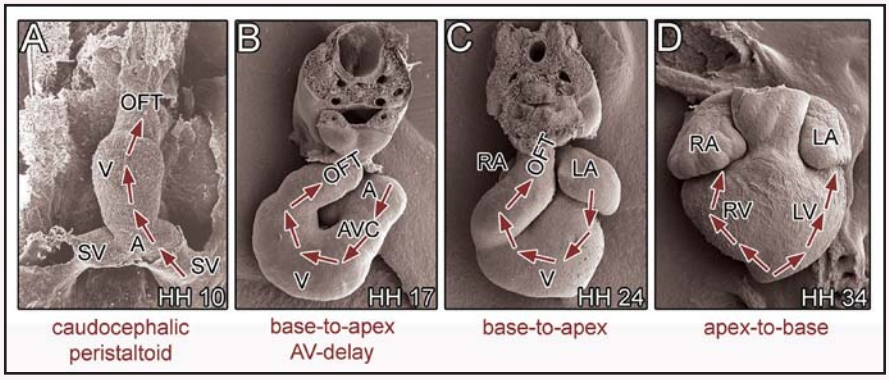


Figure 11. Transitions in ventricular activation sequences during cardiogenesis. The ventricular activation sequence changes from a slow peristaltoid caudocephalic base-to-apex pattern in the tubular heart (**A**), to a sequential base-to-apex pattern in the looped trabeculated heart due to the development of an AV delay (**B&C**) and ultimately to the mature apex-to-base sequention in the septated heart (**D**).

As described above, at the earlier stages of development the heart resembles a tube rather than an ellipsoid with a widely separated inflow and outflow tract. In the tubular heart, the primitive slow caudocephalic peristaltic contractions are sufficient to facilitate efficient slow propulsion of blood from the venous to the arterial pole.

Subsequent configuration of the alternating slow- and fast conducting segments in the looped heart, inherently subjected to progressive spatiotemporal changes in chamber arrangement, guarantees that the downstream ventricular segment does not start contracting before contraction of the upstream atrial segment is terminated. This electrical configuration also ensures that relaxation of the atrial or ventricular segment does not occur before contraction of a downstream flanking segment. This sphincter-like prolonged peristaltic contraction of the slow conducting flanking segments in a way substitutes for the adult type of one-way valves.[124]

In the septated heart, the inflow and outflow tract ultimately become more closely aligned at the top of the ventricles and a morphogenetic division between the atrial and ventricular chambers (annulus fibrosis) dissociating direct morphological coupling between the atrial and ventricular chambers is laid down, necessitating the start of ventricular activation from the apex to the base of the ventricle to efficiently propulse the blood towards the aortic and pulmonary arterial outlet. This apex-to-base sequence of ventricular activation is not only thought to increase ventricular pumping efficiency but is also used as a marker for the anatomical presence of a mature and functional His-Purkinje-System (HPS). More precisely, apex-to-base conduction functionally marks the emergence of mature “apex-first” epicardial breakthrough, near the termini of first the right (at HH stage 29) and secondly the left bundle branch.[227, 236]

The ventricles of the mammalian looped heart are however already capable to contract from apex-to-base even before ventricular septation is completed.[164, 237] In a developmental timeline, apex-to-base conduction might thus already be facilitated before completion of formation of the four-chambered heart and complete structural maturation of the His-Purkinje-System (HPS).

Furthermore, functional activation of the working muscle of the ventricle and its ensuing contraction, also proceed from the right or left ventricular apex in the primitive heart of lower vertebrates (e.g. the African lungfish, bullfrog and crocodilian) in whom the existence of an anatomically distinct organized specialized ventricular conduction system has never been demonstrated.[238-240] Coordinated contraction of the ventricular myocardium from apex-to-base or

from inflow to outflow tract, the common functional principle in the ventricular conduction system of all species, thus already seems to be realized early in vertebrate evolution, suggesting the presence of non-specialized preferential pathways of conduction.[241]

Moreover, the physiological transition in ventricular activation sequence is highly influenced by epigenetic factors affecting general hemodynamics. For instance, maturation of HPS functioning has been shown to be accelerated in the setting of increased pressure load at distinct developmental stages, an effect that is probably mediated by endothelin signaling.[178, 179, 227] Conversely, bundle branch maturation can be delayed by a decreased workload in experimental left heart hypoplasia and inhibition of stretch-sensitive cation channels by gadolinium.[193]

Despite the onset of preferential conduction through the central AV-conduction axis, the occurrence of immature base-to-apex conduction in the developing postseptated heart is not an exceptional phenomenon (*this thesis*).

1.7. Annulus Fibrosis Development: State-of-the-Art

Coincidently with maturation of the His-Purkinje system, completion of ventricular septation and the transition to an apex-to-base ventricular activation sequence, the AV myocardial continuity, which is present around the entire circumference of the slow conducting AV junction disappears as a result of annulus fibrosis formation.[237, 242] It is well established that this AV junctional myocardium is incorporated in the atrial myocardium forming the smooth walled lower atrial rim leading toward the valvular orifices.[243] A small part of the AV canal myocardium however remains in situ and contributes to the AVN and normally this structure constitutes the only site of myocardial continuity with the ventricular conduction system.[243]

