

REVIEW ARTICLE

Basics of Cardiac Development for the Understanding of Congenital Heart Malformations

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ABSTRACT

Cardiovascular development has become a crucial element of transgene technology in that many transgenic and knockout mice unexpectedly present with a cardiac phenotype, which often turns out to be embryolethal. This demonstrates that formation of the heart and the connecting vessels is essential for the functioning vertebrate organism. The embryonic mesoderm is the source of both the cardiogenic plate, giving rise to the future myocardium as well as the endocardium that will line the system on the inner side. Genetic cascades are unravelled that direct dextral looping and subsequent secondary looping and wedging of the outflow tract of the primitive heart tube. This tube consists of a number of transitional zones and intervening primitive cardiac chambers. After septation and valve formation, the mature two atria and two ventricles still contain elements of the primitive chambers as well

as transitional zones. An essential additional element is the contribution of extracardiac cell populations like neural crest cells and epicardium-derived cells. Whereas the neural crest cell is of specific importance for outflow tract septation and formation of the pharyngeal arch arteries, the epicardium-derived cells are essential for proper maturation of the myocardium and coronary vascular formation. Inductive signals, sometimes linked to apoptosis, of the extracardiac cells are thought to be instructive for differentiation of the conduction system. In summary, cardiovascular development is a complex interplay of many cell-cell and cell-matrix interactions. Study of both (transgenic) animal models and human pathology is unravelling the mechanisms underlying congenital cardiac anomalies. (*Pediatr Res* 57: 169–176, 2005)

The recent development of new molecular and developmental biologic techniques, culminating in the transgenic era, implies a rejuvenation of research in early cardiovascular morphogenesis (1). For the first time it has become possible to study normal as well as abnormal heart development in detail in a rapidly increasing number of animal models. This allows us to get a grip on genes that dictate sidedness (2) and cardiac looping (3). It has taken away the notion that the cardiac mesoderm ever has a completely symmetric bilateral origin (Fig. 1a). It also shows that cardiac segmental predetermination already takes place in the cardiogenic plate stage (4). The next step brings us to the more complicated stages of cardiovascular development, being the secondary stage of looping of the heart with tightening of the inner curvature and the completion of an arterial and a venous pole, in which a complicated

set of septa separates veins, atria, ventricles, and the great arteries. This is completed by valve formation, differentiation of a conduction system, and a heart-specific supply from the coronary vascular system. During these latter stages of heart formation, it is essential that extracardiac cellular components are incorporated into the heart. These are the neural crest cells (5–8) as well as epicardium-derived cells (EPDC) from the pro-epicardial organ (9–11). The cellular contributions are essential for cardiac development and there is cardiac dependent embryo lethality if outgrowth to the heart is inhibited. In this review, we will shortly describe the basic aspects of heart development, incorporate results of novel research technology, and make a link to human congenital heart disease where possible.

NOMENCLATURE

A major pitfall in analyzing and reading studies in this field is the confusing use of terminology. The available cardiovascular developmental literature is mainly based on a combination of chicken and mouse studies (12), which is currently

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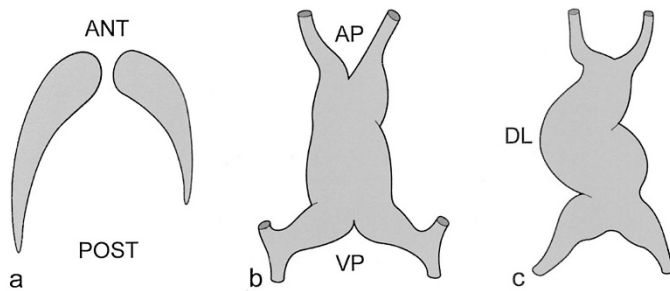


Figure 1. Schematic representation of (a) the bilateral formation of the mesoderm-derived cardiogenic plates, which are not quite symmetric. (b) After fusion, these form a straight heart tube with an arterial (AP) and a venous (VP) pole. (c) Thereafter, the tube starts its rightward (dextral) looping (DL).

extended by the relative simple anatomy of the zebra fish heart (13). The reliable studies based on human embryonic material date back to the few famous collections as exemplified by the Carnegie Collection and data from local collections such as present in the anatomy and embryology department at Leiden University Medical Center. This implies that current papers appear with a confusing variety of terms based on avian and mammalian data, which unfortunately do not overlap completely. Molecular biologists, wanting to describe their transgenic models, run into this problem. They are often annoyed and surprised that heart development has not been solved long ago and is being described in unambiguous terms, so that they can simply explain the gene expression patterns and transgenic phenotypes by using a cardiovascular developmental database. Awareness of the problem is the only help that can be offered at the moment.

