Molecular Cardiology

Three-Dimensional and Molecular Analysis of the Venous Pole of the Developing Human Heart

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Background—Various congenital malformations and many abnormal rhythms originate from the venous pole of the heart. Because of rapid changes during morphogenesis, lack of molecular and lineage data, and difficulties in presenting complex morphogenetic changes in the developing heart in a clear fashion, the development of this region in human has been difficult to grasp.

Methods and Results—To gain insight into the development of the different types of myocardium forming the venous pole of the human heart, we performed an immunohistochemical and 3-dimensional analysis of serial sections of human embryos ranging from 22 through 40 days of development. Three-dimensional models were prepared in a novel interactive portable format providing crucial spatial information and facilitating interpretation. As in the mouse, the systemic venous myocardium expresses the transcription factor TBX18, whereas the pulmonary venous myocardium expresses NKX2–5. In contrast to the mouse, a systemic venous sinus is identified upstream from the atrial chambers, albeit initially with nonmyocardial walls. From the outset, as in the mouse, the pulmonary vein empties to a chamber with atrial, rather than systemic venous, characteristics. Compared with the mouse, the vestibular spine is a more prominent structure.

Conclusion—The similarities in gene expression in the distinctive types of myocardium surrounding the systemic and pulmonary venous tributaries in man and mouse permit extrapolation of the conclusions drawn from transgenic and lineage studies in the mouse to the human, showing that the systemic and pulmonary venous myocardial sleeves are derived from distinct developmental lineages. (Circulation. 2010;122:798-807.)

Key Words: heart ■ human development ■ pulmonary vein

The venous pole of the human postnatal heart has a complex morphology, reflecting its development from several components.1,2 It comprises the union of the caval veins with the right atrium (sinus venarum) and the connection of the pulmonary veins to the developing left atrium.3,4 Various congenital malformations such as the sinus venosus interatrial communications, anomalous connection of the pulmonary veins, division of the atrial chambers,5 and many abnormal rhythms6,7 are known to originate from the venous pole.

Clinical Perspective on p 807

Descriptions of the development of the venous pole remained controversial,2,8–11 not only because of difficulties in describing the rapid temporal changes in morphology but also because of inconsistent use of terminology, with the same structures described in different ways by different investigators.4 This holds particularly true for studies on human development, for which data on gene expression are virtually nonexistent. In experimental animals, analysis of lineage and gene expression allowed distinction of the components of the atrial chambers and provided accuracy in defining the boundaries between the bodies of the atrial chambers, their appendages and vestibules, and the systemic and pulmonary venous tributaries.1,2,12,13 Connexin40, which forms high-conductance gap junctions, specifically marks the differentiation of the chamber myocardium, distinguishing it from the primary myocardium.2 Lineage studies have demonstrated distinct origins of the myocardial sleeves clothing the systemic and pulmonary venous returns, having distinct transcriptional profiles. The sinus muscle expresses transcription factor Tbx18 but not Nkx2-5, as opposed to the myocardium surrounding the pulmonary veins, which expresses from the outset Nkx2-5 but not Tbx18.12,13 These different signatures of transcription factors control, in turn, both regions differently. To date, these findings have been extrapolated to the development of the human heart. In the absence of reference molecular data for human cardiac morphogenesis, it remained uncertain whether this extrapolation is justified.
In this study, therefore, we have delineated the molecular phenotypes of the component parts of the venous pole in the early stages of human cardiac development. To facilitate the appreciation of the complex relations between different structures at the venous pole, we have presented our results in a novel 3-dimensional interactive fashion, permitting the reader to form his or her independent opinion of our findings (see the online-only Data Supplement).

Methods

Collection, Staging, and Tissue Processing of Embryos

Human embryos were collected from medically induced abortions performed for social reasons at the Gynecology Department of the Tartu University Hospital, Estonia. Collection and use of the human embryonic material for research presented here were approved by the Medical Ethics committees of the universities of Tartu, Estonia, and Amsterdam, the Netherlands. As soon as was feasible, the aborted tissues were fixed in 4% paraformaldehyde solution. Embryos were then examined under a stereomicroscope, investigated for gross anomalies, and photographed. We graded them in the Carnegie series of developmental stages on the basis of external landmarks such as limbs, retinal pigment, and general size of the embryo.14 After further fixation overnight, embryos were dehydrated in graded ethanol series, immersed in butanol, and embedded in Paraplast. We included only embryos considered normal.

