The role of the second heart field in pulmonary vein development : new insights in the origin of clinical abnormalities

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Discussion

Normal and Abnormal Development of Pulmonary Veins: State of the Art and Correlation with Clinical Entities

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Abstract

Interest for the pulmonary veins has increased in the past decade after the potential arrhythmogenicity of the myocardial sleeve surrounding these structures has been recognized. Furthermore, there are several clinical entities, such as anomalous connection pattern and pulmonary vein stenosis, that are related to abnormal pulmonary vein development.

In this review, we will describe current literature and aim to elucidate and reorganize current opinions on normal and abnormal pulmonary vein development in relation to clinical (management of) diseases. Several unresolved questions will be addressed, as well as current conceptual controversies. First, a general overview of development of structures at the venous pole of the heart, including normal development of the pulmonary vein from a primitive Anlage, will be provided. Recent insights indicate an important contributory role of the mesoderm behind the heart, the so-called second heart field, to this area. Subsequently, the formation of a myocardial and smooth muscle vascular wall of the pulmonary veins and the left atrium is described, as well as current insights in the mechanisms involved in the differentiation of these different cell types in this area. Next, developmental concepts of normal pulmonary venous drainage patterns are reviewed, and an overview is provided of clinical entities related to abnormal development at several anatomical levels. Lastly, attention is paid to arrhythmogenesis in relation to pulmonary vein development, as well the consequences for clinical management.

Introduction

In recent years, the involvement of the pulmonary veins (PVs) in several pathogenetic processes, has increasingly been recognized. The PVs play a role in the onset of atrial arrhythmias, such as atrial fibrillation[1]. Furthermore, congenital malformations, including abnormal pulmonary venous drainage patterns and PV stenosis, may warrant surgical or percutaneous interventions. Also the structure of specific components of the PVs may be involved in pathogenesis, e.g. vulnerability of the smooth muscle and myocardial wall for acquired PV stenosis.

In the light of these clinical considerations, understanding of both normal and abnormal PV development is mandatory. PV development is narrowly related to development of the venous pole of the heart, that includes the sinus venosus and atrial segment. Recent new insights have demonstrated that during cardiac development large compartments of the heart are developed in and subsequently incorporated from the mesocardium behind the heart, the so-called second heart field[2,3].

Current concepts of PV development result in several questions that seem to recirculate and ask for clarification. We should in this regard also take notice of the fact that differences in semantics, definitions and interpretations of findings might be of great influence. After giving a general overview of development, in this review we will focus on the following questions:

1. How are the different components of the venous pole defined and what are current controversies?
2. Which role does the second heart field play in the development of the venous pole, myocardialization and smooth muscle cell (SMC) formation of the PVs?
3. What, in the view of previous points, are the current developmental concepts on the origin of normal and abnormal PV connection?
4. How is PV development related to arrhythmogenesis?
5. What are the consequences of the various concepts of PV development on clinical management?

We will try to clarify these topics by describing normal and abnormal PV development, as well as the relation to clinical entities, based on our own research and current literature.

With regard to abnormal PV development, in literature different terms are used to describe the same condition. While “anomalous PV drainage or return” mainly focuses on the physiological condition, “anomalous PV connection”, in our opinion, describes most accurately the anatomic condition.

General overview of early cardiac development.

The embryo starts as an embryonic plate, in which a primitive streak area develops[4]. The primitive streak is formed as an invagination in the midline of the embryonic plate, at the initiation of gastrulation (the formation of the three embryonic germ layers). From the primitive streak, two large lateral plate mesodermal compartments are derived on both sides of the embryonic axis[4] (Fig.1a, upper panel). In each compartment a coelomic cavity develops that splits the mesoderm into a somatic layer, lining the ectoderm, and a splanchnic layer, lining the endoderm[5]. The bilateral splanchnic mesoderm contains the cardiac precursor cells. Anterior to the embryonic axis (notochord), these mesodermal compartments fuse to form a primitive myocardial heart tube (primary heart tube) that is lined on the inside with endocardium and separated from the myocardial layer by cardiac jelly[4,5]. After a complicated bending of the head region of the embryo, this heart tube obtains an arterial (anterior/cranial) pole and a venous (posterior/caudal) pole (Fig.1a, lower panel).

During further development of the heart, splanchnic mesodermal cells from the so-called second heart field will continue to be added to the heart[2,3]. The second heart field is defined as an anteroposterior extension of mesoderm, located behind the heart, from which myocardium is incorporated to the heart during further development. The contribution to the venous pole of the heart is referred to as derived from the posterior heart field[6,7], whereas the contribution at the arterial pole is referred to as derived from the anterior or secondary heart field[8,9] (Fig.1b-d). The second heart field in relation to PV development is discussed in detail in section 2.

The splanchnic mesoderm also differentiates into a vascular plexus of endothelial cells. This plexus is the source of the lining of the vitelline veins, the pharyngeal arch arteries and the initially bilateral dorsal aortae[4,5]. The splanchnic plexus is connected to the sinus venosus, being itself in connection with the common atrium, by means of a small strand of endothelial cells, the so-called midpharyngeal endothelial strand, which runs from the arterial to the venous pole of the heart[10]. This structure is the Anlage of the primitive PV (see section 3), that is situated in the mesocardium behind the heart (the dorsal mesocardium). We will focus on structures that will develop at the venous pole of the heart, being the area in which the PV develops.

1. How are the different components of the venous pole defined?

The inflow part or venous pole of the heart includes the embryological sinus venosus segment and the atrial segment. These compartments constitute the most caudal parts of the primary heart tube (Fig.1a). In this paragraph we will clarify the nomenclature of embryological structures at this site, as well as their counterparts in the adult. As will become clear, definitions and descriptions of some structures have been the subject of vivid discussions over the past decades. We will also address these controversies where appropriate.

After development from the second heart field (see section 2), the sinus venosus is connected to the common atrium and consists during the 4th week of development of a left and right part, each receiving blood from the vitelline/omphalomesenteric vein, the umbilical vein and the common cardinal vein. During early stages of development, the border between the sinus venosus and the still unseptated common atrium, can be distinguished as a fold of myocardium, the sino-atrial fold. Asymmetrical growth of the right part of the common atrium causes a rightward shift of the site of connection of the sinus venosus to the common atrium. The right part of the sinus venosus and corresponding veins also enlarge and form the caval veins that are incorporated into the future right atrium. The left part of the sinus venosus obliterates to a large extent in human development. The left common cardinal vein will form the coronary sinus. In mice, the left anterior cardinal vein (the cranial branch of the common cardinal vein) will persist as the left superior caval vein, while in human the left caval vein usually obliterates, forming the ligament of Marshall

(oblique vein of Marshall in case of incomplete obliteration). After septation, the right atrium consists of 2 parts: the smooth-walled main body derived from the sinus venosus and the right trabeculated atrial appendage. The sinus venosus part of the right atrium, receiving the caval veins and the coronary sinus (in the adult called sinus venarum) contains during development 2 muscular valves: the right and left venous valves. The inferior part of the right venous valve will form in the mature heart both the Eustachian valve, covering the inferior caval vein, and the Thebesian valve, covering the coronary sinus. The left venous valve in human fetuses fuses with the atrial septum that develops at the end of the 4th week[11], whereas in mice it can still be distinguished as a separate structure. The superior part of the right venous valve will develop into the terminal crest (or crista terminalis), that forms a border structure between the right atrial appendage and the right atrial body or sinus venarum in the adult.