The exact signaling processes that underlie atrial and ventricular myocardium dissociation are still incompletely understood and the tissues responsible for the formation of the annulus fibrosis have largely remained unknown. It is however well established that the development of this isolating structure involves several processes in which fusion of the endocardial AV cushions lining the luminal side of the primitive AV canal and the epicardially located AV sulcus tissue at the ventricular site of the AV junction play an important role.[243-245] State-of-the-art in literature postulates critical roles for bone-morphogenetic-protein (BMP) signaling and periostin (an osteoblast specific factor) expression in formation of the isolating annulus fibrosis (see also **Chapters 2-5**, *this thesis*).[246-251] Moreover, recently the important role of the

multipotent EPDCs, migrating through the developing AV dissociated border, in structural formation and electrical isolation of the annulus fibrosis was further established by electrophysiological studies in the EPDC-inhibited quail embryo (see also **Chapter 3**, *this thesis*).[68, 251]

Around the 7th week of human development, the process of AV dissociation at the primitive AV canal has started. From the 12th week of development onwards, the atrial and ventricular myocardium will be completely separated by the annulus fibrosis, through which the AV conduction axis should be the only remaining AV myocardial continuity in postnatal life (see also **Chapter 5**, *this thesis*).[245, 248]

The isolating annulus fibrosis is part of the fibrous skeleton of the heart, which additionally consists of the AV valve annuli, the arterial orifices and the central fibrous body (CFB) or trigonum fibrosum (a triangular mass of fibrous tissue), which connects the AV and aortic valve annuli. Ingrowth of tissue from the dorsal mesocardium contributes to the atrial part of the CFB and is continuous with the tendon of Todaro - a strip of connective tissue originating in the anterior CFB - directly above the junction of the AVN and bundle of His, and passing posteriorly through the atrial septum.[252]

The ventricular part of the CFB is formed by invagination of AV sulcus tissue from the posterior AV sulcus towards the dorsocaudal extension of the bulbar ridge. In this process, a small part of endocardial AV cushion tissue on top of the ventricular septum is trapped and incorporated in the CFB. The AVN passes through the CFB beneath the endocardial cushions and becomes separated from the atrial tissues and directly contacts the bundle of His.[252]

1.8. Accessory Pathway (AP) Persistence

It is certainly not uncommon for annulus fibrosis formation to be incomplete at birth, resulting in postnatal AP persistence providing a possible substrate for clinical AVRTs. During physiological embryonic development, remnants of the primitive AV connections bypassing the insulating AV groove, have morphologically been described in the post-septated embryonic and adult quail (see also **Chapters 2 & 3**, *this thesis*),[125, 225, 249, 251] mouse (see also **Chapter 4**, *this thesis*)[253] and human heart (see also **Chapter 5**, *this thesis*).[248, 254-260] Interestingly, a conducting right-sided AV myocardial continuity was demonstrated in postseptated CCS-LacZ transgenic mice, providing a possible explanation for the occurrence of functional atriofascicular bypass tracts via the moderator band, as a possible substrate for Mahaim tachycardias.[23] Additionally, another

electrophyiological study in wildtype mice has demonstrated the onset of AP mediated AVRT at early stages of mouse development[261]

The structural characteristics and electrophysiological properties of persistent myocardial APs have however not been studied systematically and will be further described in **Chapters 2-5** of *this thesis*.

1.9. Animal Models (Used in This Thesis)

Genetic pathways that dictate cardiac development are highly conserved across vastly diverse species from flies to humans.[27] Despite diversities of body structures in different species, a common genetic program for the early formation of a circulatory system seems to exist. The cardiovascular system seems to have adapted increasing complexity in order to adapt to specific environments.[262, 263]

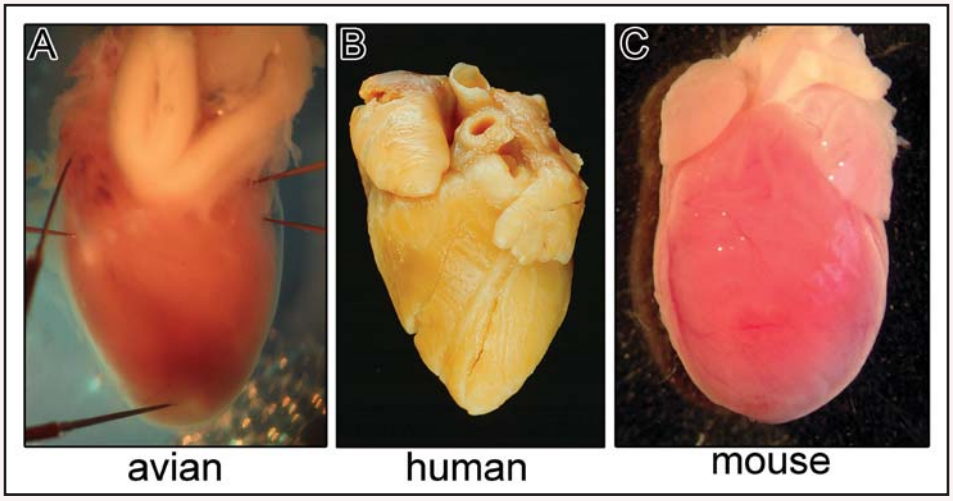


Figure 12. External shape of the embryonic avian, mouse and human heart. A. Externally, the overall shape of the avian heart closely resembles that of the human heart (**B**), while the elipsoidal mouse heart (**C**) is externally differently shaped in comparison to the more pyramidal human and avian developing heart.

1.9.1. Avians (Aves, Amniota, Diapsida)

Since the days of antiquity, the chick embryo has been a very popular model for studying morphology and (patho)physiology of the developing heart.[28, 264] Birds belong to the only class of vertebrates (*Aves*) that consists exclusively of oviparous forms, which makes the avian embryo a vertebrate model always ready at hand. Aristotle, whose work was written toward the end of the fourth century B.C., described many observations on the development of birds and was probably the first to compare the embryology of avians with that of other vertebrates.