Immunofluorescence Staining and Reconstruction

Standard procedures were used to visualize antibody binding, as detailed in the online-only Data Supplement, which also outlines the use of Amira software (Visage Imaging) to make the reconstructions and the export of the 3-dimensional (3D) objects into interactive portable document format. The color codes for the structures shown in the 3D reconstructions, along with our chosen abbreviations, are shown in Figure 1. The domain of connexin40 expression, labeled in pink, is shown only in the atria, not in the ventricles.

Technical Limitations

Our study has a number of limitations that derive from the use of human embryonic material such as the small number of available specimens and the variable time preceding fixation of the material. The limited number of available embryos did not permit complete optimization of the staining protocol or assessment of biological variation between specimens at similar developmental stages. Staining proved to be reproducible, albeit with variations in intensity between embryos for some antibodies.

Results

The reader is encouraged to read the results along with the interactive PDF file in the online-only Data Supplement.

Myocardial Markers and General Histology

In all but 3 embryos, we used troponin I as myocardial marker. This antibody recognizes both cardiac (TNNI3) and skeletal isoforms of troponin I. The slow skeletal isoform (TNNI1) is the predominant isoform in the human fetal heart.15 Because sarcoplasmic reticulum calcium ATPase2 (SERCA2a) is one of the earliest markers of cardiomyocyte differentiation,16 we verified whether anti–troponin I identified all myocardium that was recognized by the SERCA2a antibody. In 3 embryos at Carnegie stages 10 to 12, we stained consecutive sections for SERCA2a, troponin I, and cardiac troponin I. At the venous pole, the domain revealed by SERCA2a and cardiac troponin I was minimally broader compared with the domain of troponin I (Figure 2A). At the arterial pole, expression of SERCA2a was much weaker than the other myocardial markers (Figure 2B), as has been reported for the rodent embryonic heart.16 The antibody against cardiac troponin I was raised in rabbits, preventing its use in combination with other rabbit antibodies. Therefore, we chose troponin I as a reliable marker for delineation of the myocardial tissues. After the immunostaining, sections were stained with hematoxylin and azophloxin, facilitating histological analysis (Figure 2C).

Stage 10: The Prototypic Linear Heart Tube

The 2 youngest embryos studied were equivalent to Carnegie stage 10, which corresponds to ∼22 to 23 days of develop-
ment, and embryonic day 8.5 in the mouse. Folding had just been initiated, with the caudal two thirds of the embryos still flat. The neural tube was not yet closed; there was a wide anterior intestinal portal and a broad coelomic cavity; and the relatively short heart tube was beginning to loop. In the youngest specimen, the heart tube was little more than a myocardialized part of the splanchnic mesodermal layer folded into the coelomic cavity consisting of a narrow lumen lined by a single layer of endothelial cells, a thick acellular layer of cardiac jelly separating the endothelium from the outer myocardial layer (Figures 2C and 3A and 3D). The dorsal mesocardium had not yet ruptured, although the myocardium surrounded the middle portion of the tube in its entirety. At the arterial and venous poles, the tube was not yet closed, and the myocardial wall was contiguous with the nonmyocardial coelomic wall (Figure 3C). Caudally, the cardiac jelly extended outside the heart proper over the proximal unfused parts of the cardiac inflow and was bounded by the endoderm, coelomic ducts, and transverse septum (Figure 3A, 3B, and 3D). Caudally, the vitelline veins were joined to the umbilical veins and received small vessels from the venous plexus of the yolk sac.

Cranially, the border of the pericardial cavity was recognized as the reflection between the coelomic wall and myocardial heart tube, but caudally, the pericardial cavity was continuous with the bilateral coelomic ducts (Figure 3B and 3C). The coelomic wall covering the venous tributaries, except its most lateral extremes, was already myocardial (Figure 3E and 3E*). All myocardial cells present at this stage, including those at the venous pole of the heart and adjacent cells of the dorsal coelomic wall, as well as the ventral wall of the foregut, expressed NKX2–5, a core cardiac network transcription factor essential for cardiogenesis17 (Figure 3D** and E**). Connexin40 was weakly expressed in the endothelial cells of the heart tube but could not be detected in the endothelium of the veins and aortas and was not detectable in the cardiomyocytes (not shown).