Between the 4th and 5th week of development a lumenized PV can be discriminated in the dorsal mesocardium, derived from the midpharyngeal endothelial strand[10]. The PV is at this stage a single structure, that is, at the entrance into the sinus venosus (connecting to the common atrium), bordered by 2 muscular ridges, the left and right pulmonary ridges. These ridges are part of the so-called dorsal mesenchymal protrusion (DMP)[12,13], an accumulation of mesenchymal cells that encompasses the primitive PV, and that contributes to the atrial septum[14]. During atrial septation, the DMP is responsible for the shift of the PV towards the left side[13]. Thus, after atrial septation, the common PV drains to that part of the sinus venosus that is incorporated in the posterior wall of the left atrium (LA) after which the right pulmonary ridge fuses with the left side of the atrial septum[14]. Subsequently, the PV bifurcates, dilates and incorporates into the LA, contributing to the size and consistency of the smooth-walled LA body, which has histological identical characteristics to the PV, with an inner vascular layer covered on the outside by myocardium[15]. Usually, by the end of this process, two right and two left PVs enter the LA, although variations in anatomy are very common[16]. Similar to the situation on the right side, the trabeculated LA appendage can be distinguished from the smooth-walled LA body. Histologically, a transitional zone between these parts can be recognized that is similar to the sinus venosus segment of the right atrium[15].

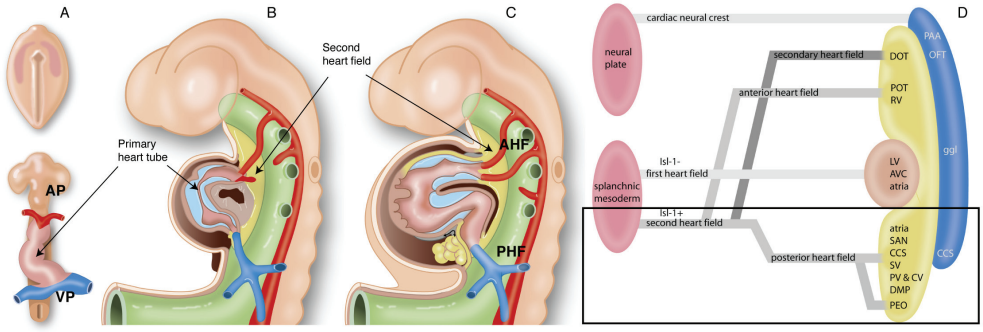

Figure 1.

Schematic representation of sequential stages of cardiac development and the contribution from first and second heart field. The primary heart tube is depicted in brown and the myocardium that is derived from the second heart field (and incorporated later in the heart) in yellow. A. The primary heart tube is formed after fusion of bilateral plates of splanchnic mesoderm in the primitive plate. This tube already has a venous pole (**VP**), and an arterial pole (**AP**). B. Lateral view of embryo (approximately 23 days in human), showing the primary heart tube, surrounded by cardiac jelly (blue), and the second heart field situated dorsally to the heart. C. Human embryo at 25 days. The primary heart tube has expanded both at the venous and at the arterial pole with myocardium derived from the second heart field (depicted in yellow). D. Scheme of the nomenclature the primary and second heart field (see also Chapter 1, Fig.1). At the venous pole of the heart, myocardium is derived from the *posterior heart field*, that contributes to the posterior wall of the atria and the interatrial septum, the sino-atrial node (**SAN**), the myocardium of the sinus venosus (**SV**), pulmonary veins (**PV**) and cardinal veins (**CV**) including the coronary sinus, part of the central conduction system (**CCS**) and the dorsal mesenchymal protrusion **(DMP)**. The second heart field contribution to the venous pole is discussed in more detail later on in this review. Modified after: Crawford and DiMarco, third edition 2009[125].

1.1 Current controversies regarding definitions

1.1.1. Drainage of the primitive PV to the sinus venosus or to the LA segment?

The question whether the common PV originally is connected to the sinus venosus segment or to the LA segment has been a topic of vivid discussion over the past decades. In literature there have been advocates of an original connection of the PV to the sinus venosus[14,17-23] as well as to the LA[24-29]. A probable explanation for

these divergent opinions in the past might be the absence of adequate techniques for a distinct demarcation of the sino-atrial fold in the region of the PV, making it difficult to decide whether the PV connected to a sinus venosus or LA structure. Other limitations were the lack of availability of human embryos in good condition, (resulting in some cases in incorrect interpretations[25] that were revised later on[30]) and the comparability of stages between different species. Although recent studies using novel molecular techniques have contributed new data, this discussion is still alive and seems at least partly to be based on different interpretations of similar findings.

On the basis of scanning electron microscopic studies it was reported by some authors that the PV is connected to the LA, cranial to the systemic venous tributaries at gestational day 10 and 11 in mouse[27], at HH 15-30 in chick[28] and at Carnegie stage 12 in human embryos[29]. In these studies, the sinus venosus segment was defined as the part of the atrium that was encompassed by the venous valves. Indeed, when the left and right venous valves are taken as border structures for the sinus venosus, then the PV does not drain in this area. A limitation of this definition is however, that in very early stages, venous valves can not be distinguished yet. Furthermore, on the basis of expression of the myocardial markers Cx40 and ANF, in combination with 3-dimensional reconstructions, it has been concluded that the venous valves themselves are not completely part of the sinus venosus[26]. According to these studies, from the outset, the myocardium surrounding the PV is distinct from that lining the systemic veno-atrial junctions based on expression patterns of molecular markers[26,31]. It should be noted however in this regard, that, there is no evidence that the PV is surrounded by myocardium from the outset, as it has been demonstrated that the PV myocardial sleeve only develops from stage E11.5 and further on[32-34].

In contrast to these studies, other studies propagate an original connection of the primitive PV to the sinus venosus segment of the heart. In the mouse, at day 9.5 (Carnegie stages 11-12), the primitive PV was recognized posterior to the sino-atrial fold, its endothelium being continuous with that of the sinus venosus[22]. Also in quail embryos, from stage HH15 onwards, expression patterns of the marker HNK-1, which is expressed in the sinus venosus myocardium, suggested that the orifice of the common PV drains to the left part of the sinus venosus[18] (Fig.2). Transient

HNK-1 expression of the embryonic myocardium surrounding the PV was also seen in rat (E 13.5)[23] and human (CRL14-23mm)[35] embryos. Consistent with these findings are more recent data published by our group on CCS-LacZ expression[32] and expression of podoplanin in the developing myocardium surrounding the PVs in mice[6]. The marker CCS-LacZ is expressed in the myocardium surrounding the PV, that becomes continuous with the CCS-LacZ positive sinus venosus area of the right atrium. The posterior heart field marker podoplanin is expressed in major parts of the cardiac conduction system and also found in the myocardium surrounding the PV. It's expression is linked to the Nkx2.5 negative sinus venosus myocardium of the posterior heart field[6,36] (see section 2).

Based on the results of our studies, we favour an original connection of the primitive PV to the sinus venosus segment of the heart.

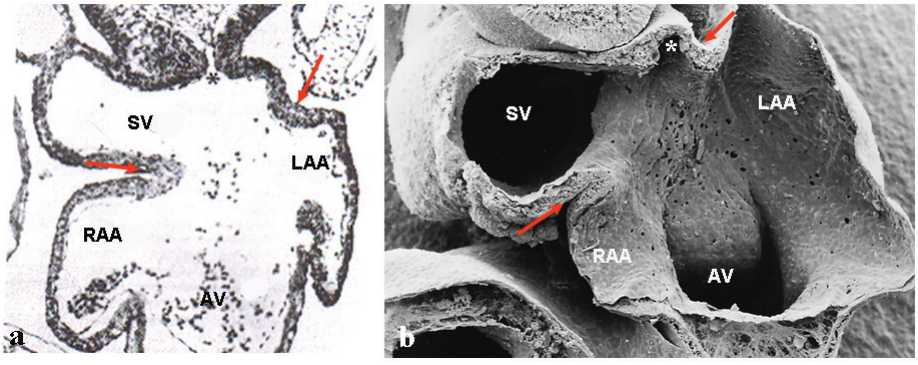

Figure 2.

a. Transverse section through the sinus venosus and left and right atrial appendage of a chick embryo (HH17).

b. Electron microscopic picture at the same level. Arrows point at the sino-atrial fold, which separates the sinus venosus from the atrial segment. The asterisk indicates the common pulmonary vein, that appears to drain in the sinus venosus. Atrio-ventricular canal (**AV),** left atrial appendage (**LAA**), right atrial appendage (**RAA**), sinus venosus (**SV**). Modified after DeRuiter et al., Anat Rec 1995[18].

