Development of the Myocardium of the Atrioventricular Canal and the Vestibular Spine in the Human Heart

Jung-Sun Kim, Szabolcs Virágh, Antoon F.M. Moorman, Robert H. Anderson, Wouter H. Lamers

Abstract—To establish the morphogenetic mechanisms underlying formation and separation of the atrioventricular connections, we studied the remodeling of the myocardium of the atrioventricular canal and the extracardiac mesenchymal tissue of the vestibular spine in human embryonic hearts from 4.5 to 10 weeks of development. Septation of the atrioventricular junction is brought about by downgrowth of the primary atrial septum, fusion of the endocardial cushions, and forward expansion of the vestibular spine between atrial septum and cushions. The vestibular spine subsequently myocardializes to form the ventral rim of the oval fossa. The connection of the atrioventricular canal with the atria expands evenly. In contrast, the expression patterns of creatine kinase M and GlN2, markers for the atrioventricular and interventricular junctions, respectively, show that the junction of the canal with the right ventricle forms by local growth in the inner curvature of the heart. Growth of the caudal portion of the muscular ventricular septum to make contact with the inferior endocardial cushion occurs only after the canal has expanded rightward. The atrioventricular node develops from that part of the canal myocardium that retains its continuity with the ventricular myocardium. (Circ Res. 2001;88:395-402.)

Key Words: atrioventricular junction ■ atrial vestibule ■ vestibular spine ■ tendon of Todaro ■ atrioventricular node

The primary heart tube loops¹ and balloons² to develop its atrial and ventricular compartments. Initially, the atria and ventricles are arranged in series, being joined via the atrioventricular canal and the interventricular foramen. Because of this, the developing right atrium at first communicates only indirectly with the putative right ventricle.³ Therefore, the establishment of a direct communication between right atrium and right ventricle requires not only physical separation of the left and right sides of the initially common atrioventricular junction but also remodeling of the canal myocardium to produce the right atrioventricular connection. Previous studies suggested that the formation of this connection might involve rightward expansion of the canal⁴ or, alternatively, leftward remodeling of the primary ventricular septum.⁵ Although simple conceptually, these hypotheses have not thus far been tested by accurate observations of the changing junctional architecture.

Some time ago, we showed that the carbohydrate epitope recognized by the GlN2 (HNK-1, Leu7) antibody was a marker for the developing interventricular myocardium.⁶ This allowed us to establish that the entire right-ventricular myocardium, including its inlet portion, was developed from the distal ventricular component of the primary heart tube⁷ and that the canal myocardium becomes sequestered as an atrial structure.⁷,⁸ We did not establish, however, which parts of the adult atrium are derived from the canal myocardium. Furthermore, we did not elucidate the formation of the right-sided part of the atrioventricular junction, nor are we aware of other studies that have addressed this issue. We have now studied the changing shape of the atrioventricular canal musculature, using the lack of expression of creatine kinase M (CKM) as its phenotypic marker.⁹ By combining the expression patterns of CKM and GlN2, we show that the junction of right atrium and ventricle forms concomitant with local expansion of the inner curvature of the heart. We additionally show that the canal myocardium itself eventually forms smooth-walled vestibules of both atria, the atrioventricular node persisting as a specialized portion of this myocardium. Finally, we show that the vestibular spine, a mesenchymal mass derived from the extracardiac dorsal mesocardium, plays a pivotal role in septation of the atrial side of the developing junctions. These findings elucidate several of the developmental disturbances that underlie malformations involving the atrioventricular canal.

Materials and Methods

Tissue Sources, Preparation, and Staining
We studied human embryos from 4.5 weeks to 10 weeks of development. The embryos and their immunohistochemical staining
The atrioventricular canal myo-cardium is characterized by its lack of CKM expression, in contrast to the ventricular myocardium.9 The interventricular ring expresses the GIN2 epitope.4 The area of colocalization of the CKM-negative atrioventricular myocardium and the GIN2-positive interventricular myocardium in the inner curvature delineates the area of formation of the right atrioven-tricular junction. This CKM-negative but GIN2-positive area occupies only about one fifth of the atrioventricular junc-tional circumference.