Stage 12: Appearance of the Systemic Venous Sinus and Pulmonary Pit
We analyzed 3 human embryos at Carnegie stage 12, which spans the time from 26 to 30 days after conception and is equivalent to mouse embryonic day 9.5. In all embryos, the

Figure 2. Expression of the myocardial markers at the venous (A) and arterial (B) poles of the stage 10 embryonic heart. Sections were incubated with antibodies as indicated. Arrowheads show the extent of staining with the different antibodies (see text for further description). Scale bars=100 μm. C, General histology of the cardiac tissues at the stages as indicated. For abbreviations, see Figure 1.
heart tube had looped, and evidence was found of ballooning of the atrial appendages and the apical ventricular trabecular components. An extensive and discrete atrioventricular canal was positioned to the left of the midline (Figure 4B and 4E). The pericardial cavity remained in communication with the peritoneal cavity through the bilateral pericardio-peritoneal canals, and no epicardial layer was seen on the surface of the heart.

By now, the wall of the common atrial chamber was positive for connexin40, a marker for differentiating working myocardium in the mouse embryo at comparable stages (Figure 4A). A small but discrete systemic venous sinus was present upstream relative to the atrial chamber, being more obvious in the older specimens (Figure 4E and 4F) than in the younger one (Figure 4B and 4G). The right- and left-side venous tributaries joined into bilateral common channels, which emptied into the venous sinus. The lumens of these common veins were bordered ventrally by the mesenchyme of the transverse septum and dorsally by the mesothelial lining of the pericardio-peritoneal canals (Figure 4H). The 2 common veins themselves were separated by mesenchymal tissue, forming the so-called sinus septum.

The left- and right-side appendages had already ballooned from the common atrium, but as yet there was no overt sign of formation of a primary atrial septum. The junction of the venous sinus with the atrium, the sinu-atrial foramen, was already located at the right side of the common atrial chamber. The ventral side of this junction was marked by the so-called sinu-atrial fold (dashed lines in Figure 4B, 4E, and 4F). At the dorsal aspect, there was no distinct morphological landmark marking the junction between the venous sinus and the atrial chamber (Figure 4C and 4D). At this stage, all myocardium was still NKX2-5 positive (Figure 4H and 4J). The larger part of the walls of the venous sinus was negative for myocardial markers, with only the most proximal part adjacent to the sinu-atrial foramen acquiring a primary, connexin40-negative, myocardial phenotype (Figure 4K).

TBX18, a marker for the tissues making up the systemic venous sinus in the mouse embryo, was observed in the mesenchymal tissues bordering the venous sinus and in the proepicardium but was absent from myocardium and tissues bordering the pulmonary pit (Figure 4L).

Dorsally and in the midline, the reflections between the atrial and coelomic walls formed obvious ridges, which

Figure 3. Reconstructions and sections of the prototypic linear heart from a stage 10 embryo. Note the direct continuation of the pericardial cavity into the coelomic ducts (red arrows in B and C). In C, the proximity of the arterial and venous poles, separated by only a small portion of the dorsally fused myocardium, is striking. Sections shown in D* and D** and in E* and E** were incubated with antibodies as indicated and correspond to the cross section of the 3D model shown in D and E, respectively. Note the broader domain of NKX2-5 expression compared with the expression of troponin I (TnI; arrowheads). See text for further description. Scale bars=100 μm. For abbreviations, see Figure 1.
Figure 4. Reconstructions and sections of a stage 12 embryonic heart and related structures. D through F are from a slightly older embryo for which no connexin40 expression data were available; the other panels are from a younger specimen. Dashed lines in B, E, and F represent the sinu-atrial fold, and dotted lines in C and D indicate the pulmonary ridges surrounding the pulmonary pit (arrowheads). H and I show expression of PECAM1 in the endothelium and troponin I (TnI) in myocardium, both in pink owing to the use of the same fluorochrome. To allow visualization of the more weakly stained endothelial cells, the myocardial staining was overexposed. The sections shown in H, J, K, and L were incubated with antibodies as indicated and approximately correspond to the cross section of the 3D model shown in G. I shows the region from a more cranial section corresponding to the boxed area in H. Note the pair of PECAM1-positive capillaries at the ventral surface of the foregut (arrowheads in I), which were continuous with the endothelial cells of the pulmonary pit (arrowhead in H). The asterisk in G and H points to a bigger right pulmonary ridge; arrows in H point to veins joining the venous sinus; arrowheads in L point to mesenchymal cells expressing TBX18. See text for further description. Scale bars=100 μm. For abbreviations, see Figure 1.
enclosed the pulmonary pit (Figure 4G and 4H). The space between the pulmonary ridges was filled with a jelly-like substance, which was lined ventrally by endocardium and dorsally by the loose mesenchyme surrounding the foregut. The primordium of the pulmonary vein was already identifiable in the pulmonary pit as a blind endocardial evagination into the jelly located ventral to the foregut (arrowhead in Figure 4H), the ventral surface of the foregut itself being covered by loose mesenchyme containing capillaries, the so-called midpharyngeal endothelial strands19 (Figure 4I). As in the working myocardium of the atrial chambers, NKX2.5 was strongly expressed in the mesenchyme and myocardium of the ridges, less so in the more prominent right ridge, but not in the mesenchymal wall of the venous sinus (Figure 4J). The myocardium of the ridges was also positive for connexin40 (Figure 4K). The enlarged right ridge contained a mesenchymal mass, which appeared to shift the primordium of the pulmonary vein slightly to the left (Figure 4H).