The *Gallus domesticus* (chick) and *Coturnix coturnix japonica* (Japanese quail) are among the most frequently used avian strains in experimental research. Externally, the overall shape of the avian heart closely resembles that of the human heart (**Figure 12**). While birds do not have a diaphragm, the relatively large size of the liver leaves little free space in the pericardial and peritoneal cavity, which explains the similar pyramidal shape of the avian heart in comparison to the human heart, which rests on the diaphragm.

Despite the obvious differences in size, the morphological plan of the avian (nonmammlian vertebrate heart) and the mammalian vertebrate heart is quite similar.[35] In comparison to the mammalian heart, the avian heart has valuable advantages as a research model: 1) experimental work with avian embryos (eggs) is much more practical than with mammalian embryos, 2) avians have a very rapid reproduction cycle (5 generations/year) and a very high offspring production (80-90 per 100 days) and 3) the avian embryonic heart has a relatively large size compared to hearts of mammalian embryos at equivalent developmental stages.

Furthermore, the anatomy of the avian CCS is well characterized,[142, 226, 265-268] since the components of the conduction system of the birds heart can be more easily recognized histologically in contrast to the conducting cells in the mammalian heart.[269] Additionally, the electrophysiology of the avian CCS has also been extensively characterized using microelectrodes as well as optical mapping with voltage sensitive dyes.[124, 131, 179, 227, 237, 266, 270, 271] Functionally, the ventricular activation patterns in the avian and mammalian model show remarkable spatiotemporal homology.[227, 253, 272, 273]

As is the case with all animal models, there are however also some differences in anatomy between the avian and mammalian heart: 1) the interventricular septum (IVS) of the bird is an entirely muscular bulky structure, while the upper part of the IVS in the human heart is a thin fibrous structure, 2) the right AV valve (tricuspid valve) of avians is not a fibrous, cusped valve as

in mammals, but consists of a large, single, sickle shaped muscular flap and 3) the bird heart has a very generous subendocardial and intramural distribution of diffuse Purkinje cells throughout the walls and septa of both the atria and ventricles, which is not seen in the mammalian heart.[226]

1.9.2. Mouse (Mus, Amniota, Synapsida)

Due to the increasing availability of tools for genetic manipulation, the mouse has become one of the most popular and most used animal models for studying normal and abnormal cardiac development. Enormous advances in mouse genetics have led to the production of numerous mutants with cardiac abnormalities resembling those seen in human congenital heart disease.[35] Apart from differences in heart rate (adult mouse 300-800 bpm vs. adult human 80-100 bpm) and size (adult mouse heart 0.5 grams vs. adult human heart 250-400 grams), the most pronounced difference between the mouse and human heart is found in their overall shape (**Figure 12**).

As described above, in humans, the heart rests on the diaphragm, which is reflected by a more pyramidal shape and a flat dorsal (or inferior) shape. The heart of the four-legged mouse, in comparison, does not rest on the diaphragm and has more room in the pericardial cavity to more freely move around which is reflected in a more ellipsoidal shape. Moreover, the atria in the human heart are very prominent, whilst in the mouse heart the atrial chambers and atrial appendages are very small.[35]

While the cardiac anatomy of the mouse and human heart is considered remarkably similar, there are however small variations: 1) the AV septum (AVS) of the mouse is a relatively thick and mostly muscular structure, while in the human heart the AVS is a thin fibrous structure known as the membranous septum, 2) the muscular part of the IVS in the human heart is a massive and compact muscular structure, while this structure in the mouse is not quite as compact or massive, 3) in the human but not in the mouse, there is a pronounced difference in the morphology of the trabeculae in the right (coarse) versus left (relatively thin) ventricle, 4) whereas the left atrium of the human heart receives four pulmonary veins, in the mouse heart the pulmonary veins join in a pulmonary confluence behind the left atrium and 5) in the mouse heart the left superior cardinal vein (LSCV) persists into postnatal life, while this structure regresses in the human heart and becomes the ligament of Marshall and oblique vein.[35]

1.10. Immunohistochemical Markers (Used in This Thesis)

1.10.1. Atrial Myosin Light Chain 2 (MLC2a)

Atrial Myosin Light Chain 2 (MLC2a) is a protein, which is predominantly expressed in atrial myocardium and to a lesser extent in the ventricular myocardium and outflow tract of the heart.[274] Embryonic and adult cardiac muscle express two major isoforms of myosin light chain 2, MLC2v (MYL2 – Mouse Genome Informatics) and MLC2a (MYLC2A – Mouse Genome Informatics).

During cardiogenesis, MLC2v is expressed exclusively in ventricular and AV junctional myocardium. Ablation of MLC2v results in disruption of ventricular function at embryonic day 11.5 and embryonic lethality at E 12.5.[275] MLC2a is initially expressed throughout the heart at E 7.5 (human ~E 18) and becomes restricted to the atria after E 12.5 (human ~E 40).[274] In the embryonic and adult avian heart however, MLC2a expression does not become restricted to the atria but demonstrates an expression gradient in which the atria express more MLC2a in comparison to the ventricular myocardium.