At 5.5 weeks, the junction of the atrioventricular canal musculature with the atria has become wider than its ventric-ular connection, so that its shape changes from a tube into a funnel (Figures 2 and 3C), being most pronounced on the right side. As viewed from the ventricles, the primary atrial septum is positioned over the center of the canal (Figure 2A, 5.5 weeks). At this stage, the vestibular spine has penetrated the heart between the atrial connections of the caval veins and the pulmonary vein to abut the inferior atrioventricular cushion (Figure 3A). The left venous valve inserts on its right side, whereas the leading edge of the muscular primary atrial septum inserts on a thin, finger-like extension from its cranial end (Figures 2A [5.5 weeks] and 3C). The spine and its cranial extension do not express the 9G9 epitope in contrast to the tissues of the endocardial cushions (Figures 3B and 3D).

When viewed from the atrial aspect, the atrioventricular canal retains its position over the left ventricle (Figure 2B, 5.5 weeks). The muscular ventricular septum is prominent ventrally but not yet seen separating the caudal part of the ventricles. The developing branches of the atrioventricular conduction bundle, however, can be distinguished as small GIN2-positive twigs in the caudal wall of the ventricles (Figure 2B, 5.5 weeks). The adjacent part of the GIN2 ring belonging to the canal myocardium has widened as the precursor of the atrioventricular node.

Period of Septation (Seventh Week): Differential Growth of the Connection of the Canal With the Right Ventricle

In this period, the atrioventricular cushions and outflow-tract ridges fuse to separate the left and right sides of the heart (Figure 2C, 6.5 to 7 weeks) and the lateral endocardial cushions of the canal become evident. In the beginning of the 7th week, fusion of the vestibular spine with the atrial surface of the cushions closes the primary atrial foramen (Figure 3E). In this process, the body of the spine expands cranially to incorporate its spur on the free rim of the primary atrial septum (Figure 2A, 6 to 6.5 weeks). After closure of the primary atrial foramen, the primary septum itself inserts on the left and dorsal margin of the spine, with the well-developed left and right venous valves inserting on its right-sided dorsal and ventral margins, respectively. The tissue of the spine now contains myocytes near the insertions of atrial septum and venous valves (Figure 3E).

The atrioventricular canal itself has retained its character-istic funnel shape in this period of septation. Whereas its connection with the atria continues to expand evenly, the expansion of its connection with the ventricles is distinctly uneven. Marked rightward growth is limited initially to the

![Figure 1. Tubular atrioventricular canal at 5 weeks. A, Reconstruction of a heart that is opened in the midsagittal plane, as shown in panel B. GIN2-positive interventricular myocardium (red) and CKM-negative AVC myocardium (yellow) are highlighted. Note that both structures are reconstructed in toto and, hence, protrude partly from the plane of sectioning. Additionally note colocalization of both rings in the lesser curvature. C, Ring complex is rotated over 60° relative to panel A along the dorso-ventral axis. D, Right-sided atrioventricular junction can develop as a result of local growth at the site of colocalization (upper variant) or a generalized rightward expansion of the AVC (lower variant). The staining patterns of GIN2 and CKM revealed local growth at the site of colocalization. Bar=250 μm. RA indicates right atrium; PAS, primary atrial septum; CS, coronary sinus (left sinus venosus); LA, left atrium; PAF, primary atrial foramen; OFT, outflow tract; VS, ventricular septum; RV, right ventricle; and LV, left ventricle.](http://circres.ahajournals.org/)

Three-Dimensional Reconstruction

Reconstructions were made using a computer-aided method.14 The contours of the atria, atrioventricular canal, ventricles, outflow tract, endocardial tissues, vestibular spine, and CKM and GIN2 staining pattern, as observed in serial sections of the embryos, were traced onto acetate sheets. The vestibular spine was distinguished from the endocardial cushions by its higher density of cells and the absence of staining with antibody 249-9G9.