Stage 14: Shift of the Systemic Venous Sinus and Formation of the Pulmonary Vein
We examined 3 embryos at stage 14, which represents the period of 31 to 35 days after conception and is equivalent to mouse embryonic day 10.5. By this stage, the atrial appendages and ventricular apical components were well formed, and the primary atrial and ventricular septal structures were evident. Connexin40 was expressed in the ventricular trabeculations and walls of the atrial chambers but was absent from the myocardium of the atrioventricular canal (Figure 5G). The primary atrial septum was sickle shaped, with a mesen-
Myocardialization of the Venous Tributaries
Sinus Into the Right Atrium and
Stage 16: Incorporation of the Systemic Venous Sinus Into the Right Atrium and
Myocardialization of the Venous Tributaries
We studied 3 embryos at Carnegie stage 16, ~37 to 42 days after conception, equivalent to mouse embryonic day 11.5. By this stage, the atrial appendages were obvious (Figure 6A), with pectinate muscles now recognizable on their luminal surfaces. The sepal structures had also developed further, and a secondary foramen was present in the primary atrial septum (Figure 6C and 6D). The cushions in the atrioventricular canal had grown together, separating the canal into right and left channels. In contrast, at the venous pole, there had been relatively small morphological changes. Compared with the size of the right atrial cavity, there had been a relative decline in size of the systemic venous sinus, reflecting the initiation of its incorporation into the morphologically right atrium (Figure 6C). As at stage 14, its myocardial walls remained thick and porous (not shown).

The coronary sinus was now completely myocardial (Figure 6B), expressed connexin40 and TBX18, but was still devoid of NKX2-5 (Figure 6F through 6H). It opened between the venous valves into the right atrium, adjacent to the atrioventricular junction (Figure 6C), but its continuation as the left superior caval vein had become much narrower (arrowhead in Figure 6B).

The pulmonary venous plexus continued to drain into the morphologically left atrium through a solitary channel, which joined the heart still adjacent to the atrioventricular junction but had now acquired a myocardial sleeve (Figure 6C and 6E). The myocardial surrounds of the orifice were positive for connexin40 and NKX2-5 and negative for TBX18 (Figure 6F through 6H). The vestibular spine, now forming the base of the primary atrial septum, interposed between the orifice of the pulmonary vein and that of the systemic venous sinus. The diameter of the individual pulmonary veins had increased, so they could be traced from the bronchopulmonary buds, which were surrounded by an extensive platelet endothelial cell adhesion molecule 1 (PECAM1)–positive capillary network (Figure 6E). The mediastinal mesenchymal tissues remained contiguous with the dorsal cardiac mesenchyme, which in turn fused with the inferior atrioventricular cushion (Figure 6D).