CARDIOGENIC PLATE AND HEART TUBE FORMATION

The heart is derived from the anterior splanchnic mesoderm. It forms from two crescent-like cardiogenic plates (Fig. 1a) that already early on express cardiac-specific genes like *Nkx 2.5* (3) and *GATA4* (14). After fusion of these plates in the midline, a primary heart tube (Fig. 1b) is formed (15) that shows peristaltic contraction at 3 wk of development in a human embryo. The genes expressed in the cardiac tube already show an anterior (ventricular) and posterior (atrial) specification, but the differentiation in chamber myocardium and myocardium of the transitional or intersegmental zones takes place during the rightward looping process (Fig. 1c). This latter process is regulated by a cascade of genes that are essential for left-right programming (3,16). Disturbances will lead to abnormal looping that can vary from random, anterior to leftward looping. The consequences for human development are found in the abnormal atrial situs (situs inversus or isomerism), dextrocardia, and ventricular inversion. It is important to realize that only the atria of the heart may present with isomerism, whereas the ventricles never show heterotaxia patterns.

Differentiation of the Primary Heart Tube into Cardiac Chambers and Transitional Zones. The formation of the cardiac chambers from the primary heart tube has recently

received a great deal of attention. The ballooning concept of chamber expansion (17) sets an example here. In the myocardium of the looped heart tube, we discern several transitional zones (TZ) and intervening primitive cardiac chambers (CC). The transitional zones deserve special attention as they will become part of the septa, valves, conduction system, and fibrous heart skeleton. Furthermore, they will be partly incorporated in the cardiac chambers during formation of the definitive right and left atrium and their ventricular counterparts. We distinguish from the venous to the arterial pole: the sinus venosus and the sinoatrial ring (TZ), the primitive atrium (CC), the atrioventricular canal or ring (TZ), the primitive left ventricle (CC), the primary fold or ring (TZ), the primitive right ventricle (CC), and the ventricular outflow tract with a proximal and a distal part, also referred to as the ventriculoarterial ring (TZ) (Fig. 2). Of the transitional zones, the sinus venosus and primary fold do not develop endocardial cushions, whereas the atrioventricular canal and outflow tract present with cushions. The looping process brings all transitional zones together in the inner curvature of the heart tube whereas the trabeculated primitive chambers stand out on the outer curvature. The morphologic characteristics of the inner curvature can explain why many genes, when disturbed, have a deficient remodeling of the inner curvature as a result. This will lead to a deficient leftward movement of the outflow tract over the atrioventricular canal, resulting in a spectrum of outflow tract abnormalities. The most extreme case is a double-outlet right ventricle with an obligatory ventricular septal defect or, in less extreme cases, just a ventricular septal defect. These malformations are the most common in transgenic mice.

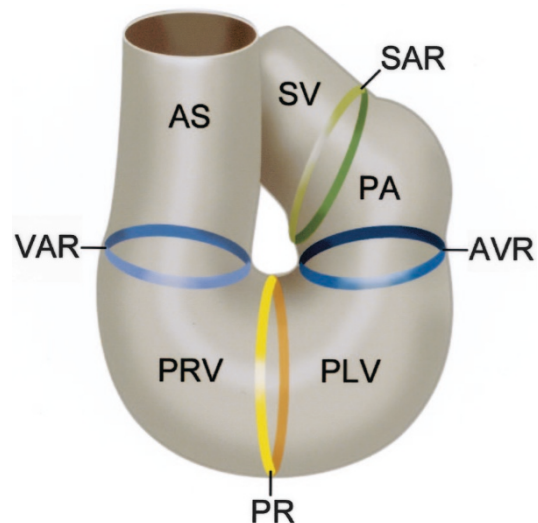


Figure 2. Schematic drawing of the looped heart tube with the cardiac chambers and the transitional zones. Following the blood flow from venous to arterial, we can distinguish the sinus venosus (SV), the sinoatrial ring (SAR), the primitive atrium (PA), the atrioventricular ring (AVR) encircling the atrioventricular canal, the primitive left ventricle (PLV), the primary fold or ring (PR), the primitive right ventricle (PRV), the outflow tract ending at the ventriculoarterial ring (VAR), and the aortic sac (AS).

CARDIAC SEPTATION AND CHAMBER FORMATION

Septation has to take place at three levels: the atrium, the ventricle, and the arterial pole. For normal septation, correct looping is essential. In the septation process, specifically of the outflow tract of the ventricle, the extracardiac contribution of neural crest cells also plays an essential role. With proper septation of the various levels, transitional zones are incorporated into the primitive chambers leading to the formation of the definitive cardiac atria and ventricles. The three levels will be discussed separately and, where necessary, integrated.