1.1.2. Is there a direction of growth of the primitive PV in relation to the heart?

Already since the middle of the nineteenth century there have been different opinions about the direction of growth of the PV in relation to the heart, in other words: does the primitive PV grow out from the heart towards the lungs, or vice versa? In former reports by Buell in chick[17], Schornstein in pig[37], Auër in rat and rabbit embryos[38], and Los in human embryos[25], the stem of the PV was considered to be an outgrowth of the dorsal wall of the atrium, whereas by Brown in domestic cats[39], and by Davies in a human neonate[40], the primordium of the PV was considered to grow out of the splanchnic plexus of veins towards the heart. The splanchnic plexus is not species-specific[17,39,41]. In quail embryos, this vascular plexus arises at HH8 by differentiation of splanchnic mesodermal cells into endothelial precursor cells (vasculogenesis). The midpart of this splanchnic plexus is the earlier mentioned midpharyngeal endothelial strand. Immunohistochemical studies performed in avian embryos, using antibodies against quail endothelial cells and precursors, showed that this midpharyngeal endothelial strand from early stages on connects the endocardium of the primitive heart tube to the splanchnic vascular plexus around the foregut and the lung buds and contains the Anlage of the non-lumenized common PV[10]. This is supported by studies in mouse, chick and human embryos by Webb et al.[27-29]. This implies that, in contrast to the concepts of PV ingrowth[39,40] and outgrowth[17,25,37-39], the endothelium of the PVs has been connected to the endocardium of the heart right from the beginning[10,18].

2. Which role does the second heart field play in the development of the venous pole?

As mentioned earlier, there is increasing attention for the role of the second heart field in cardiac development. Different lineage markers have been used to study the final destination of cells in the heart[2,7,42-44]. Islet (Isl)1 is a marker for cells recruited from the second heart field and is expressed at the arterial as well as the venous pole of the heart during differentiation[2] (Fig.1d). There are several markers expressed in the posterior heart field, including, Shox2[45], Tbx18[42], Pitx2[46], podoplanin[6,7], but excluding (lack of expression of) Nkx2.5[6,46]. The posterior heart field, as a part of the second heart field, contributes to the sinus venosus, the cardiac conduction system and the

atrial septum[6,7] (Fig.1d). A subset of cardiac progenitor cells in the posterior heart field receives Hedgehog (Hh) signals, likely from pulmonary endoderm, resulting in migration of these progenitor cells through the dorsal mesocardium to form the atrial septum as well as the DMP[47]. Similar to Hh signaling, also, PDGF-signaling is known to play a role in the development of second heart field derived structures of the venous pole[48].

Next to a contribution to these aforementioned structures, in recent years, second heart field contribution to the vascular wall and myocardium surrounding the PVs[36,49] has been established.

2.1 Second heart field contribution to formation of a PV myocardial sleeve.

Several theories exist about the origin of the myocardium surrounding the PVs. The three most important processes described are: First the process of myocardialization which refers to *migration* of atrial cardiomyocytes into the extracardiac mesenchyme and extending towards the PV[34,50-52]. Between species, differences exist in the extension of the myocardial sleeve. In mice, this sleeve extends intrapulmonary[49,53], while in the chick this intrapulmonary (i.e.peripheral) myocardial extension is very limited[54]. The second process is *recruitment*, which refers to differentiation of splanchnic mesenchymal cells into cardiomyocytes[6,33,55,56] and the third process is a *combination* of the two aforementioned[46]. The recruitment of mesenchymal cells fits smoothly with earlier research in cardiac development underlining the addition of myocardium to the primary heart tube[57], from the second heart field[2,3].

In the context of the origin of myocardium surrounding the PV, as aforementioned, the myocardial markers Nkx2.5[6,46], Pitx2[46], Shox2[45], Tbx18[42] and podoplanin[6,7] are of interest for their expression in cells that contribute to the venous pole of the heart. Here, a distinction must be made between the *mesoderm* that surrounds the primitive PV in early stages (when formation of a myocardial sleeve around the PV has not taken place yet), and that is Nkx2.5 positive already in early stages; and the newly added *myocardial sleeve* that develops around the PV at stage 12.5 and further on[32-34]. Nkx2.5 expression in this newly forming myocardium around the PV initially is absent during a brief time period[6] (like in the sinus venosus myocardium), rapidly turns into a mosaic pattern with positive and negative cells, and finally is completely Nkx2.5

positive, indicating newly added myocardium that has differentiated. Compared to the cardinal veins that become Nkx2.5 positive at a later stage as well, this was considered as the result of a higher proliferation rate of myocardium of the PVs[7,49], which is in line with previous findings by other groups of a high proliferation rate of the PV myocardium as compared to the proliferation rate of the atrial myocardium[46]. Several markers expressed in putative PV myocardium, including the myocardial marker MLC2a and the posterior heart field marker podoplanin[6], are initially expressed at the left side of the dorsal mesocardium, indicating that PV myocardium is preferentially added from the left side of the posterior heart field, regulated by progenitor cells that play a role in left-right patterning, as was reported for Pitx2c[46,58,59]. Podoplanin is a transmembrane protein that was originally described in the kidney and in later studies was found to be expressed in the heart. Podoplanin knockout embryos show marked hypoplasia of the posterior heart field derived myocardium of the sinus venosus, including the sinus node and the myocardium surrounding the PVs and covering the posterior atrial wall[36]. Interestingly, in this model also a reduced number of SMCs was observed, indicating that the posterior heart field has a role in myocardial and SMC contribution at the venous pole of the heart[36,49]. This will be further addressed in section 2.2. Analogous to podoplanin mutants, chick, mouse and human embryos with DMP abnormalities caused by defective Hh or PGDF-signaling, also show hypoplastic PV myocardium, indicating a role of the DMP in proper PV development[48].

2.2 Second heart field contribution to smooth muscle cell (SMC) formation in the PVs and LA.

SMCs are another important source of cells that contribute to the vascular layer of the PVs and the inner layer of the LA[15]. It has been described that SMCs can differentiate from mesenchymal cells[60]. At the arterial pole SMCs have been described to originate from the splanchnic mesoderm, the neural crest[61] or endothelial cells[62]. At the venous pole however, not much is known about the origin of SMCs. As was implied above, several hypotheses consist about the origin of the SMCs in the LA body. Initially, SMCs were considered to migrate from the PVs towards the heart and into the LA[34]. SMCs in the LA body have also been considered to be the result of PV incorporation in the

LA[15]. The above mentioned data[60-62] have suggested differentiation as a mechanism by which the presence of SMCs in the LA can be explained, which has been supported by studies using the posterior heart field marker podoplanin[49]. In *podoplanin* knock-out embryos there is a significantly reduced myocardial cuff around the PVs as well as a reduced number of SMCs. Similar findings were reported by Bleyl et al. in embryos with DMP abnormalities[48]. These SMCs do not extend in the complete smooth-walled LA body. These findings indicate that a process of differentiation rather than incorporation is the mechanism responsible for SMC formation in the LA (Fig.3; compare with Chapter 1, Fig.2, adapted to new insights). These findings are in line with observations that have been made in the inhibitor of differentiation (Id2) mouse model. Id2 knockout mice seem to have small-sized LA bodies -probably caused by impaired or delayed PV incorporation-, while the amount of differentiated smooth muscle cells in the LA did not differ from wild type mice (Jongbloed et al., unpublished results).
These data suggest that *incorporation* and *differentiation* are two autonomous independent processes.

3. What are the current concepts on the origin of normal and abnormal PV drainage?
In the paragraphs above, we have described the origin of the PV Anlage from the midpharyngeal endothelial strand, that is connected to the heart from the beginning. During development different drainage patterns of the primitive PV can be distinguished (Fig.3a-g). We will describe here the drainage patterns according to Rammos[30], who discriminated three periods with different drainage patterns in **normal** human PV development: 1. A *peripheral period* (CRL 4.4-7mm) during which there is no open connection yet between the primitive atrium and the developing lung vessels. The mid-pharyngeal endothelial strand has not lumenized yet. The primitive lung drains via the splanchnic venous plexus into the systemic venous circulation (cardinal and umbilical veins). 2. An *intermediate period* (CRL 7-11mm) during which the lumenized common PV connects to the LA as well as to the lung plexus. The lungs can drain their blood either centrally to the LA, or peripherally to the systemic venous system. 3. A *central period* during which the only possible drainage is via the common PV to the heart (Fig.4a).