Discussion

The recent expansion of molecular technology has revealed functions of the genetic networks driving the development of the vertebrate heart. Although the findings in model organisms have been extrapolated to human development, in the absence of reference molecular data for human cardiac morphogenesis, it is unclear whether such extrapolation is justified. Furthermore, the scarcity of molecular data, along with the uncertainty relative to the morphological landmarks in the early stages of development of the human heart, has hampered the full understanding of this complex region. We have now used triple immunofluorescently labeled serial sections to investigate in the human embryo the patterns of expression of multiple genes known to be essential for mouse cardiac morphogenesis and function. This has permitted us, as in the mouse, to delineate different types of myocardium and to construct accurate morphological models of the developing human heart. We have supplemented these findings with data on the expression of relevant genes. The 3D models are presented also in an interactive format (see the online-only Data Supplement), which allows the observer to form an independent opinion on the topic under discussion. Our results show that, as in the mouse, the systemic and pulmonary tributaries of the human embryonic heart are surrounded by distinct types of myocardium, supporting the notion that they are derived from distinct lineages. In contrast to the situation in the mouse, we have been able to recognize the presence of a discrete systemic venous sinus at early stages, which subsequently becomes incorporated into the morphologically right atrium.

Systemic Venous Return

In the mouse, evidence from gene expression and lineage analyses, along with morphological studies, has shown that the systemic tributaries connect directly to the atrial chamber at early stages without the formation of a discrete systemic venous sinus. In contrast to the mouse, we observed, first at stage 12 (mouse embryonic day 9.5) and thus before the formation of the venous valves, a discrete systemic venous...
sinus, which probably is excavated from the body wall and transverse septum into the pericardial cavity earlier in human than in mouse.

The systemic venous tributaries in mouse acquire myocardial sleeves, having a molecular phenotype different from that of the atrial chambers and pulmonary vein. Our current data show that, as in the mouse, the myocardium of the human systemic venous tributaries does not express NKX2-5 but does express TBX18, thus distinguishing it from the pulmonary venous myocardium (see below).

The myocardium of the systemic venous sinus contains the primordium of the sinus node, responsible for normal pacemaking. We noted a thickened porous structure at the junction of the right superior caval vein and right atrium in Figure 6.
embryos at stage 14 and beyond (Figure 5E and not shown). This cuff-like structure represents the primordium of the sinus node, which we are currently analyzing in detail.

Two additional structures, the venous valves and the proepicardium, deserve additional attention. The morphogenesis of the venous valves, demarcating the sinus-atrial junction, has been well described in human embryos. Of the 2 layers of the embryonic right valve, which remains in the postnatal heart as the Eustachian valve, the layer facing the lumen of the systemic venous sinus had the same molecular makeup as the sinus muscle, whereas the other layer had the atrial phenotype (Figure 5G and 6G). TBX18 identified the sinus myocardium and adjacent mesenchyme, including the proepicardium. This mesenchymal precursor pool has been shown in chicken to separate early into the pericardial and myocardial lineages.

Pulmonary Venous Return

The principal debate remaining in the development of the cardiac venous pole concerns the origin of the pulmonary orifice and its relation to the systemic venous sinus. When assessing this ongoing debate, we need to distinguish between the development of the venous lumen and the formation of the myocardial sleeve around the lumenized vein. The lumen of the pulmonary vein forms within the endothelial strand, which at the early stages of cardiac development is part of a common splanchnic capillary plexus facing the ventral aspect of the foregut. The venous primordium acquires its lumen only after its leftward shift, concomitant with both the formation of lung buds in the mediastinal mesenchyme and the division of the common atrial chamber into right and left components by the growth of the primary atrial septum.

At the stage of formation of a discrete systemic venous sinus, there is no evidence of lung buds, and hence there can be no pulmonary venous return to the heart. At this stage, nonetheless, the primordium of the orifice of the pulmonary vein is already recognizable as a blind evagination of the endocardium into the jelly between the pulmonary ridges, the so-called pulmonary pit (Figure 4H). As in the mouse, this primordium was located cranially relative to the opening of the systemic venous sinus and was surrounded by atrial working myocardium. We also found that in subsequent stages of human development, the right pulmonary ridge enlarged significantly, accumulating more mesenchymal cells than the left. We correlate this mesenchymal accumulation with the development of the vestibular spine, or the spina vestibuli as originally described by His. Unlike the situation in the mouse, the spine in the human protrudes into the atrial lumen, thus facilitating the entry of the common pulmonary vein to the left atrium. Only after acquiring its lumen does the wall of the pulmonary vein become myocardialized through differentiation of the surrounding mesenchyme.