For the description of the specimens and reconstructions, we used cranial, caudal, dorsal, and ventral as indicators of orientation, with the apex of the heart always pointing ventrally. It should be noted that in the postnatal human heart, these terms correspond to superior, inferior, posterior, and anterior, respectively. For description of the atrioventricular endocardial cushions, we have retained the commonly used terms superior and inferior.

Results

Period Before Septation (Fifth and Sixth Weeks): Expansion of the Atrioventricular Canal

At the end of the 4th week of development, the trabecular portion of the ventricles and the atrial appendages begin to balloon from the outer curvature of the primary myocardial tube.3 In the ventricles, the original lumen of the tube remains temporarily recognizable as an area free of trabeculations in the inner curvature that is continuous with the atrioventricular canal proximally and the outflow tract distally (see Figure 15-5 in Reference 13) At this stage, the canal is positioned exclusively above the inlet to the developing left ventricle.

Reconstruction of a 5-week-old heart shows the canal to be straight and tubular (Figure 1). Because atria and ventricles are substantially wider than the canal, their respective bound-aries are easily delineated. The atrioventricular canal myo-
cranial part of the canal, so that the circumference of the canal resembles that of a boomerang (Figure 2A, 6 to 6.5 weeks). Toward the right ventricle, the developing right-sided atrioventricular junction is continuous with a myocardial gully that develops at the junction of the atrioventricular canal and the right ventricle, funneling atrial blood toward the middle of the right ventricle.11 Because the right side of the atrioventricular canal is covered with epicardial tissue (Figure 3E) whereas the gully is a purely myocardial structure (Figure 3F), the boundary is well-defined.

The 7th week is characterized by a pronounced caudal expansion of both the ventricular connection of the canal and the right ventricle (Figures 2A through 2C, 6.5 weeks). This growth accompanies formation of the caudal portion of the muscular ventricular septum, which expands toward the base
of the heart to become attached to the endocardial cushions (Figures 2 [5.5 to 6.5 weeks] and 3F). The topography of the bundle branches parallels the newly gained prominence of the caudal portion of the muscular septum, achieving a position on the crest of the muscular septum at 6.5 weeks (Figure 2B, 6.5 weeks). This positional change is accompanied by an attenuation of the diameter of the GlN2 ring between the node and the bifurcation of the bundle branches (Figure 2B).

Concomitant with the expansion of the ventricular connection of the right side of the canal, the floor of the muscular gully cavitates to form the part of the tricuspid valve lying caudal to the right bundle branch (Figure 2B, 6.5 weeks), permitting expansion of the initial valvar orifice, which persists cranially.

The right-sided expansion of the atrioventricular canal is reflected in the growth of the GlN2-positive ring (Figure 2B, 6 to 6.5 weeks). Approximately one third of the circumference of this ring is now also CKM-negative and occupies the frontal instead of the original sagittal plane. This portion of the ring, which is part of the right atrioventricular junction, is called the right atrioventricular ring bundle.15 The nodular portion of the ring bundle at its junction with the ventricular septum has increased in size but retains its position in the protruding right-sided portion of the developing canal.

**Figure 4. Relation of atrioventricular node to spine, central fibrous body, and ventricular myocardium, Transverse (A through D) and sagittal (E through H) sections of 10-week-old hearts. A, C, and E were stained for the presence of α-myosin heavy chain; B, D, and F, GlN2 epitope; G, CKM; and H, α-smooth muscle actin. Plane of E through H is indicated in panel A; plane of A through D is indicated in panel E. Note topographic relation between AVN, coronary sinus, and vestibular spine, including tendon of Todaro. Furthermore, note topographic relation of the AVN with the central fibrous body. Finally, note the segmentation of the AVN, as reflected in the expression patterns of GlN2, CKM, and myosin heavy chains. Arrow (E through H) indicates junction between the atrioventricular node and bundle; arrowheads (A and C), atrioventricular canal myocardium; and asterisk (C and E through H), His bundle. Bars = 250 μm. TT indicates tendon of Todaro; CFB, central fibrous body; and AO, aorta. Other abbreviations as in Figures 1 and 3.**