1.10.2. Nk2 transcription factor related locus 5 (Nkx 2.5)

Nk2 transcription factor related locus 5 (Nkx2.5), also known as Cardiac specific homeobox protein (Csx), is a homeodomain-containing transcription factor of the Nkx-2 gene family. Nxk2.5 is expressed early during embryogenesis, and although not exclusively found in embryonic regions destined to be heart tissue, it helps to define the cardiogenic field.[276] In many organisms, its expression persists in the heart throughout development. The function of Nkx2.5 and its relatives has been examined in a variety of ways. For instance, the loss of the fly homologe *tinman* gene prevented the development of the dorsal vessel in flies.[277] Mice without functional Nkx2.5 formed small hearts that failed to loop, failed to septated, had underdeveloped ventricles and malformed AV canals.[278] These null studies are complemented by overexpression studies carried out in Xenopus that showed that increased Nkx2.5 levels lead to enlarged hearts in early embryos.[279]

Mutations in the Nkx2.5 gene have also been reported in humans with congenital heart disease. Human individuals were found to be heterozygous for the mutations, indicating that the mutations are either dominant or the result of haploinsufficiency. The phenotypes of patients are varied. Two specific Nkx2.5 mutations (Nkx2-5 Gln170ter and Gln198ter) have been found to lead to problems that included atrial septal defects, AV heart block (conduction system defects), and less commonly, tetralogy of Fallot, mitral valve defects, left ventricular

hypertrophy, pulmonary atresia, and ventricular septal defects.[188] Interestingly, recent studies have identified the contribution of a Nkx2.5 negative myocardial population to the developing sinus venosus and SAN.[47, 192]

1.10.3. Periostin

Periostin was originally isolated as an osteoblast-specific factor that functions as a cell adhesion molecule involved in osteoblast recruitment, attachment and spreading. Expression of periostin mRNA was later also found in the embryonic mouse and chicken heart in the endocardial cushions that ultimately divide the primitive heart tube in a four-chambered heart.[246, 250] Additionally, periostin expression was found to be maintained within the valves of the adult mouse heart.[250] Periostin is secreted during cushion mesenchym formation.[280] Expression of periostin is significantly increased in response to BMP and TGF-β signaling in mesenchymal cells undergoing differentiation.[281, 282] Induction of periostin expression has also been shown following myocardial infarction in the adult heart.[283] In a recent study, a 40-fold increase in periostin mRNA expression in mouse hearts subjected to cardiovascular overload was described.[284] Moreover, in a rat cardiac dilatation model, a decrease in periostin expression was correlated with an increased survival rate and left ventricular function.[285] In literature, periostin has been suggested to induce myocardium to transform into mesenchym of a mixed phenotype, which can subsequently transdifferentiate into cells with a fibrous identity, possibly in response to shear stress during cardiac development,[246, 280] while at late stages of development, periostin may also serve to maintain the integrity of the fibrous tissues of the heart.[280] At the boundary where myocardial cells directly interface endocardial cushion tissue at the AV junction, periostin expression is enhanced and myocardial cells are replaced over time by dense fibrous periostin-positive tissue.[286]

1.10.4. Connexin 43 (Cx43)

Connexin 43 (Cx43) is one of the 4 major connexins in the mammalian heart: connexin 40 is expressed in fast conducting cardiac tissues and in the atria,[287] connexin 45 is expressed in slow conducting pathways and in the myocardium of the primary heart tube,[135, 136, 168] connexin 30.2 is expressed in the AVN and contributes to slowing of propagation of excitation in the AVN[288] and connexin 43 is expressed in the slower conducting working myocardium of the atria and ventricles and in the distal part of the CCS.[289] The expression of connexins is highly variable between species and varies during different stages of development.[287]

Connexin 43 expression in the avian heart is subject to considerable controversy in literature. Expression of connexin 43 in the developing avian embryo has been demonstrated in the smooth muscle cells in the media of the vessel walls of the arterial outflow tract of the heart (aorta, pulmonary arteries, brachiocephalic arteries) and the smooth muscle cells of the coronary arteries.[290, 291] Additionally, persistent Cx43 expression throughout development and in the mature avian heart has been reported in a Northern blotting study of the developing chick heart and persisted at significant levels.[292]

In contrast, complete absence of Cx43 expression in the myocardial tissues of the developing and adult chick heart has also been described in literature[290, 291] and can possibly be explained by the use of mammalian antibodies and slight differences in the Cx43-isoform between avians and mammals.[292] Freeze-fracture studies of avian embryonic myocardium have indicated that its gap-junctions are very tiny and infrequent,[293] which might have a restrictive effect on the ability to properly detect Cx43 in the developing avian heart. Moreover, gap-junctional expression seems to follow an ontogenic sequence since a developmental increase in the density of gap junctions in prenatal rat hearts has been observed[294] and electrophysiological studies of isolated cell pairs from developing avian hearts have noted a change in the regulation of gap junctions between embryonic days 4 (~HH 24) and 18 (~HH 44).[295]

1.10.5. Sodium Channel, voltage-gated, type V, alpha subunit (SCN5a)

The Sodium Channel, voltage-gated, type V, alpha subunit (SCN5a) gene encodes for the Nav1.5 sodium ion channel protein and is responsible for the rapid influx of sodium ions (inward sodium current, I_{Na}) that initiates and propagates the cardiac action potential in the heart.[296, 297] The SCN5A gene is located on the short (p) arm of chromosome 3 at position 21. The encoded protein provides instructions for making a sodium channel that is abundant in heart muscle and is responsible for the initial upstroke of the action potential. These channels open and close at specific times to control the flow of sodium ions into cardiac muscle cells.[298-300]

SCN5a mRNA can first be detected at stage E 9.5 of mouse heart development, peaks at E11.5, than decreases and steadily increases from E17.5 onwards. Mutations in the SCN5a gene are associated with diverse channelopathies, such as long QT syndrome type 3 (LQT3), Brugada syndrome, and idiopathic ventricular fibrillation.[301]

1.10.6. Periodic-Acid-Schiff (PAS)-Staining

Periodic Acid Schiff (PAS) is a staining method used for histology in Pathology. This method is primarily used to identify glycogen in tissues. Glycogen is a high-molecular-weight polysaccharide that serves as a repository of glucose units for utilization in times of metabolic need. In PAS staining, the reaction of periodic acid selectively oxidizes the glucose residues and creates aldehydes that react with the Schiff reagent and than creates a purple-magenta color.