Atrial Formation, Septation, and Sinus Venosus Contribution. The atria consist of a main smooth-walled compartment and trabeculated appendages, which have a characteristic shape differentiating the right atrium from the left atrium (18). The primitive atria are connected to the dorsal body wall by the dorsal mesocardium, in which the sinus venosus and its tributary veins are lodged. During heart looping, the main veins feeding the sinus venosus, being the right anterior and posterior cardinal vein as well as the left anterior cardinal vein, become incorporated in the posterior wall of the right atrium. In this process, a right and a left sinus venosus valve are formed, creating a sort of funnel for the entrance of blood into the atrium (Fig. 3a). Anteriorly, the left and right venous valves fuse to form the septum spurium, which is connected to the anterior part of the atrioventricular canal. On the basis of immunohistochemical data in several species such as quail (19), mouse (20), rat (21,22), and human embryos (23,24), it is likely that the pulmonary venous anlage belongs to the embryonic sinus venosus. During atrial septation, the pulmonary veins will not remain embedded in the right atrium, but instead relocate to the dorsal wall of the left atrium. This opinion is contested by the group of Anderson and colleagues (25), who claim a separate midline origin of the so-called pulmonary pit in the dorsal mesocardium. Novel findings in the development of the primitive conduction system do not favor this opinion.

From the studies of development of the conduction system, it also became obvious that a ring of sinus venosus-derived

tissue encircles the mitral orifice (Fig. 3b). This implies that the majority of the smooth-walled atrium will be sinus venosus-derived, whereas the appendages are atrium proper. The primary septum of the atrium will develop a fenestration, confusingly referred to as the ostium secundum. The ostium primum of the atrium is an embryonic right-to-left connection that is normally closed by fusion of the cushion-like under-rim of the primary septum with the atrioventricular cushions. When this process does not take place, a primary atrial septal defect will develop, mostly in combination with insufficient fusion of the superior and inferior atrioventricular cushions as well. The resultant malformation is called an atrioventricular septal defect or atrioventricular canal defect. It is encountered in, e.g. the transforming growth factor (TGF)- β 2 knockout mouse (26). In human patients, it is often found in trisomy 21 or Down syndrome patients (27,28).

In human development, we see the formation of a secondary atrial septum in which the left venous valve is incorporated. This valve is actually a fold in the roof of the right atrium, which we consider to be pulled down by the sinus venosus-derived septum spurium (24). In mouse embryos, however, the left venous valve remains a separate structure and chicken embryos lack a septum secundum altogether (19). Deficient formation of the primary atrial septum (valve of the foramen ovale) as well as insufficient formation of the secondary septum lead to atrial septum secundum defects (ASD II), a common abnormality in human neonates. Familiar forms have been shown to be linked to a mutation in the *Nkx2.5* gene (29).

Ventricular Formation and Septation. The primary heart tube after looping shows an atrioventricular canal, a primitive left ventricle, and a primitive right ventricle that are separated by the primary fold or ring (Fig. 2). In the process of ventricular septation, we are basically dealing with two components. First, the ventricular inflow septum and, second, the ventricular outflow septum.

Ventricular inflow septation. The ventricular inflow septum is mainly formed from the primary fold or ring that rises up from the ventricular apex, in fact as a resultant of the outgrowth of the primitive right and left ventricular chambers (Fig. 4, a and b). The process is complicated by subsequent positioning of a tricuspid orifice above the right and a mitral orifice above the left ventricle. This is accomplished by the secondary formation and outgrowth of the right ventricular inflow tract that starts as an atrioventricular gully in the mass of the primary fold (30–32). This process results in the formation of a definitive right ventricle (Fig. 4, c and d) with an anterior trabeculated part leading to the outflow tract, separated from the also trabeculated inflow tract by the septomarginal trabeculation that continues into the moderator band, both derived from primary-fold myocardium. Definitive closure of the primary “interventricular” foramen is achieved by fusion of the superior and inferior atrioventricular cushions, which will also receive a contribution from the outflow tract cushions. The cushion material will mainly condense into what is known as the membranous part of the atrioventricular septum. We have recently shown in human trisomy 21 fetal hearts, studied for their collagen VI distribution, that the membranous septum is exceptionally large in these cases (27).