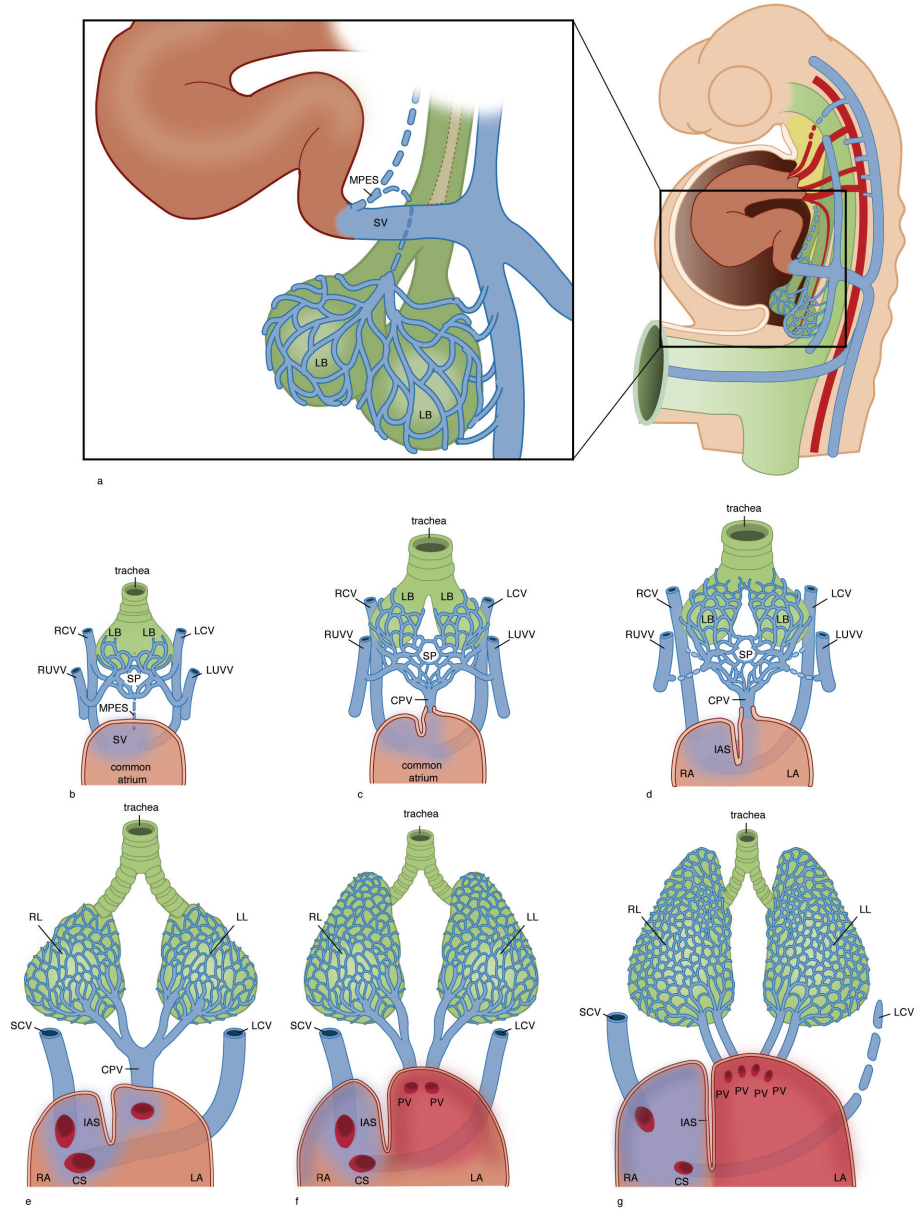
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Figure 3a-g. Schematic depiction of pulmonary vein development, adapted to new insights.

a,b. Peripheral period, lateral (a) and frontal (b) view. The splanchnic vascular network surrounds the two lung buds (**LB**) and drains to the systemic circulation by means of primitive pulmonary-to- systemic connections to the right and left cardinal veins (**RCV/LCV**) or the right and left umbilical and vitelline veins (**RUVV/LUVV**). In the region of the heart, this splanchnic plexus (**SP**) is connected to the sinus venosus segment of the heart (**SV**, blue) by means of the midpharyngeal endothelial strand (**MPES**), which is the remaining part of a strand of endothelial cells that initially runs from the arterial to the venous pole in the dorsal mesocardium.

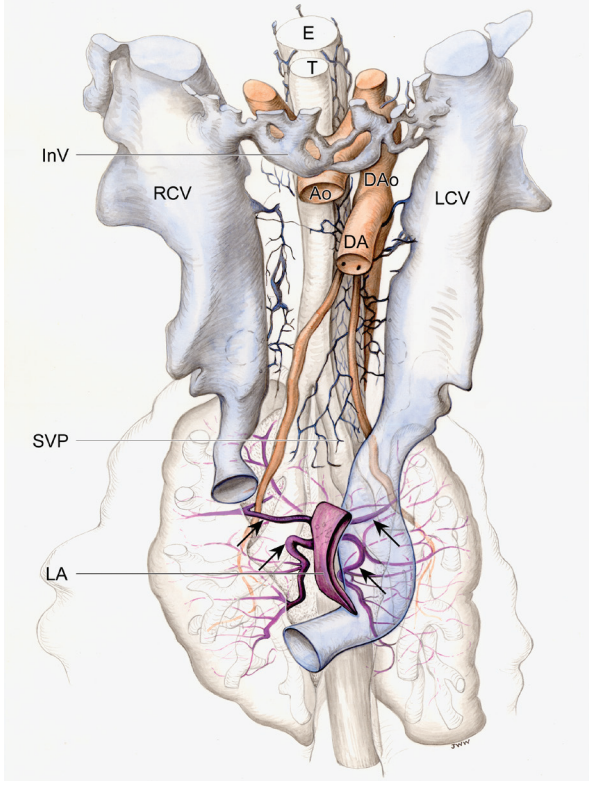
c. Intermediate period. When the endothelial strand, which is the anlage of the future common pulmonary vein (**CPV**) lumenizes, the splanchnic plexus can drain its blood to the systemic circulation as well as to the heart. Although atrial septation has started, the CPV still drains to the sinus venosus segment that is connected to a common atrium.

d. Central period. The CPV grows and dilates, becoming the main route for drainage of pulmonary venous blood. The primitive pulmonary-to-systemic connections are not necessary anymore and regress. After atrial septation has completed (**IAS**), the part of the sinus venosus containing the CPV is placed to the left, becoming part of the left atrium (**LA**).

e. The CPV has bifurcated, so that the right and left lung (**RL/LL**) drain to the heart by means of two left and two right pulmonary veins. The right cardinal vein becomes the superior caval vein (**SCV**) and the left cardinal vein gives rise to the coronary sinus (**CS**), both connecting to the sinus venosus part of the right atrium (**RA**).

f,g. By incorporation of the CPV, initially, one right and one left CPV drain to the LA (f). After incorporation has completed, four separate pulmonary vein (**PV**) ostia can be identified on the inner side of the LA (g). Independent on PV incorporation, smooth muscle cells (red) can be identified in the LA as a result of differentiation. The extracardiac part of the LCV regresses, becoming the so-called ligament of Marshall.