The first glycogen in the muscular tissue of chick embryos becomes recognizable at about the time that cardiac contraction begins (9 somite stage).[302] Unlike in mammals, little or no glycogen is found in the specialized CCS tissues of the avian heart, while the atrial and ventricular myocardium have a high glycogen content.[303-306]

CLINICAL ASPECTS OF SUPRAVENTRICULAR TACHYCARDIAS IN CHILDREN AND ADULTS

1.11. Clinical SVTs in Children and Adults

Supraventricular tachycardia is the most common cardiac arrhythmia in both children and adults, with an estimated prevalence of 2.25 per 1000 in the normal population.[1, 2] The prevalence of SVTs in children is estimated at one in 500 children worldwide.[307] In adults, AV Nodal Reentrant Tachycardia (AVNRT) accounts for approximately 80% of all SVTs,[308] yet it accounts for only 5% of SVT cases in infants and toddlers and comprises only 13–16% of SVTs in children and adolescents.[309] Conversely, macroreentry through an accessory AV pathway (Atrioventricular Reentrant Tachycardia, AVRT) accounts for ~30 % of SVT cases in adults, but is by far the most common (80%) mechanism of SVT in children.[2, 310] Much less prevalent forms of SVTs include Permanent-Junctional-Ectopic-Tachycardia (PJET), Ectopic-Atrial-Tachycardia (EAT) and Intra-Atrial-Reentrant-Tachycardia (IART) or Atrial Flutter.

1.12. Symptoms of SVTs in Children and Adults

Symptoms of SVT in infancy differ from those in childhood or adolescence. Newborns may present with a history of fetal tachycardia, signs of left ventricular dysfunction or even with hydrops foetalis representing severe heart failure from persistent rapid fetal tachycardia (see also **Chapter 9**, *this thesis*).[311, 312] Neonates can also present with new onset incessant and difficult to treat tachycardia at birth with no history of any fetal tachycardias.[307] Moreover, neonates with SVTs are at high risk for sudden cardiac arrest, since cardiac reserve in neonates is very small and typical SVTs with heart rates exceeding 200/min. can lead to life threatening myocardial dysfunction within several days. [313]

In infants, symptoms of SVTs are inconspicuous and masquerade those of many other common illnesses in infancy and include irritatebility, poor feeding, tachypnea, diaphoresis and poor color. Most infants with SVT have structurally normal hearts, while in 15% of patients tachycardia is associated with heart disease, drug administration or febrile illness.[307] Older children and adolescents complain of palpitations (in excess of 150/min.), general malaise, indistinct pressure or discomfort in the throat, isolated headaches, fatigue, chest discomfort, shortness of breath or lightheadedness. Syncope is unusual and may indicate life-threatening arrhythmia. In adolescence, typical SVTs are characterized by a sudden onset (often in rest) and sudden termination of tachycardia.

1.13. Treatment of SVTs in Children and Adults

Regardless of SVT type, maneuvers that increase parasympathetic (vagal) tone, slow down conduction through the AVN and break the reentry circuit responsible for SVT, apply to all patients. Different measures may be successful in different children and include placing an ice-bag around the nose and mouth or abdomen, immersion in ice-cold water, applying the Valsalva maneuver (take a deep breath and bear down), pressure on the abdomen, carotid sinus massage or blowing on a thumb.[307]

Acute treatment with intravenous administration of adenosine as a rapid bolus is safe in children of all ages and usually breaks the SVT. When symptoms however resume, beta-blockers (e.g. propranolol), procainamide, a calcium blocker (e.g.verapamil) or amiodarone should additionally be administrated.[314]

Chronic management of SVTs should be individualized, but in general prophylactic treatment with antiarrhythmic medication is prescribed to infants younger than 1 year of age. At the age of 1 year, this prophylactic treatment is temporarily discontinued in order to see if tachycardia recurs.[315] Elimination of the arrhythmogenic substrate and permanent cure from almost all forms of SVT can be achieved by percutaneous radiofrequency catheter ablation (RFCA) or surgery.[307, 316, 317]

During a RFCA procedure, the cardiac tissue is locally heated to 50-60 ˚C by alternating current (350 kHz to 1 MHz) delivered by the small metal tip of a RF catheter, producing a permanent small scar measuring approximately 4 mm in diameter and 4 mm in depth.[318] The initial success rates of RFCA exceed 90%.[312] In **Chapter 8** of this thesis the therapeutic issues in pediatric SVT are further outlined.

1.14. AV Reentrant Tachycardias in Children and Adults

AV Reentrant Tachycardia (AVRT) is by far the most common (80%) mechanism of SVT in children,[1, 2] and accounts for ~30% of SVTs in adults.[310]

1.14.1. Arrhythmogenic Substrate in AVRT

AV Reentrant Tachycardia (AVRT) involves the presence of an accessory myocardial AV pathway (AP) that bypasses the annulus fibrosis. The overall incidence of APs in the general population is 0.1-0.3% and 3.4% in first degree relatives of patients with ventricular preexcitation on ECG.[319]

Commonly, the APs are “concealed” bypass tracts, which are capable of conducting solely in the retrograde direction (from the ventricles to the atria).