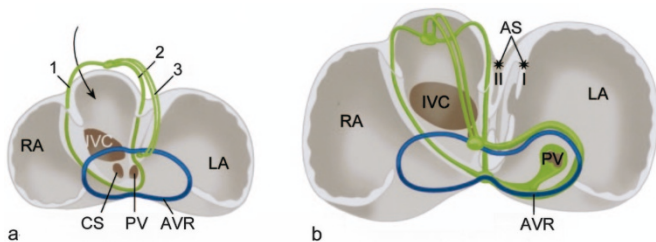


Figure 3. (a) Schematic representation of the incorporation of the sinus venosus into the right (RA) and left (LA) atrium. The sinoatrial ring is depicted in green and consists of three components: 1) the right venous valve, 2) the left venous valve, and 3) the fused right and left valve that anteriorly connect to the atrioventricular ring (AVR, blue) as the septum spurium. The superior caval vein enters from above at the site of the arrow. The other venous tributaries are the inferior vena cava (IVC), the coronary sinus (CS), and the pulmonary venous pit (PV). (b) During atrial septation, a primary atrial septum (AS I) and a secondary atrial septum (AS II) are formed, the latter incorporates the left venous valve. The entrance of the pulmonary veins (PV) is now relocated to the posterior wall of the left atrium.

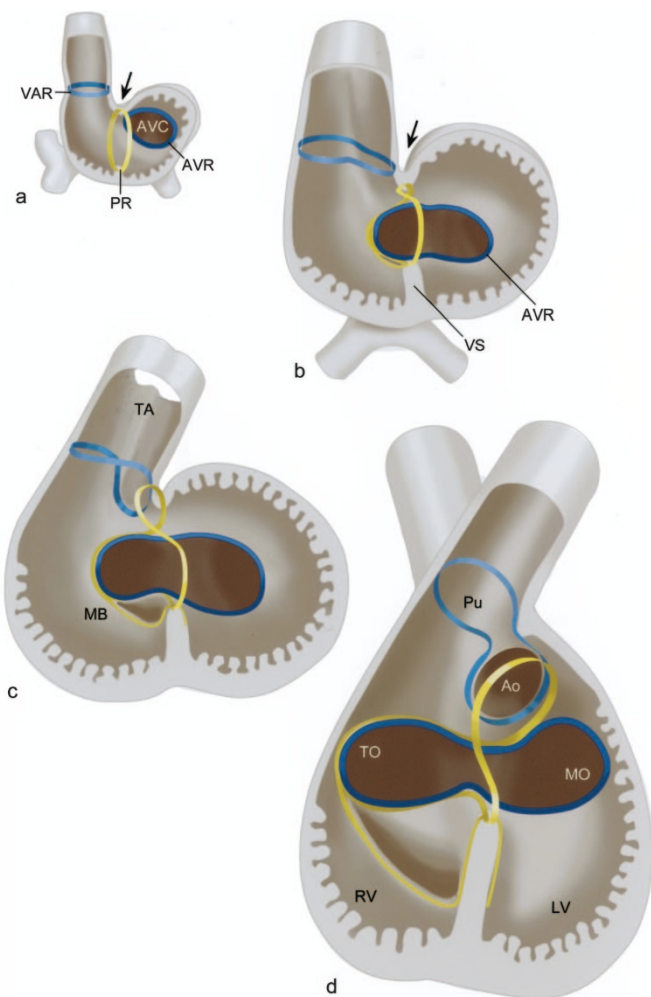


Figure 4. Schematic representation of the remodeling of the cardiac chambers and the transitional zones at the ventricular level. (a) internal view (compare with Fig. 2) of the looped heart tube. The transitional zones are, going from the venous to the arterial pole, the atrioventricular ring (AVR, dark blue), the primary ring (PR, yellow), and, at the distal end of the myocardial outflow tract, the ventriculoarterial ring (VAR, bright blue). (b) During looping, with tightening of the inner curvature (arrow), the right part of the AVR moves to the right of the ventricular septum (VS). (c) Start of formation of the inflow tract of the right ventricle by excavation of the PR. The lower border is formed by the moderator band (MB). (d) Completion of the process with formation of a tricuspid orifice (TO) above the right ventricle (RV) and the aortic orifice (Ao) and the mitral orifice (MO) above the left ventricle (LV). It is easily appreciated that there is aortic-mitral continuity, whereas the distance between the TO and the pulmonary orifice (Pu) is marked.

From these developmental steps, a number of heart malformations in both human and mouse can be explained relatively easily. For instance, with insufficient outgrowth of the right ventricular inflow tract the tricuspid orifice remains connected to the embryonic primitive left ventricle, leading to a spectrum of overriding and straddling tricuspid valve to double-inlet left ventricle (DILV). Cases with tricuspid atresia belong most often to the DILV category. In this case, the right ventricle lacks the inflow tract and only consists of an outflow chamber derived from the right ventricular outflow tract. The trisomy 16 mouse (33) is a model in which all components of this inflow tract malformation are found, as well as a number of outflow tract anomalies.