A



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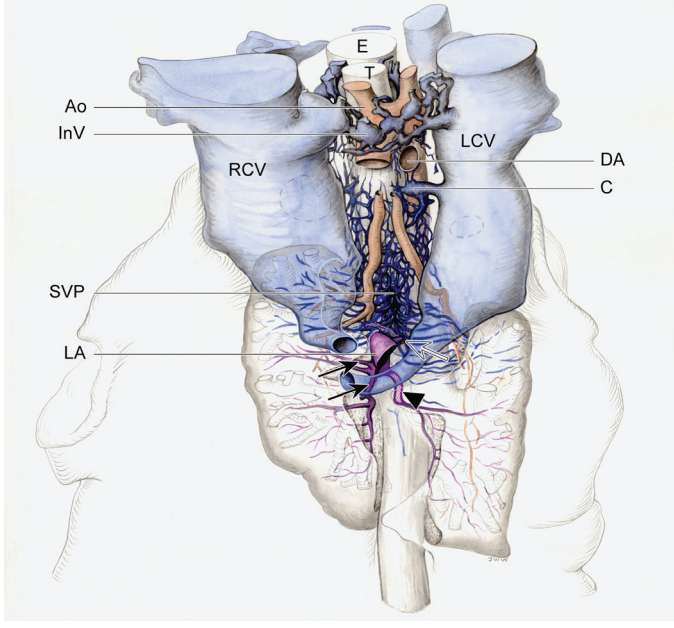

Figure 4a,b.

a. 3-D Reconstruction of the thoracic contents of a normal human embryo of 18 mm crown-rump length (approximate gestational age 8 weeks; Carnegie stage 14). Frontal view, after resection of the heart including part of the left atrial dorsal wall. Four pulmonary veins (arrows**)** are connected to the lung region and to a short common pulmonary vein that has an open connection with the left atrial (**LA**) dorsal wall. At this stage there is no connection anymore of the splanchnic plexus (**SVP**) to the lungs, but still very small communications of the SVP with the right (**RCV**) and left cardinal veins (**LCV**) exist caudal to the innominate vein (**InV**). Aorta (**Ao**), descending aorta (**DAo**), ductus arteriosus (**DA**), esophagus (**E**), trachea (**T**).

b. 3-D Reconstruction of an abnormal human embryo of 18mm crown-rump length (approximate gestational age 8 weeks; Carnegie stage 14). Frontal view, after resection of the heart, including part of the left atrial dorsal wall. Posteriorly to the left atrial (**LA**) dorsal wall three individual pulmonary veins, originating from a common pulmonary vein run towards the lungs. On the left side the left upper PV is lacking (open arrow), the left lower PV does not contain blood (black arrowhead). The two right PVs (black arrows) are normal. The splanchnic vessels (**SVP**) extend a great deal further than in the normal embryo and with numerous connections to both lungs. A large communication analogous to the vertical vein (**C**) exists between the splanchnic plexus and the left cardinal vein (**LCV**). Aorta (**Ao**), ductus arteriosus (**DA**), esophagus (**E**), innominate vein (**InV**), right cardinal vein (**RCV**), trachea (**T**).

The primitive connections of the lung to the systemic veins are not necessary anymore at this stage and regress[30,63] by a process called remodeling, analogous to the developmental processes at the arterial pole[64]. Persistence of pulmonary-to-systemic communications is the likely substrate of *extracardiac* anomalous PV connections, probably related to abnormal development of the common PV (TAPVC) or one of its branches (PAPVC)[30,63,65]. On the other hand, *cardiac* anomalous PV connections seem to result from absent or impaired mesenchymal contribution to atrial septation[14,48].

Insights in **abnormal** PV development historically have mainly been based on post-mortem studies leading to classifications and tentative embryological explanations of the encountered anomalies[63,66]. Total anomalous PV connection was explained by a shift of the common PV with regard to the atrial septum, leading to a complete connection to the right atrium, or, when the PV connection with the atrium was lost, to the use of the nearest systemic vein for definitive drainage[66].

Abnormal PV development can be based on various processes that take place at different developmental stages and at different anatomical levels. These are summarized in Table 1. These processes lead to different congenital heart anomalies (clinical entities), that we will subsequently describe in relation to embryonic development.

Noteworthy is that, regularly, inflow and outflow tract abnormalities are found to co-exist[67,68]. Since the second heart field contributes to incorporation of mesenchymal cells at both the venous as well as the arterial pole of the heart[2], second heart field impairment may be the underlying cause for this co-existence.

3.1. Clinical entities (also see Table 1).

3.1.1 Total anomalous pulmonary venous connection (TAPVC).

In this condition there is no open connection to the LA because of non-lumenization of the midpharyngeal endothelial strand (PV Anlage) or atresia of an initially lumenized common PV. If the latter takes place at a late embryonic stage, the condition is lethal, with 4 PVs draining to a blind ending confluence (Table 1). Usually, all the PVs drain to the systemic venous circulation (extracardially), but rarely, they have a cardiac connection as a consequence of impaired mesenchymal contribution to atrial septation[14,48]. Four types of TAPVC can be discriminated: A *supracardiac* type accounting for 45% of cases; an *infracardiac* type for 25%; a *cardiac* type for 25%; and a *mixed* type for 5%[69].

The supra- and infracardiac type both belong to the extracardiac group of TAPVC which means that, in absence of an open connection to the LA, the nearest extracardiac systemic vein is used for PV drainage. The preserved primitive pulmonary-to-systemic connection is represented by the so-called vertical vein. In *supracardiac* TAPVC the (left) vertical vein usually connects to the innominate (brachiocephalic) vein, less often to the superior caval vein -at the cavo-atrial junction-, and rarely to the azygos vein. These systemic veins are representatives of the peripheral part of the former cardinal veins. The commonest sites of connection in patients with *infracardiac* TAPVC are the portal vein (65%) and the embryonic ductus venosus, that has persisted in these patients. Less common are connections to the gastric vein, the hepatic veins and the inferior caval vein. These systemic veins originate from the former umbilicovitelline veins[11].

Tabel 1. Abnormal pulmonary vein development in relation to anatomical level, time and clinical entities. On the left side, the developmental impairments and different anatomical levels are depicted, and on the right the clinical entities that result from this abnormal development. These clinical entities are described in the text in section 3.1. Abbreviations: atrio(ventricular) septal defect (**A(V)SD**); mid-pharyngeal endothelial strand (**MPES**); partial anomalous PV connection (**PAPVC**); pulmonary vein(s) (**PV(s)**); total anomalous PV connection (**TAPVC**); second heart field (**SHF**).

Anatomical level	Anomaly	Developmental impairment	Time relation (related to atrial septation)	Pulmonary-to-systemic connections	Clinical entity
common PV/ MPES	absence	absent connection PV-LA (absent MPES)	before	will persist	TAPVC (extracardiac type)
	atresia	non-lumenization of the MPES (PV Anlage)	before	will persist	TAPVC (extracardiac type)
	stenosis	secondary stenosis of an initially lumenized common PV	during/after	will disappear	cor triatriatum
	atresia	secondary obliteration of an initially lumenized PV	after	have disappeared	TAPVC (lethal)
first or second PV bifurcation	atresia	non-lumenization of 1 or more individual PV(s)/tributaries	during	will persist	PAPVC (extracardiac type)
	stenosis	secondary stenosis of an initially lumenized individual PV	during/after after	will disappear have disappeared	congenital PV stenosis acquired PV stenosis
	atresia	obliteration of 1 or more initially lumenized PVs	after	have disappeared	(solitary) PV atresia
veno-atrial junction	variant number of PVs	abnormal PV incorporation	after	have disappeared	
	<4	incomplete			unilateral common PV ostium*
	>4	extreme			>4 PV ostia*
dorsal mesenchymal protrusion	hypoplasia	abnormal mesenchymal contribution from SHF	during	will disappear	sinus venosus defect with PAPVC (cardiac type) A(V)SD
	absence	absent mesenchymal contribution from SHF	during	will mostly disappear	TAPVC (cardiac type) A(V)SD

* Note: not regarded as pathological but rather as anatomical variations

In the *cardiac* type of TAPVC, the PV confluence drains directly to the right atrium, or more often, directly to the coronary sinus. Drainage to the coronary sinus is the result of persistence of pulmonary-to-systemic venous connections to the left common cardinal vein (which becomes the coronary sinus). The etiology of the direct connection to the right(-sided) atrium is a topic of discussion since this condition is very rare in case of situs solitus (i.e. in normal atrial arrangement), and almost always associated with (right) atrial isomerism[70]. Leftward displacement of the atrial septum has been proposed[71], although this was also considered a secondary phenomenon to the abnormal expansion of the developing right atrium due to incorporation of the PVs[72]. The fact that this condition is more often seen in case of right atrial isomerism suggests that inhibition of left-sided structures is a more probable explanation. This would fit in the concept of a bilateral Anlage (left and right pulmonary pit) with development of the right pulmonary pit and inhibition of the left part as was suggested by Männer[72]. Such a bilateral Anlage was never identified by our group. We hypothesize that in case of right atrial isomerism, the unilateral Anlage or common PV connects to one of the "right" atria or maintains a midline position, connecting to both atria, since atrial septation is also disturbed in this condition[73]. Another component that might play a role in the onset of cardiac TAPVC to the right atrium is the dorsal mesenchymal protrusion, which role in atrial septation and leftward displacement of the PV has already been established[12,13]. Defective development of this dorsal mesenchymal protrusion might lead to a rightward shift of the PV, resulting in PV drainage to the sinus venosus part of the right atrium[14,48].