Previous studies, largely morphological, have become polarized, arguing that the pulmonary vein was initially connected to either the atroioventricular (AV) valve or the systemic venous sinus. These variations in opinions may represent differences in interpretation rather than observation. At stage 12 of human development, a capillary plexus is present within the mesenchyme ventral to the foregut, connected through nonlumenized endothelial strands with both the caudally expanding systemic venous sinus and the more cranially located pulmonary pit. At stage 14, the pulmonary venous primordium acquires its lumen and becomes connected to the morphologically left atrium, being separated from the systemic venous sinus by the vestibular spine. Only after this connection do the walls of the pulmonary vein become myocardial.

It has been suggested, on the basis of the expression of the HNK-1 antigen and the CCS-lacZ transgene in the myocardium of both the systemic venous sinus and the pulmonary vein, that the myocardial sleeves around the pulmonary veins originate from the sinus venosus. This presumption has then been used to explain why cardiac arrhythmias originate so frequently from the pulmonary myocardium. Gene expression and lineage analyses in the mouse, however, unambiguously demonstrated that the musculatures of the systemic venous sinus and the pulmonary myocardium have distinct lineages and molecular signatures. As we now show, the genetic profiles of the systemic and pulmonary venous myocardial walls in the developing human heart are also fundamentally different from the onset of their development. As in the mouse, the musculature of the systemic venous sinus expresses TBX18 and lacks NKX2-5, whereas the pulmonary myocardium expresses NKX2-5 but does not express TBX18. The similarity of these transcriptional signatures in mice and humans suggests evolutionary conservation of the genetic program for the formation of these compartments at the venous pole, lending no support to the notion that the pulmonary myocardium originates from the systemic venous sinus.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

The morphologically complex venous pole of the postnatal human heart, consisting of the union of the caval veins with the right atrium, the coronary sinus, and the connection of the pulmonary veins with the left atrium, is a clinically important entity. Although rare, many forms of congenital heart disease locate at the venous pole of the heart and influence the hemodynamics by changing the quantity and direction of blood inflow. In addition, many abnormal rhythms such as focal atrial tachycardia and paroxysmal atrial fibrillation have been shown to originate from the myocardium making up part of the venous pole. In particular, the hypothesis that the myocardial sleeves of the systemic and pulmonary venous returns have a common origin has been used to explain the nonrandom location of the arrhythmogenic foci at the venous pole. In agreement with gene expression and lineage studies performed in mice that have shown that the myocardium of the systemic and pulmonary veins develops from genetically distinct cellular populations, the present study in human embryonic hearts reveal distinct transcriptional profiles in the myocardium surrounding the systemic and pulmonary returns. The similarity of these transcriptional signatures in mice and humans suggests evolutionary conservation of the genetic program for the formation of these compartments at the venous pole, lends no support to the notion that the pulmonary myocardium originates from the systemic venous sinus, and suggests another mechanism for the origin of the electric impulses initiating paroxysms of atrial fibrillation within the pulmonary venous sleeves.
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This PDF file is designed to interactively assess the morphology of the human embryonic heart and related structures in three dimensions. For the descriptions see the paper.

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**How to use this PDF file**

Every following page contains on the left the preset view buttons (with references to the figures of the paper) to enable the user to (re)set the 3D model into the orientation shown on the particular button.

Below the buttons for preset views there is a list of the structures ("myocardium", etc) with three buttons per structure to permit to:

To further interact with the 3D model:

* **rotate** : click and hold the left mouse button and move the mouse
* **zoom** : click and hold the right mouse button and move the mouse
* **translate** : click and hold the left+right mouse buttons and move the mouse
Stage 10 human embryo (22-23 days of development)

views

fig. 3a  fig. 3b
fig. 3c  fig. 3d

structures

myocardium
lumen
jelly
coeleomic wall
foregut
s. transversum
neuro-ectoderm

http://3d.hfrc.nl
### Stage 12 human embryo (26-30 days of development)

#### Views

- fig. 4a
- fig. 4be
- fig. 4cd
- fig. 4fg

#### Structures

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**Stage 14 human embryonic heart (31-35 days of development)**

**views**

- fig. 5a
- fig. 5b
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**structures**

- myocardium
- Cx40-pos myo
- arteries
- veins
- cushion tissue
- dorsal mesench.
- trachea/bronchi
- pulmonary vein

http://3d.hfrc.nl
Stage 16 human embryonic heart (37-42 days of development)

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