Ventricular outflow tract septation. During outflow tract septation, the aortic orifice becomes physically connected to the left ventricular outflow tract while the pulmonary orifice remains above the right ventricle. This is in some way comparable to the designation of the mitral and tricuspid orifice to their respective ventricles. It can be appreciated that, in outflow tract septation, the aorta has to undergo the most marked change in position.

The sequence of events can be described in the following way. In the looped heart tube, the outflow tract is considered as a transitional zone lined on the inside by two endocardial outflow tract cushions. In humans and mammals, these cannot be easily distinguished as having a proximal and a distal part. In avian embryos, this distinction is clear and has led to the recognition of bulbar (proximal) and truncal (distal) cushions. The distal cushions will take part in semilunar valve formation whereas the proximal cushions will become the muscular outflow tract septum (34).

Initially, the cushions start to fuse from distal to proximal and during this process it is essential that an extracardiac contribution of neural crest cells enters the heart. These neural crest cells present as a central mass of condensed mesenchyme between the fourth and sixth pharyngeal arch arteries and extend two prongs deep down into the proximal cushions. This phenomenon was first described in the avian embryo (35) and confirmed in human embryos (36).

We have reconstructed the outflow tract of two human embryos to show the disposition of the condensed mesenchyme relative to the mesenchymal vessel wall and the arterial orifice level, the cushion tissue and the myocardium (Fig. 5, a–d). By use of animal models such as quail-chick chimera (8) and retroviral lacZ neural crest cell tracing (6,5,37) in chicken and recently by neural crest reporter mice (38–40), it was confirmed that the earlier described condensed mesenchyme (Fig. 5, a–c) consists mainly of neural crest cells.

The exact role of the neural crest cells in outflow tract septation is much debated. Initial studies by the group of Kirby (41) in which the neural crest cells were ablated showed severe outflow tract abnormalities. More recent studies in various animal models such as the TGF- β 2 knockout mouse (26), chicken neural crest ablation (42), and the RxR α knockout mouse (43) show that neural crest cells can still be present in the outflow tract in case of severe outflow tract malformations. An explanation for this phenomenon will be provided later.

In normal outflow tract septation, neural crest cells are found in a condensed mass between the aortic and pulmonary orifice, where they most probably play a part in the formation of the conal tendon, initially connecting both orifices (6,36). At the level of the proximal outflow tract cushions, the neural crest cells take up a position in close contact with the myocardial cells and in part invade the myocardium. The majority of these cells have been shown to go into apoptosis (6). Study of the human outflow tract myocardialization showed apoptosis in a similar manner. We have postulated that neural crest cells are essential in releasing factors that induce activation of TGF- β 2. *In vitro* studies have shown that TGF- β 2 is important for myocardialization of the outflow tract cushions (17), which supports the results of our *in vivo* TGF- β 2 knockout mouse