The ECG of a patient with a concealed AP during sinus rhythm is normal (no signs of ventricular preexcitation). Accessory pathways in patients with the Wolff-Parkinson-White (WPW) syndrome are however usually capable of both antegrade and retrograde conduction and give rise to ventricular preexcitation on ECG recordings during sinus rhythm.[307]

Other, less frequently encountered APs include substrates with decremental (nodal) properties mostly located in the septal or inferior half of the tricuspid valve, Mahaim fibers producing ventricular preexcitation resembling left bundle branch block and slow conducting APs giving rise to Permanent Junctional Reciprocating Tachycardia (PJRT).[307]

1.14.2. Natural Course of AVRTs

In approximately 60% of pediatric patients, the first episode of AVRT occurs before birth or in infancy and appears to spontaneously resolve completely in two thirds of cases before the age of 1 year, while more than 80-90% of patients become asymptomatic after the first year of life.[1, 2] Relapse during follow-up is however observed in 20-30% of these cases.[320] A second new onset AVRT incidence peak is seen around the age of 8-12 years, in which case spontaneous regression of symptoms is only observed in approximately 20% of cases.[321]

1.14.3. Arrhythmia Mechanism in AVRT

Orthodromic AVRT is the most common tachycardia mechanism in AVRT in both children and adults. In orthodromic AVRT, antegrade conduction occurs through the normal AV conduction system, while retrograde conduction to the atria occurs via the AP. Antidromic AVRT is far less common and utilizes the AP as the antegrade pathway of circus movement conduction. Reentrant tachycardia may also involve multiple APs, providing both antegrade and retrograde conduction.[322]

Besides an anatomical substrate two additional conditions are required for functional preexcitation during sinus rhythm to occur: 1) electrical coupling between adjacent ventricular and atrial myocytes, and 2) a higher conduction velocity through the AP than in the normal ventricular conduction system. Reentry tachycardia can occur when: 1) at least two functionally distinct conduction pathways are present, 2) unidirectional block is induced in one pathway and 3) conduction time is slow enough over the nonblocked pathway to allow recovery of excitability in the blocked pathway, thereby permitting retrograde conduction over the blocked pathway and completing the reentry circuit.[322, 323]

1.14. The Wolff-Parkinson-White (WPW) Syndrome

1.14.4.1. Epidemiology of WPW Syndrome

The estimated prevalence of typical WPW syndrome is 0.1 to 3.1 per 1000 persons.[324, 325] Because of its intermittent pattern, the precise incidence of WPW is unknown.[326] The incidence of WPW in males is more than twice that in females.[324, 326-328] Additionally, in female patients, an incidence peak around the age of 7 years has been reported, while men are significantly younger at first presentation than women.[326]

The AP in WPW, known as the bundle of Kent, is usually located in the lateral rings of the annulus fibrosis and consists of a thin muscular segment that does not posses decremental properties.[254, 329-331] Approximately 10% of patients may have two or more AV bypass tracts. Most patients with typical WPW syndrome demonstrate isolated ventricular preexcitation in a structurally normal heart.[332] In a small percentage of patients with WPW, APs however occur in association with other cardiac abnormalities or congenital heart disease.[333] WPW is more prevalent in children with Epstein anomaly of the tricuspid valve, AV septal defects and ventricular septal defects, while occasionally the WPW syndrome may be inherited.[328, 334] Moreover, coronary artery disease was found to be associated with WPW in 6% of patients.[326]

1.14.4.2. Genetics in WPW Syndrome

The inherited form of WPW syndrome is an autosomal dominant trait of which the gene has been identified on chromosome 7q34-q36.[335, 336] A point mutation in the PRKAG2 gene, which encodes the regulatory γ-subunit of AMP-activated protein kinase (AMPK), results in the substitution of glutamine for arginine (R302Q).[335] These mutations cause glycogen-storage hypertrophic cardiomyopathy (HCM) associated with Wolff-Parkinson-White ventricular preexcitation syndrome and progressive cardiac conduction system disease.[337] In other hereditary forms of ventricular preexcitation associated with glycogen storage hypertrophic cardiomyopathy, mutations in the Lysosome associated protein 2 (LAMP2) and α-galactosidase A (GLA) have been identified.[338-340]

14.4.4.3. Arrhythmia Mechanism in WPW Syndrome

The macroreentry circuit in WPW syndrome is produced in the atrial muscle, AVN, ventricular muscle and the AP itself. This circuit facilitates continuous alternate depolarization of the atrial and ventricular myocardium.