**Figure 5. Components of the atrioventricular junctions. Reconstruction of a 10-week-old heart as seen from dorsal and cranial. Yellow indicates CKM-negative atrial and junctional myocardium; red, remains of GlN2-positive ring with right atrioventricular ring bundle, node, and bifurcation; green, myocardialized spine; blue, tendon of Todaro; and purple, lumen of coronary sinus. Stippled line indicates insertion of primary atrial septum. Note right-sided wedging of junctional myocardium below the spine to form the node.**

**Stages Subsequent to Septation (Eighth to Tenth Weeks): Formation of Figure 8–Shaped Atrioventricular Junctions**

Although the remodeling that accompanies septation is complete at the end of the 7th week, the separated right and left atrioventricular junctions do not become equal in diameter until the end of the 8th week. Concomitant with this increasing diameter of the right junction, the CKM-negative canal myocardium, containing the GlN2-positive ring bundle, grows to occupy half of the circumference of the canal (Figure 2B). The ventricular connections of the canal myocardium gradually assume the configuration of a figure 8, indicating that both the separated atrioventricular junctions grow faster than the muscular ventricular septum (Figures 2A and 2B, 7.5 weeks). The growth of the right junction is
accompanying an increase in size of the caudal valvar orifice in the floor of the muscular gully (Figure 2B, 7 to 7.5 weeks). As a result, the myocardial band that marked the cranial border of the gully and that contains the right bundle branch becomes prominent as the septomarginal trabeculation.

Meanwhile, the vestibular spine has become muscularized to become the major myocardial constituent of the base of the atrial septum. MyocardIALIZATION spreads from 2 sites, namely, the insertion of the primary atrial septum and of the venous valves (Figures 3G and 3H). Both expand so that at 8 weeks, the vestibular spine is largely muscular. Two weeks later, these myocytes express relatively high levels of CKM. A nonmyocardial component containing cells that abundantly express α-smooth muscle actin remains present between the myocardializing foci throughout the length of the spine (Figures 4H and 5). This structure persists as Todaro’s tendon, which runs from the sinus septum to insert in the central fibrous body, the latter now formed from the fused endocardial cushions.

By 10 weeks, the atrioventricular canal myocardium itself has become sequestered within the atria as the smooth-walled vestibules of the tricuspid and mitral valves (Figures 4A, 4C, and 5). The primary atrial septum is continuous with the left, dorsal side of the bulky, myocardialized, vestibular spine (Figures 4A, 4C, and 5). The spine, in turn, rests cranially on the central fibrous body (Figure 4C) and caudally on the canal myocardium (Figures 4A and 5). As a result of the differential growth in the atrioventricular connection, the medial part of the canal has become wedged beneath the spine (Figures 4A, 4B, and 5). It remains folded, nonetheless, around that part of the developing central fibrous body derived from the inferior endocardial cushion (Figure 4E, compare with Figure 6), with the coronary sinus draining into the right atrium between the spine and the vestibule (Figures 4A, 4B, and 5).

The atrioventricular node persists as the apical portion of the infolded canal musculature (Figures 4 and 5), with its caudal, or inferior, extension representing the continuity of nodal tissue with the adjacent part of the canal. The transitional cells of the node are formed in part from the myocardialized spine. The node itself continues cranially as the penetrating atrioventricular bundle of His. The boundary is marked by the transition of the α-myosin heavy chain–positive (Figure 4E), CKM-negative (Figure 4G) canal myocardium into the β-myosin heavy chain–positive (not shown) and CKM-positive (Figure 4G) myocardium of the bundle. This transition from CKM-negative to CKM-positive myocardium coincides with the junction of the node with the bundle of His at the end of the 8th week but is positioned distally in the bundle in younger embryos (Figure 2B). The myocytes in the bundle of His also lose their GIN2 expression (Figures 4F and 5).

Discussion
The atrioventricular canal is usually perceived as a simple tube connecting the septating atria and ventricles. Instead, we have shown that its pattern of growth is complex, with marked differences in the evolution of its atrial and ventricular connections.