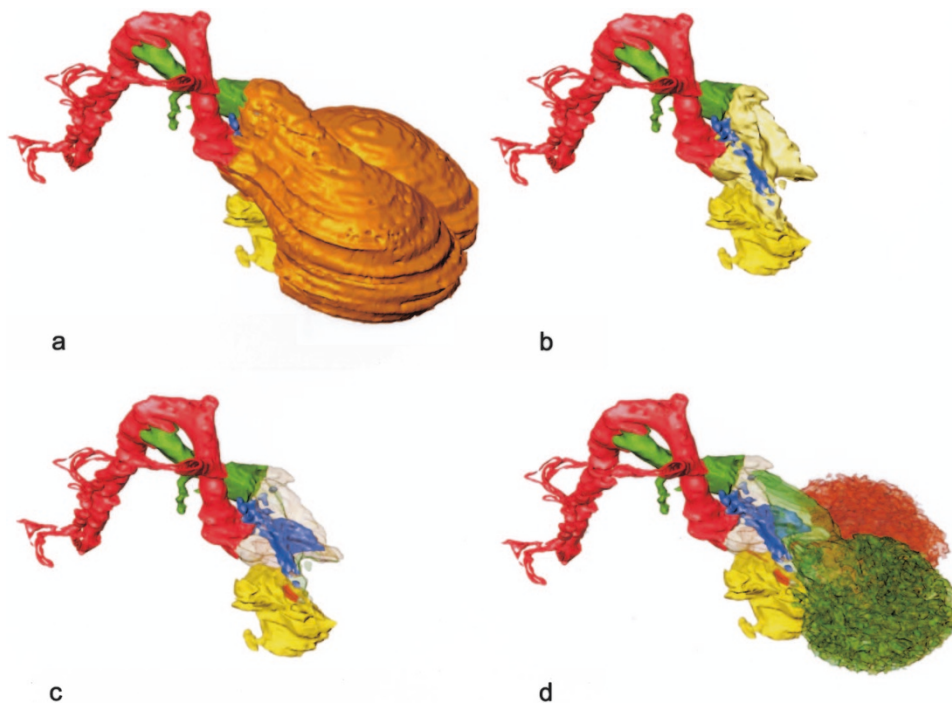


Figure 5. Reconstruction of a the heart of a human embryo of 5 wk development. (a) The myocardium (*brown*) of the ventricles reaches up to the arterial orifice level. The pulmonary trunk (*green*) arises anteriorly from the right ventricle and the aorta (*red*) originates at a more caudal and posterior level. (b) The myocardium has been removed and the endocardial outflow tract cushions (*light yellow*) and the atrioventricular cushions (*dark yellow*) become visible. The condensed mesenchyme (*blue*), consisting mainly of neural crest cells, is incorporated within the outflow tract cushion mass. It is visible, however, at the entrance site between the arterial orifices (see also *a*) and as a lateral streak (right side visible, left side not) where the condensed mesenchyme connects to the outflow tract myocardium (removed, see *a*) At these sites, the myocardialization of the outflow tract septum will take place. The right lateral outflow tract cushion is connected to the atrioventricular cushion mass, whereas the left lateral cushion is not connected to this mass. (c) By making the outflow tract cushions translucent, the complete condensed mesenchyme becomes visible extending way into the cardiac outflow tract. (d) Insertion of the right (*green*) and left (*red*) ventricular lumen shows how the condensed mesenchyme and thus the outflow tract septum mainly borders the right ventricular pulmonary infundibulum.

(26). There are various models like the trisomy 16 mouse (33) and the avian venous clip model (44) in which this myocardialization does not take place, resulting in a mesenchymal outflow tract septum. Neural crest cells are present in the outflow tract in these cases, but several mechanisms may interfere with proper interaction of the neural crest cell with the myocardium.

The primary role of the neural crest cell is challenged by research of the 22q11 deletion syndrome. Intensive research of several groups regarding the genes of the deleted region has shown *Tbx1* to be a primary candidate gene (45). However, this gene is expressed in the embryonic mesoderm surrounding the pharyngeal arch arteries and not in the neural crest cell population. An interaction with *Fgf8* and *Fgf10* (46), which are both expressed in the pharyngeal mesoderm and are under control of *Tbx1*, could explain a defective neural crest cell function in aortic arch and outflow tract septation. There are exciting new data linking the above information on *Tbx1* and *Fgf8* and *Fgf10* expression to cardiac outflow tract development. Based on observations dating back to the 1970s and 1980s, there is now ample evidence of the existence of a so-called secondary (47) or anterior (48) “heart field.” From this heart field, positioned in the pharyngeal mesoderm and expressing the aforementioned genes, myocardium is added to the outflow tract of the heart up to 8 d of development in the mouse and stage 24 in the chicken. This information links

unequivocally the concept that disturbed interaction of the pharyngeal arch mesoderm (*Tbx1* positive) and adjoining neural crest cells will result in aortic arch and cardiac outflow tract malformations. Recently, *Tbx1* mutations have been observed in a small group of patients from a population with a DiGeorge phenotype lacking the full 22q11 deletion (49). This proves at least that *Tbx1* mutations can lead to a DiGeorge phenotype in human patients. We have shown in chicken embryos using antisense retroviral technology that two genes of the DiGeorge critical region being *Ufd1L* (50) and *DGCR6* (unpublished results), which are both expressed in neural crest cells, directly influence pharyngeal arch and outflow tract formation, giving rise to a DiGeorge-like phenotype. Both genes have been found to be mutated in a single human patient in a large screen. So it is expected that several genes and gene-cascades can lead to a DiGeorge phenotype.

For cardiac outflow tract malformations, insufficient looping and remodeling of the inner curvature may also hamper the positioning of the aortic orifice toward the left ventricle. This can lead to a spectrum of double-outlet right ventricle and subaortic ventricular septal defects. If the outflow tract septum is muscular in these hearts, neural crest cells have at least played their role. The developmental disturbance that leads to tetralogy of Fallot with pulmonary stenosis still needs to be sorted out. There are two animal models for this anomaly, a transgenic vascular endothelial growth factor (VEGF) 120/120

mouse (51) and a maternal diabetic rat model (52), which are currently studied in our lab for this purpose. It has been *en vogue* for some time to study all neonates with outflow tract anomalies for a 22q11 deletion, including cases with transposition of the great artery (TGA). It has been shown that TGA and the hypoplastic left heart syndrome do not fall within the 22q11 deletion phenotype. Until now, there have been two models for TGA, a retinoic acid-induced rat model (53) and the recently published perlecan-deficient mouse (54). Interestingly, both molecules are known to have a role in laterality determination of the embryo.