In typical orthodromic reciprocating AV tachycardia, the electrical impulse proceeds from the atria to the ventricles through the AVN and retrograde back up to the atria from the ventricle via the AP. In this type of AVRT, the effective refractory period of the AP exceeds that of the normal AV Nodal His-Purkinje pathway.[341] In orthodromic AVRT, the QRS complexes are narrow and a retrograde P wave can be seen embedded in the early portion of the T wave (best seen in leads II and V2).[307]

Infrequently, the reentry circuit in WPW can proceed in the opposite direction and produce antidromic AVRT. In this type of AVRT the effective refractory period of the AP is shorter than that of the normal HPS pathway.[341] The ECG demonstrates very abnormally wide QRS complexes resembling those seen in ventricular tachycardia.[307]

14.4.4. The WPW Syndrome ECG

The typical ECG recorded during sinus rhythm in a patients with WPW syndrome shows ventricular preexcitation manifested by: 1) a short PR interval, 2) a wide QRS complex, and 3) initial slurring (delta wave) of the QRS complex. Ventricular preexcitation during sinus rhythm is produced by a fast wave of depolarization, which prematurely enters one of the ventricles through the AP. In most patients, ventricular preexcitation is present at all times and all heart rates, while in some cases preexcitation is intermittent. In the later, preexcitation is usually only present at lower heart rates.[322]

14.4.5. Atrial Fibrillation in WPW Syndrome

Episodes of atrial fibrillation are reported in 20% to 30% of adult patients with WPW syndrome, while atrial fibrillation is uncommon in children.[342, 343] These episodes are clinically important, since extreme rapid rates may occur over the bypass tract, leading to hemodynamic deterioration or ventricular fibrillation. In case of atrial fibrillation, the AP conducts the atrial rate to the ventricles, which in this case can exhibit extremely rapid rates, possibly resulting in ventricular fibrillation and cardiac arrest. Patients are considered to be at high risk for ventricular fibrillation when the shortest interval between two subsequent preexcited ventricular beats during atrial fibrillation is less than 220 ms .[344, 345]

14.4.6. Treatment Options in WPW Syndrome

Currently, conservative treatment with class I anti-arrhythmic drugs, beta-blockers (class II) and/or amidarone (class III) achieves a 70-80% efficacy in prevention of arrhythmia relapse.[346] Patients with WPW syndrome should not be treated with medications which shorten refractoriness along the bypass tract, such as calcium channel blockers and digoxin.[326]

After thorough evaluation of the risk/benefit ratio, radiofrequency (RF) ablation has been shown to also be a highly effective definitive treatment in the pediatric age group.[346] Since the risk of fatal complications is estimated to be up to 0.3% in children less than 4-5 years of age, the Pediatric Radiofrequency Catheter Ablation Registry advises to restrict the indication for RF ablation to children older than 10-12 years.[346] Currently, main concerns in pediatric RF ablation focus on X-ray exposure and possible damage to the CCS,[346] in which respect new systems of 3D mapping or the 'non-contact' system and the introduction of cryoablation are promising.[347-349]

1.14.4.7. Sudden Infant Death in WPW Syndrome

Sudden death (SD) in ventricular preexcitation syndrome is rare with an overall risk rate of 0.006 per patient-year, and mostly occurs in young and otherwise healthy individuals and can be the first manifestation of the WPW syndrome in previously asymptomatic patients.[256, 324, 328, 350-354] In a clinicopathological series on 273 SDs in children and young adults (aged < 35 years), a 3.6% prevalence of ventricular preexcitation syndrome was reported.[355] The fatal event was not preceded by warning symptoms in 40% of patients and death almost invariably occurred at rest and often during sleep. In 50% of these SD patients additional isolated acute atrial myocarditis was found on histological examination.[355]

Pathophysiologically, in most cases SD results from a rapid ventricular response to atrial fibrillation over an AP with a short refractory period.[354] In these cases, atrial fibrillation might be triggered by a primary atrial pathology or be secondary to AV reentrant tachycardia (AVRT).[354] Additionally, strenuous physical activities shorten the refractory period of the bypass tract and may precipitate atrial fibrillation or flutter.[326]

Syncope in patients with WPW might indicate high risk of sudden death. Successful ablation of the AP eliminates the risk of sudden death from WPW.

1.14.4.8. Animal WPW-Models

Most electrophysiological characteristics of APs and their role in causing AVRT have been obtained from clinical studies in humans. Extensive electrophysiological experiments in dogs have provided additional detailed insights in types A and B ventricular preexcitation.[341] Moreover, since the mutation for familial WPW syndrome was identified transgenic technology has been used to generate transgenic models for WPW. [335, 336]

Transgenic mice overexpressing the mutated PRKAG2 gene, were recently found to nicely recapitulate the human phenotype of familial WPW syndrome and glycogen storage cardiac hypertrophy.[335, 338] In this model, the mutated PRKAG2 gene, which encodes for the γ-2 subunit of AMP-activated-protein-kinase (AMPK), was cloned along side a powerful alpha-myosin heavy chain promoter. Histopathology demonstrated glycogen filled cardiomyocytes disrupting the annulus fibrosis and functionally giving rise to ventricular preexcitation. Additionally, in a similar transgenic mouse model overexpressing the PRKGA2 mutation, the preexcitation phenotype was reproduced and SVTs could be induced.[356]

Furthermore, deletion of the ALK3 gene (BMP receptor type IA), which encodes for the type 1a receptor for bone morphogenetic proteins (BMPs), in the AV canal cardiomyocytes during development causes ventricular preexcitation, possibly indicating an important role for the ALK3 gene in the etiology of the WPW syndrome.[247, 357]

1.15. AV Nodal Reentrant Tachycardias in Children and Adults

1.15.1. Epidemiology in AVNRT

AV Nodal Reentrant Tachycardia (AVNRT) is the most common form of SVT in adolescence and adulthood, while its very unusual in infants and toddlers (<5 %) and only school-aged children may present with AVNRT.[2, 308]

1.15.2. Arrhythmogenic Substrate in AVNRT

AV Nodal Reentrant Tachycardia (AVNRT) is based on the concept of AV Nodal conduction dichotomy, which implies that AV Nodal conduction is longitudinally dissociated in a slow and fast pathway. Functionally, the slow (α) and fast (β) pathways have distinct conduction velocities and refractory periods, while their precise anatomic boundaries are unknown. The fast pathway conducts rapidly and has a relatively long refractory period in the antegrade direction, while the slow pathway conducts relatively slowly and has a shorter refractory period than the fast pathway.[358] The polemic in dual AV nodal pathways concentrates on confinement of the slow and fast pathway to the AVN itself or the presence of an upper common pathway in the adjacent atrial tissues to complete the reentry circuit.