Formation of the Right Atrioventricular Connection
Initially, the orifice of the canal is positioned exclusively above the developing left ventricle. The temporally distinct pattern of growth of the ventricular connections of the canal musculature and the increasing proportion of the circumference of the canal possessing a CKM-negative but GIN2-positive phenotype shows that the right-sided part of the junction forms as the result of a local expansion of myocardium with phenotypic features shared by both the atrioventricular and the interventricular junctional myocardia. Only after the canal has expanded rightward does the caudal portion of the muscular ventricular septum rise to make contact with the inferior endocardial cushion. This last process completes muscular ventricular septation, ensuring...
that blood crossing the right atrioventricular junction enters exclusively to the right ventricle.

The growth of the morphologically right ventricle, a structure barely identifiable at 4 weeks of development yet the same size as the morphologically left ventricle by 7 weeks, corresponds temporally with the rightward expansion of the atrioventricular canal. This can be correlated with congenital malformations such as straddling tricuspid valve, in which the size of the right ventricle corresponds with the proportion of the right junction connected to it. Indeed, the formation of the right junction is intimately linked with the development of the tricuspid valve. Concomitant with expansion of the ventricle, there is delamination of a muscular gully from the ventricular trabeculations.11 This gully guides atrial blood to the cranial part of the ventricle (see Figure 1 in Reference 11). The tricuspid orifice is initially formed cranially, with its margins demarcated by the rim of the gully containing the right bundle branch, and by the fusing ridges of the outflow tract. Only after 6.5 weeks of development is the caudal orifice of the valve developed in the floor of the gully, the septomarginal trabeculation demarcating the junction of these developmental components of the definitive valve.

**Fate of the Canal Myocardium and Location of the Atrioventricular Node**

The definitive fibro-fatty atrioventricular junction is formed at the site where the canal myocardium itself initially made contact with the ventricular myocardium.7,8 Hence, the initial myocardium of the canal is sequestered as an atrial structure, forming the smooth-walled atrial vestibules. The atrioventricular node, along with its caudal extension,16 is also an integral part of the right atrial vestibule. This is because the node is no more than the only part of the canal, which retains its muscular continuity with ventricular myocardium (Figure 6). Accordingly, the node retains the slow conduction, which initially characterizes all of the canal myocardium.17 During formation of the node, the canal myocardium thickens but does not increase in length, in accordance with its very low mitotic activity.18 The canal itself remains folded around the central fibrous body, the structure that develops locally from the endocardial cushions.19 The atrial margin of the node is apposed to the myocardialized vestibular spine, containing the tendon of Todaro. Its vestibular margin is continuous with the GIN2-positive ring remaining on the crest of the muscular ventricular septum as the bundle of His. The slowly conducting component derived from canal musculature17 is characterized by a lack of expression of CKM. In contrast, the rapidly conducting ventricular components of the conduction axis are distinguished from the surrounding working myocardium by their high expression of CKM. This high expression is initially confined to the bundle branches and trabeculations, expanding into the bundle only between 8 and 10 weeks of development (Figure 2). It remains to be established whether the increased CKM expression reflects maturation concomitant with an increase in velocity of conduction.

**Vestibular Spine Functions as an Anchor in Atrial Septation**

On its atrial side, the septation of the expanding canal is achieved by the downgrowth of the primary septum. It is growth of the vestibular spine, nonetheless, that completes atrial septation. This mass of extracardiac mesenchymal tissue, first described by His,20 penetrates the heart via the caudal hiatus in the myocardial wall of the atrium, the so-called dorsal mesocardium. It then becomes wedged between the primary atrial septum dorsally, and the fused endocardial cushions ventrally. Its development is linked topographically with that of the pulmonary vein.12,21 The mesenchymal tissue of the spine and its spur on the rim of the primary atrial septum share some structural and biochemical properties with the cushions.22,23 But unlike endocardial tissue, spinal tissue does not express the 9G9 epitope.12 Furthermore, the vestibular spine and its spur share a common origin,24 and both become myocardialized. These findings suggest that the primary atrial foramen is closed as a result of cranial expansion of the body of the spine over the endocardial cushions,25,26 its finger-like cranial extension serving as a conduit. Indeed, this extension is often absent in animal models with persistence of the primary atrial foramen.27,28 Therefore, the role of the vestibular spine during septation of the venous pole of the heart is remarkably similar to that of the aorto-pulmonary septum, derived from the neural crest, in the arterial pole of the heart, all the more because the spine also serves as a conduit for migration of cells from the neural crest.29 The myocardialization of the spine also resembles that found occurring within the outflow tract concomitant with formation of the subpulmonary infundibulum.30 It starts near the sites of insertion of the primary atrial septum and the venous valves on the spine, largely replacing the mesenchymal component (the mesenchymal caps in Figure 12 of Reference 31) with myocardium and covering the atrial aspect of the atrioventricular node to form part of the so-called transitional cells.31,32 The center of the spine (the mesodermal core in Figure 12 of Reference 31), in contrast, does not myocardialize but remains as the tendon of Todaro.