DEVELOPMENT OF THE CONDUCTION SYSTEM

The primary heart tube already shows peristaltic contractions propelling the blood from the venous to the arterial pole. With remodeling of the heart into a four-chambered structure, a differentiation of the myocardium takes place into a working myocardium and a conduction system myocardium. The processes and genes involved are currently being investigated in detail.

Several markers such as GIN (55) and HNK1 (22) have been used to depict the development of the embryonic to the mature conduction system. Using the HNK1 antibody, the development of both the sinoatrial and the atrioventricular conduction system in the human embryo was described (23). This study revealed a major role for two transitional zones, namely the sinus venosus and the primary ring. The incorporation of the sinus venosus into the atrium (described under “Atrial formation, septation, and sinus venosus contribution,” above) showed the embryonic existence of three internodal pathways between the sinoatrial and the atrioventricular node (Fig. 6a) and the relation of the pulmonary venous system in the dorsal left atrial wall with the primitive conduction system. More recently, we were able to study the CCS lacZ mouse (56,57) and have complemented the above findings by showing the development of the interatrial Bachmann’s bundle and the continuation of the right ventricular moderator band with the right atrioventricular ring bundle (58,59) (Fig. 6b). The factor that directs myocardial development toward either a working myocardium phenotype or a conduction system phenotype still has to be elucidated. On the basis of positional clues, we have postulated a role for neural crest cells in the induction of the central conduction system (5) and for the epicardium-derived cells in differentiation of the Purkinje network (10,58). At the molecular biologic level, endothelin-1 might be the signaling molecule (60).

The clinical importance of understanding the differentiation of the conduction system associated with gene expression patterns in the embryo is found in a link to sites of origin of atrial arrhythmias increasingly seen in the adult population (59). Furthermore, Nkx2.5 mutations are associated with conduction system abnormalities in both mouse and man. For the understanding of familial genetically determined arrhythmias such as the long QT syndrome, the underlying gene pathways still need to be elucidated.

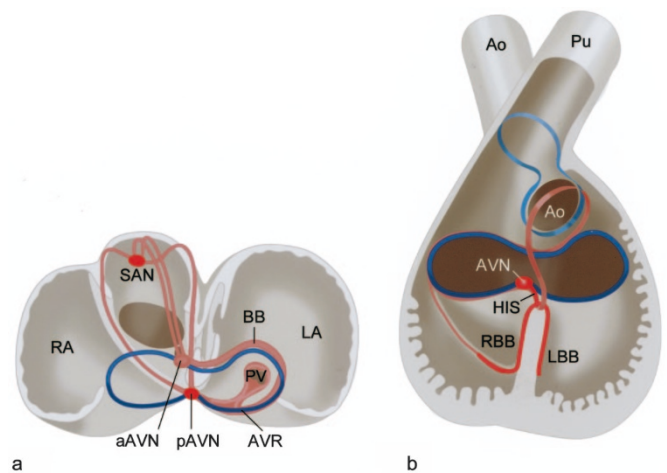


Figure 6. Schematic representation of the transitional zones that are relevant for the development and differentiation of the conduction system. (a) The sinoatrial ring and derivatives (pink) form the sinoatrial node (SAN) and three internodal pathways that connect the SAN to the posterior atrioventricular node (pAVN). This sinoatrial tissue also encircles the left atrioventricular ring (AVR), forming the Bachmann’s bundle (BB) and part of the left atrial posterior wall surrounding the pulmonary veins (PV). The pathways depicted in pink have not been proven to be functional under normal circumstances in the mature heart, in contrast to the structures in red. (b) The atrioventricular conduction system is mainly derived from the primary ring that was positioned between the primitive left and right ventricle (see Figs. 2 and 3). The tissue of this ring encircles the right atrioventricular canal and the aortic orifice. As the inflow tract of the right ventricle is a newly formed structure, the moderator band all the way up to the right atrioventricular ring bundle contains elements of the primitive conduction system. Only the structures depicted in red—the atrioventricular node (AVN), the bundle of His (HIS), and the right (RBB) and left (LBB) bundle branches—are functional in the mature heart.