The dual input to the AVN seems to be a normal, physiological finding. Large studies on the inducebility of echoes or repetitive reentry in normal hearts do not exist. It is said that dual pathways existing in response to atrial extrastimulation may be found in up to 25% of patients without SVT.[325] In arrhythmia free children with congenital heart disease, the prevalence of dual antegrade AV Nodal pathways was 35%.[359] Moreover, dual AV Nodal physiology was found to be present in 62% of pediatric patients with AVNRT.[349]Interestingly, dual AV Nodal conduction pathways have been identified in up to 12% of patients with WPW-syndrome.[360]

The slow pathway is predominantly posteroinferiory located between the ostium of the CS and the septal leaflet of the tricuspid valve, while the fast pathway allegedly starts anterosuperiorly in the interatrial septum. These pathways converge onto the AVN at sites known as the posterior and anterior nodal inputs.[361] In addition, left-sided atrial inputs were implicated and proved in the structurally normal human heart.[362]

1.15.3. Arrhythmia Mechanism in AVNRT

In patients with AVNRT, the physiological conduction properties of the slow and fast pathway are such that they allow for a microreentry circuit at the entrance of the AVN.[307]

Subforms of AVNRT based on the location of earliest atrial activation include: 1) the most common subform, slow-fast conduction (antegrade conduction over the slow pathway and retrograde conduction over the fast pathway, 81,4%), 2) the slow-slow form (both antegrade and retrograde conduction over a slow pathway, 13,7%) and 3) the fast-slow form (antegrade conduction over the fast pathway and retrograde conduction over the slow pathway, 4,9%).[363]

1.15.4. The ECG in AVNRT

In patients with AVNRT, the ECG recorded during sinus rhythm is normal. An ECG recorded during an episode of AVNRT however shows normal but usually narrow QRS complexes. In these ECGs, the P waves are not clearly seen since activation of the atria and ventricles occurs at the same time and P waves are thus embedded in the QRS complexes.[307]

On electrophysiological study, the classic clinical finding of AV Nodal dual pathway electrophysiology – the 50 ms jump in the AH (atrium-His) interval for a 10 ms decrement in AA (atrium-atrium) interval during atrial stimulation- is applicable to 60-85% of cases with AVNRT.[364, 365] A second but far less specific parameter to show the presence of a slow AV Nodal pathway is a PR greater than or equal to RR during atrial overdrive pacing. In adults with AVNRT, this finding is present in 93% of cases.[366]

1.15.5. Treatment Options in AVNRT

In the initial attempts of RF ablation for AVNRT, the fast pathway was targeted by application of RF energy superior and anterior to the His bundle region, with success rates of 80-90% but with AV block induction in as many as 21% of patients. Currently slow pathway ablation, first introduced by Jackman et al., is targeted to cure AVNRT. With this technique the slow pathway is targeted by placing the catheter over the posteroinferior septum in the region of the CS and has reached success rates of 99% in experienced centers.[365, 367-371] In **Chapter 8** RF ablation in pediatric AVNRT is further outlined.

Aim and Outline of the Thesis

This thesis focuses on structure-function relations in cardiogenesis in relation to clinical arrhythmia etiology. The aim of the first two parts of this thesis is to correlate the results of basic experimental studies in the developing avian, mouse and human heart to the etiology of clinical AV Reentrant Tachycardias (AVRT) and AV Nodal Reentrant Tachycardias (AVNRT) in both children and adults. In the third part of this thesis, therapeutic clinical issues in pediatric supraventricular tachycardia (SVT) will be outlined.

In **PART I,** Chapter 2 describes the physiological development of the isolating annulus fibrosis and the persistence of functional APs in the postseptated embryonic avian heart in relation to the etiology of AVRTs in neonates and children. In Chapter 3, the pathological development of the isolating annulus fibrosis was studied in an experimental avian model in which the outgrowth of Epicardium-Derived-Cells (EPDCs) was delayed by an *in-ovo* microsurgical technique. In this model the persistence of APs was analyzed in relation to the etiology of AVRTs in adolescents and adults. Chapter 4 subsequently extrapolates the acquired avian data to mammalian heart development and describes the persistence of APs in mouse heart development as a possible anatomical substrate for perinatal SVTs. In Chapter 5**,** the postulated etiological considerations described in the previous 'basic experimental' chapters are extrapolated to humans by a systematical immunohistochemical analysis of the physiological development of the annulus fibrosis in serial sections of the developing human heart at consequent stages of embryonic development.

In **PART II**, Chapter 6 provides a historical review of the different theories on the ontogenic development of the AVN in relation to AVNRT etiology. In Chapter 7, the developmental origin of the AVN was subsequently analyzed in an experimental study in the avian embryo, providing a new concept on the origin of the adult AV nodal region.

Chapter 8 in **PART III**, describes the treatment options, success-, complication- and recurrence rates for pediatric SVTs. Finally, in Chapter 9 an illustrative case report of incessant AP mediated SVT in a premature neonate with hydrops foetalis, is presented.

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