The *mixed* type usually is a combination of the cardiac and supracardiac type of TAPVC, in which the left PVs drain to a vertical vein and the right PVs to the coronary sinus, as already explained. Also this type finds its origin in an absent or atretic PV Anlage.

3.1.1.1. TAPVC and immunohistochemical findings.

On the basis of comparative immunohistochemical studies of the LA dorsal wall and the PVs between normal human hearts and hearts with TAPVC, we know that, in contrast to normal hearts, in hearts with TAPVC a. PVs have no myocardial sleeve b. the LA has no inner smooth muscle cell layer and c. the LA body is very small and thin-walled because of non-compact hypoplastic myocardium[15,49,67].

a.: *Lack of a PV myocardial sleeve in TAPVC*. It is noteworthy that in this condition, also the vertical vein, i.e. the persisted pulmonary-to-systemic connection, lacks myocardial covering. In both normal hearts and hearts with situs inversus, the PVs are normally myocardialized which means that myocardialization is not dependent on left-sidedness in the body but probably on left second heart field signaling. In hearts with partial anomalous pulmonary venous connection (PAPVC), only the normally connecting PVs are myocardialized, from which we hypothesized that a connection of a PV to the LA is necessary for myocardialization to occur[67] (Fig.5).

The fact that left-right patterning seems to play a role in myocardialization of the PVs is supported by studies using the differentiation marker Pitx2[46,58,59,74-76]. Pitx2 is a homeodomain transcriptional protein, which Pitx2c isoform is exclusively expressed in the heart, driving asymmetrical cardiac morphogenesis. Initially, Pitx2c is asymmetrically expressed in the left lateral plate mesoderm and subsequently in the cardiogenic precursors of the left second heart field, supplying both the venous[46] and the arterial pole[77]. At the venous pole, Pitx2c is present in the posterior heart field in mesenchymal cells, that will later on differentiate into myocardial cells. PVs of Pitx2c mutants have no myocardial sleeve[46]. Pitx2 represses the transcriptional pathway of right atrial identity, also suppressing the SA node transcriptional program on the left side[75]. So, Pitx2c mutants show right atrial isomerism[46]. Since both Pitx2c mutants and TAPVC patients lack a PV myocardial sleeve, there might be a connection between Pitx2c and TAPVC. This possibility is supported by the fact that right atrial isomerism as well as TAPVC frequently go together with asplenia[78]. Until now, a relation between Pitx2c and asplenia, was not reported in literature.

b.: *Lack of LA inner smooth muscle cell layer in TAPVC*. As mentioned above, *differentiation* rather than *incorporation* is the mechanism responsible for SMC formation in the LA[49]. The podoplanin knockout mouse model, shows an impaired formation of SMCs in the LA body, similar to human TAPVC[67], suggesting that lack of a SMC layer in TAPVC is also based on a differentiation impairment.

c.: *Small LA size*. The small size of the LA in TAPVC is the consequence of non-incorporation of the PVs. In fact, the LA body has not fully developed and the main part of the LA is formed by the LA appendage. Additionally, reduced hemodynamic fetal flow may contribute to the small size of the LA.

Non-compact, hypoplastic LA myocardium in TAPVC. Impaired formation and differentiation of posterior heart field derived myocardial and smooth muscle cells in the PVs and the LA dorsal wall are findings both observed in knockout models of PHF markers and in TAPVC, suggesting that impairment in the posterior heart field contributes to TAPVC. A developmental explanation for the spongious LA myocardium in TAPVC may be the impaired formation of epicardium derived cells (EPDC's) and altered epicardial-myocardial interaction, which are known to be responsible for abnormal atrial and ventricular myocardium[7,79].

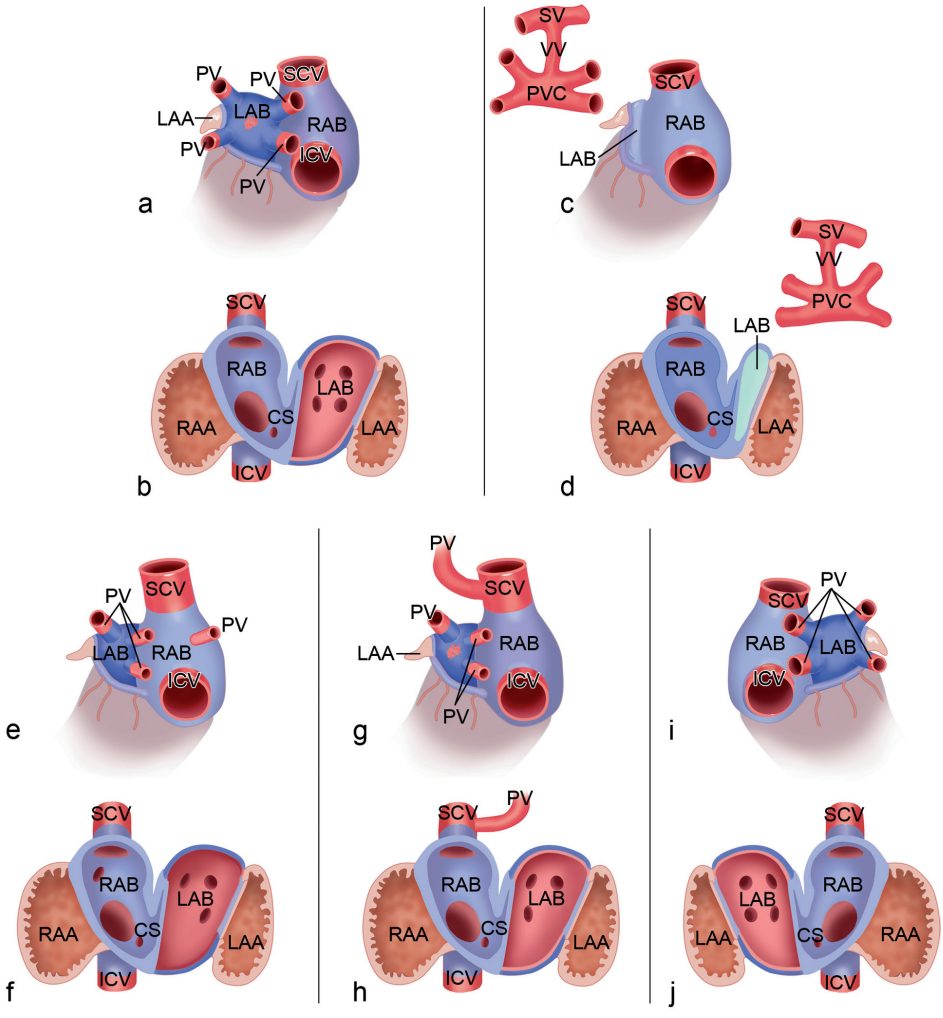

Figure 5a-j.