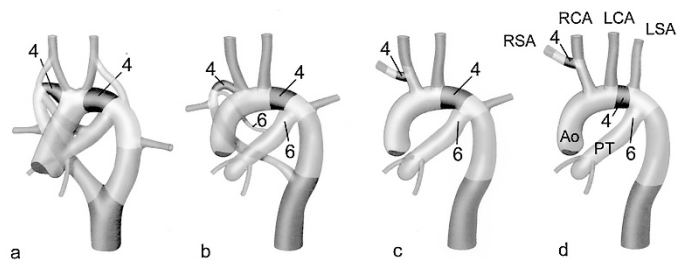


Figure 7. Schematic representation of the development of the bilateral pharyngeal arch artery system into a left-sided aortic arch (a–d). Special attention is paid to the 4th and 6th arch arteries. The right 6th arch artery disappears during early development only followed by left 6th arch artery (ductus arteriosus) closure after birth. The left and right 4th arch segments normally remain persistent, the left one as part of the aortic arch and the right one as part of the right subclavian artery. The 4th arch artery has morphogenetic characteristics that make it specifically vulnerable for the development of aortic arch abnormalities as, e.g. in the 22q11 deletion syndrome.

DEVELOPMENT OF THE PHARYNGEAL ARCH ARTERIES INTO THE MATURE AORTIC ARCH

As soon as the cardiogenic plates fuse, they connect to the primitive vascular system (15,61). The latter develops through a process of vasculogenesis (formation of isolated pro-endothelial cells that merge into vessels) and angiogenesis (sprouting of the existing endothelial vessel network). The heart tube contacts the bilateral dorsal aortae through the first bilateral set of pharyngeal arch arteries. These are subsequently

followed in time by a second, third, fourth, and sixth set (the fifth does not develop) (62). This system is remodeled into a left-sided aortic arch in mammals (63; Fig. 7) and a right-sided system in birds (7). Recent studies of abnormal aortic arch development in TGF- β 2-/- mouse embryos has shown that apoptosis plays a role in normal development and is altered during abnormal development. The most common abnormalities are the result of a regression of fourth arch segments leading to interruption of the aortic arch type B. This is often seen in combination with an abnormal persistence of the right dorsal aorta leading to a retroesophageal right subclavian artery (63). The reason for the enhanced vulnerability of the fourth arch segment, as also seen in other knockout mice, is not known, as smooth muscle cells of all arches derive from neural crest cells. It is remarkable, however, that the fourth arch segment shows specific morphologic characteristics (64).

In human infants, aortic arch anomalies are distinguished in those related to the B-segment (located between the left carotid and the left subclavian artery) and the A-segment or isthmus (located between the left subclavian artery and the ductus arteriosus). B-segment abnormalities are often seen in relation to the 22q11 deletion syndrome and are thought to relate to neural crest defects. The latter is under discussion since the finding of Tbx1 as a candidate gene (45, 50, 64a). In the human infant, hypoplasia or coarctation of the aorta at the isthmus site is not specifically linked to neural crest-related syndrome or a specific gene. In this respect, it is noteworthy that a mouse lacks the isthmus of the aorta seen in the human.

DEVELOPMENT OF THE CORONARY VASCULAR SYSTEM

Finally, the heart develops its own circulatory system necessary for nutrition and oxygenation of the myocardium. Coronary vascular formation is relatively late in development because it can only take place after proper covering of the myocardium by epicardium. The latter grows out from the pro-epicardial organ (9,65). In the subepicardial space, an endothelial network is formed (66,67) that grows into the myocardium, connects to the aortic root (68,69), and finally enters the anterior part of the right atrium (66). After these connections have been established, arteriogenesis follows with formation of a media and an adventitia. These consist of smooth muscle cells that originate from the EPDC (70,71). Coronary vascular abnormalities are encountered when the epicardial outgrowth is either inhibited mechanically (72) or by inhibiting epithelial-mesenchymal transformation, *e.g.* by Ets1 and Ets2 transcription factors (73). The abnormalities range from partial to complete absence of the main coronary arterial stems. The latter situation is always complemented by the presence of ventricular coronary arterial communications (VCAC) or fistulae. These findings can be transposed to the human situation in which, *e.g.* in pulmonary atresia without ventricular septal defects, we find VCAC with serious coronary vascular pathology already in the fetal period (74,75). We postulate that defective interactions between myocardium and epicardium are the basis for these malformations.

CONCLUSION

Current molecular developmental studies of heart development unravel the basics of both normal and abnormal cardiovascular development. The majority of malformations found in the human population, however, seem to be based on complex gene-environment interactions, including influence of diabetes (76) and hyperhomocysteine (77).

Further studies in these complex mechanisms are needed to back-up the molecular data that are currently our main body of information.

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