The location of the spine also accounts for the purported formation of the secondary septum of the atrium. Shown in many textbooks as a complete muscular partition, the cranial margin of this purported septum is no more than a deep infolding between the systemic and pulmonary venous sines. Its ventro-caudal margin, forming the ventral rim of the oval fossa, in contrast, is a true septal structure. It is derived from the myocardialized vestibular spine, with the venous valves attached to its right atrial margin and the tendon of Todaro extending through its core.

**Implications for Cardiac Malformations**

Congenital malformations (Figure 7), as explained by embryologists, are often considered to arise from developmental arrest. Our analysis of the development of the junctions elucidates the pathogenesis of several well-known malformations of this region. The spine, atrioventricular canal myocardium, and endocardial cushions are all crucial in development of the atrioventricular junctions and their environs. The spine closes the primary atrial foramen and the canal myocardium forms the atrial vestibules, whereas the endocardial cushions form the smooth atrial aspect of the atrioventricular valvar leaflets11 along with the central fibrous body.19
As previously argued, persistence of the situation existing before septation (in other words, absence of the remodeling of the inner curvature to establish the right atrioventricular connection) leads to double-inlet left ventricle. Normal expansion of the atrial connections of the canal but failure of expansion of its ventricular connections results in tricuspid atresia of the classic type, which is closely related to double-inlet left ventricle. In this malformation, the smooth myocardial floor of the blind-ending atrium represents the vestibule or, in other words, the canal myocardium. This condition resembles the heart in the 6th week of development. The normal closure of the primary atrial foramen and relatively normal position of the atrioventricular node indicate that development of the vestibular spine is normal in the setting of tricuspid atresia. Incomplete expansion of the ventricular connections of the canal muscle produces overriding and straddling of the tricuspid valve, intermediate between double-inlet and the normal heart. Significantly, the tricuspid valve overrides the caudal part of the muscular ventricular septum, this being the part of the septum that is last to form. In Ebstein malformation, the development of the junctional myocardium is almost certainly normal. The valvar complex, however, fails to delaminate properly from the ventricular trabeculations, with failure of sculpting of the papillary muscle from the trabeculations. Possibly as a consequence, the caudal postpapillary opening of the valve does not develop in the floor of the muscular gully.

The atrioventricular cushions themselves can fail to fuse to different degrees. Clefting of the aortic leaflet of the mitral valve is an example of partial fusion. Because the primary atrial foramen is closed and the muscular junction is normally separated, it can be considered an isolated malformation. Failure of fusion of the atrioventricular cushions, however, can also be seen together with a common atrioventricular junction and deficient atrioventricular septation. However, in this situation, as seen in the embryo at 7 weeks, the aorta has yet to be transferred to the left ventricle. In most definitive examples with atrioventricular septal defect, in contrast, the aorta is exclusively connected within the left ventricle. Thus, atrioventricular septal defect coexisting with common atrioventricular junction is much more than simple arrested development. In mouse models, incomplete fusion of the cushions and persistence of the primary atrial foramen also occur in conjunction with double-inlet left ventricle. These combinations are usually associated with deficient development of the vestibular spine. The malformations seen in mice and humans, therefore, suggest that the spine is needed not only for the closure of the primary atrial foramen but also for proper morphogenesis of the adjacent atrioventricular canal.

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References


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