Schematic depiction of outer (a,c,e,g,i) and inner (b,d,f,h,j) side of the atrial chambers and the pulmonary veins (**PV**) in normal hearts (a,b), hearts with total anomalous pulmonary venous connection (TAPVC;c,d**),** hearts with (extra)cardiac partial anomalous pulmonary venous connection (PAPVC; e-h) and hearts with situs inversus (i,j).

a,b. Normal heart. a. The left atrial body (**LAB**) and pulmonary veins (**PV)** are covered by myocardium derived from the posterior heart field (***dark blue****).* In the area between the PVs the myocardium can be discontinuous or absent. The right atrial body (**RAB**) is covered by sinus venosus myocardium (***light blue****).* b. The inner aspect of both LAB and RAB is smooth-walled. In the LAB vessel wall tissue (***red***) is found. The left and right atrial appendages (**LAA, RAA**) consist of trabeculated myocardium (***brown****)*.

c,d. TAPVC, extracardiac type. The PVs drain via a vertical vein (**VV)** into a systemic vein (**SV**) and are not covered by myocardium. The LAB consists of smooth-walled hypoplastic myocardium, derived from the posterior heart field. Because of the absence of a PV connection and incorporation into the LA, the LAB is small. Moreover, the LAB does not contain vessel wall tissue, probably because of impaired differentiation of smooth muscle cells.

e,f. PAPVC, cardiac type. The abnormally connected PV drains directly into the right atrial body (**RAB**), but (as in the normal situation) no vessel wall tissue is found in the RAB. In contrast to the PVs that are connected to the LAB, the abnormal PV is not covered by myocardium.

g,h. PAPVC, extracardiac type. The abnormally connected PV drains (in)directly into the superior caval vein **(SCV)** and is, similar to the cardiac type of PAPVC, not covered by myocardium. The LAB and its incorporated PVs are myocardialized and, also comparable to the normal heart, vessel wall tissue is present in the LAB.

i,j. Situs inversus. The LAB is located on the right side of the heart and receives the four PVs, which are covered by myocardium. In the LAB vessel wall is found parallel to normal PV incorporation.

Coronary sinus (**CS**), inferior caval vein (**IVC**), pulmonary venous confluence (**PVC).** Modified after Douglas et al., Int J Cardiol 2009[67].

3.1.1.2.

TAPVC and genetics. Maternal exposure to environmental teratogens as lead, paint or pesticides have been described to cause a familial susceptibility for TAPVC, mostly in the presence of a positive family history of cardiac and non-cardiac malformations[80,81]. Until now, familial incidence of TAPVC appeared not to be related to ethnic background and was seen in siblings, twins and cousins of the same family with a male preponderance[80,82]. Moreover, TAPVC has been described in association with genetic syndromes, such as the Holt-Oram syndrome, cat eye syndrome and craniofacial and skeletal dysmorphias[80,83], so that the question has raised whether there is a heritable component. Since then, various genetic hypotheses have been proposed. Multifactorial inheritance[84] seemed unlikely in families with two or more positive members, as well as autosomal recessive inheritance in the absence of consanguinity in positive families[85]. More likely is an X-linked inheritance[86] and autosomal dominance with variable expressivity and incomplete penetrance[83,87]. As beforementioned, in 70-80% of patients with the asplenia syndrome TAPVC is the most characteristic heart defect[78]. Both of these laterality disorders are suggested to be different expressions of a single genetic disorder[78]. Because of scarcity of multigeneration TAPVC families, identification of candidate genes for TAPVC is difficult. Until now, two candidate genes have been proposed. The *TAPVR1* gene, playing a role in vasculogenesis, maps to chromosome 4q12, which centromeric region contains receptor tyrosine kinase genes as kinase domain receptor (KDR)[88]. Recently, the *TAPVR1* susceptibility locus was specified to the PDGFRA-KIT intergenic interval of chromosome 4q12[48]. The *ANKRD1/CARP* gene, encoding a cardiac transcriptional regulator, was localized proximally to the breakpoint of a previously found translocation site on chromosome 10[89,90]. Investigations to limit the *TAPVR1* gene interval and specify the role of the *CARP* gene in the pathogenesis of TAPVC are on their way.

Another unsolved issue is the etiology of primary PV stenosis. Until now, in literature, a genetic basis for this entity has not been reported.

3.1.2. PAPVC.

In this condition, two or more PVs connect normally to the LA and two or less PVs do not lumenize or become atretic after initial lumenization. The corresponding lung(segment) drains its blood to the systemic venous circulation, usually extracardially by means of persistence of connections to the superior caval vein (Fig.4b) or to the inferior caval vein (Scimitar syndrome[91]). However, two or more PVs can also connect cardially, i.e. to the sinus venosus part of the right atrium, which is the consequence of defective mesenchymal contribution to atrial septation, obligatory coexisting with a sinus venosus defect[14] (section 3.1.5).

3.1.3. Cor triatriatum.

This condition is based on stenosis of the common PV, leading to non-incorporation (Table 1). The PVs connect to a separate chamber that either drains to the left or (rarely) to the right atrium, dependent on initially normal or abnormal connection of the common PV[92]. The LA body is very small due to absence of PV incorporation. Moreover, cor triatriatum in combination with left or right atrial isomerism can be found[70].

3.1.4. PV stenosis.

This condition can be congenital or acquired[93].

3.1.4.1 Primary PV stenosis.

This is the consequence of abnormal remodeling of individual PVs. It can be found in hearts with normally[94] as well as abnormally connected PVs[95]. The stenosis can be present in either all the PVs, or in individual PVs, localized either at the veno-atrial transition or more peripherally towards the lung. In hearts with normal PV connection, the PV return to the LA will be obstructed, whereas in case of anomalous PV connection, pulmonary venous return to the right atrium is obstructed. Both situations can lead to uni- or bilateral pulmonary hypertension on the long-term.

3.1.4.2. Acquired PV stenosis.

Whereas the exact etiology of congenital PV stenosis is unknown, acquired PV stenosis can be the consequence of radiofrequency ablation[96], surgery[97,98] or by a so-called pulmonary veno-occlusive disease[99,100]. Pulmonary veno-occlusive disease is characterized by extensive and diffuse occlusion of PVs by fibrous tissue. Usually the (eccentric) intimal thickening involves venules and small veins but, occasionally, also larger veins are involved. Clinically, patients have pulmonary arterial hypertension without elevation of the pulmonary arterial wedge pressure. Infectious, genetic, and toxic agents as well as a thrombotic diathesis and autoimmune disorders may be responsible for the etiology of pulmonary veno-occlusive disease[99,100].

3.1.5. Sinus venosus superior or inferior defect.

Normally, during atrial septation, mesenchyme of the dorsal mesocardium protrudes in the dorsal wall of the primary atrial segment (dorsal mesenchymal protrusion[12] or spina vestibuli[13]) being responsible for leftward displacement of the common PV. When this mesenchymal contribution to atrial septation from the posterior heart field is impaired, a so-called sinus venosus defect can develop, often resulting in anomalous connection of one or two right PVs (PAPVC) to the right-sided sinus venosus: the sinus venarum of the right atrium, one of the atrio-caval junctions or the superior or inferior caval vein[14]. Similarly, severe hypoplasia of this DMP (may) lead to TAPVC to the sinus venosus part of the right atrium (cardiac type)[48].

3.1.6. Variant number of PVs.

This condition is based on variations in PV incorporation which can be caused by PV stenosis at the veno-atrial junction. Incomplete PV incorporation can result in a unilateral common PV ostium, mostly seen on the left side[15,16,101]. Extreme PV incorporation can lead to more than four PV ostia, more frequently seen on the right side as an additional right PV draining the right middle lung lobe[16] (Table 1). In our opinion, both common ostia and additional PVs should not be regarded as an abnormality but rather as normal anatomical variations.

4. How is PV development related to arrhythmogenesis?

4.1. Normal PV development.

The majority of atrial paroxysms of arrhythmia start with earliest activation at the PV ostium or within the PVs[1]. Characteristics of the PV myocardial sleeves are held responsible for this potential to generate arrhythmias. These myocardial sleeves are thickest at the veno-atrial junction and become thinner peripherally, towards the lung[101,102]. The myocardial architecture in normal PVs is highly variable[102]. Usually, the sleeves are better developed in the superior than in the inferior PVs, specifically at the inferior side of the superior PVs and at the superior side of the inferior PVs[53,102]. At the endocardial (inner) side of the vessel a circumferentially oriented myocardial fibre arrangement is described, while at the epicardial (outer) side this arrangement is longitudinally[103]. These transitions in fibre arrangement in combination with interpositions of fibrous tissue, separating myocardial fibres, can lead to activation delay and conduction block, so creating a milieu for reentry[102,104]. Another predisposing factor to reentry in normal development may be the myocardial discontinuity in the area between the PVs, as well as in the transitional zone between the LA body and the LA appendage[15].

Next to these architectural characteristics of the PVs, several other mechanisms may contribute to the arrhythmogenic capacities of the PVs. Morphologic studies have described cells resembling pacemaker cells in the myocardium surrounding the PVs[105,106]. These observations would explain the possible independent pacemaker activity that was observed in the PVs in otherwise mechanical silent hearts of rabbits and cats, described as early as 1874. It is tempting to speculate that the presence of node-like cells would also be responsible for the initiations of paroxysms of atrial arrhythmias described later on[1].

In this light, it is interesting to note that several immunohistochemical and molecular markers related to the developing cardiac conduction system are (transiently) expressed in the myocardium surrounding the developing PV, including HNK-1, CCS-LacZ, and podoplanin, suggesting that areas related to the occurrence of arrhythmias correspond to areas derived from the embryonic cardiac conduction system[32,35]. It was hypothesized that embryonic remnants or re-expression of embryonic genes could be related to clinical arrhythmias in neonates and adults, respectively.

These findings are strengthened by several electrophysiological studies that have demonstrated specific electrophysiological capacities of the PVs as compared to the atria[107-109], with a reduced resting membrane potential, action potential amplitude, a smaller phase 0 upstroke velocity and a shorter duration of the action potential resulting in shorter refractory periods and slowed conduction. These cellular characteristics in combination with the anisotropic arrangement of the PV myocardial fibres favour the occurrence of reentry and probably the maintenance of initiated arrhythmias[103,104].

Furthermore, spontaneous or enhanced pacemaker activity (abnormal automaticity) in the PVs has been reported[110,111].

Recently, human genetics studies identified two sequence variants on chromosome 4q25 that were strongly associated with increased risk for atrial fibrillation[112]. The gene closest to these sequence variants is Pitx2, which is critical for the development of PV myocardium, making Pitx2 a likely candidate locus for atrial fibrillation[112,113]. Moreover, the presence of a sequence variant increased the risk for both early and late recurrence of atrial fibrillation[114].

4.2. Abnormal PV development.

In patients with TAPVC, conduction abnormalities, including sinus node dysfunction (sick sinus syndrome, tachy- and bradyarrhythmia) are reported[115-117]. In hearts with TAPVC, the PVs lack a myocardial sleeve and the myocardium of the LA dorsal wall is hypoplastic, with discontinuities[67]. The combination of hypoplastic myocardium and sinus node dysfunction could be related to an abnormal contribution from the posterior heart field, as studies in podoplanin knockout mice demonstrate both hypoplastic and discontinuous myocardium surrounding the PVs, as well as an hypoplastic sino-atrial node[36,49]. Hypoplasia of the sinus node in TAPVC would provide a plausible explanation to the observed conduction disorders in TAPVC. However, further research is needed to reveal a possible co-existence of pulmonary venous myocardial hypoplasia and hypoplasia of the sinus node in TAPVC[67]. Although myocardial discontinuities of the LA dorsal wall could form a substrate for generation of arrhythmias in TAPVC hearts, total absence of a PV myocardial sleeve may explain the low incidence of atrial arrhythmias originating from the PV observed in this patient group[117].

After surgical repair of TAPVC, compared to other atrial surgery, significant atrial arrhthymias as atrial flutter and reentry tachycardias are uncommon, although sinus node dysfunction (sick sinus syndrome, tachy- and bradyarrhythmia) has been reported[115-117].

In general, in this patient group, arrhythmias are asymptomatic and not related to the type of surgery[118] (and Douglas et al. unpublished results). Therefore, it is arguable whether the reported arrhythmias are related to the operation or to the pathological findings of TAPVC itself. Given the possible relation with sinus node dysfunction, for this patient category a careful follow-up including Holter registration is warranted.

5. What are consequences of the various concepts of PV development on clinical management?

For surgical correction of TAPVC, the PV confluence has to be connected to the LA. This can be performed by an anteroposterior right-to-left atrial incision after median sternotomy, or by a single LA incision after a left lateral thoracotomy. Subsequently, an anastomosis is made between the PV confluence and the LA after ligation of the former connection to the systemic circulation, the vertical vein. Since the LA in TAPVC is very small[67], in the past, surgeons have tried to augment the LA with a patch, however this appeared to be the substrate for arrhythmias[95,98]. In hearts with TAPVC, the PVs have no myocardial layer resulting in an enhanced susceptibility for internal and environmental influences making them more prone to congenital and acquired PV stenosis[93,96], which is still one of the main unravelled problems. For postoperative PV stenosis at the site of the anastomosis surgeons successfully developed and applied so-called sutureless techniques, leaving the PVs untouched[119,120]. Primary PV stenosis is still a major cause of death in this patient group[97,98].

In PAPVC, frequently presenting as part of a sinus venosus defect, drainage of the abnormally connecting PVs has to be “rerouted” to the LA which usually is performed by an atrial patch that is fixed around the abnormally connecting PV ostia, so directing drainage to the LA. If this is anatomically not possible, the PVs have to be reimplanted in the LA. Surgery of one abnormally connecting PV usually will not be performed because of absence of hemodynamic complaints and consequences.

In hearts with normal as well as abnormal PV connections, myocardial discontinuities are found in the LA dorsal wall, which can form a substrate for the onset of arrhythmias[15,67]. Interventional cardiologists performing ablational techniques for these arrhythmias should be aware of the increased vulnerability to damage of these areas in the LA where a myocardial wall is lacking, specifically in the proximity of the esophagus. Recent studies have emphasized the risk of atrio-esophageal fistula due to the application of radiofrequency current at this site[121,122]. Lower dosage of radiofrequency current is therefore recommended when ablating in this area.

Future perspectives and conclusions.

In this review normal and abnormal PV development is described and updated based on the current literature. We have described how normal PV development starts with lumenization of a midpharyngeal endothelial strand, that provides a connection to the endocardium of the heart right from early development on. Although discussion on whether the primitive PV after lumenization drains into the atrial segment or the sinus venosus segment of the heart is still ongoing, based on the results of our studies, we favour an original connection of the primitive PV to the sinus venosus segment of the heart. From the posterior heart field, as part of the second heart field, SMCs and myocardial cells are contributed to the PV and LA dorsal wall. Normally, pulmonary-to-systemic connections disappear during development. Abnormal PV development may lead to persistence of pulmonary-to-systemic connections which is a substrate of anomalous PV connection(s), related to different clinical entities. In normal PV development the myocardium surrounding the PVs has specific characteristics creating a milieu for arrhythmias and reentry. In abnormal PV development, we hypothesize that rhythm- or conduction disorders might be explained in some patients by an insufficient contribution of posterior heart field myocardium to the sinus node and LA dorsal wall.

Surgical procedures have been developed to correct anomalous PV connections and to manage surgery-related complications.

There are still a number of topics that remain unresolved and have to be addressed by future research. Additional cell tracing studies are necessary to unequivocally determine the origin of SMCs in the PVs and LA.

With regard to arrhythmia treatment, currently mainly conventional anti-arrhythmic therapies and ablational strategies have been applied to control atrial arrhythmias. Development of new ablation techniques, and the expanding possibilities to integrate imaging techniques with electrophysiological mapping data and genetic data will improve patient selection and selectivity of application sites in order to decrease the recurrence rate of the arrhythmias[114,123]. Acquired PV stenosis as a consequence of surgical correction or resulting from ablation therapy needs further improvement of surgical techniques and refinement of useful energy sources. Pre-interventional imaging by echocardiography or computed tomography may also helpful in localizing areas at risk and preventing this potentially serious disorder.

The underlying mechanisms leading to primary PV stenosis are still unknown. Future investigation of a potential genetic basis is an important next step in understanding and manipulating this serious disease.

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