Mechanisms of Deficient Cardiac Septation in the Mouse With Trisomy 16

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Abstract—It used to be thought that the atrioventricular septum was predominantly the product of the atrioventricular endocardial cushions. In a previous study, we have shown that multiple developmental primordia are of importance in its formation. With this in mind, we have evaluated cardiac morphogenesis in the mouse with trisomy 16, an animal model with a high incidence of atrioventricular septal defects. Normal and trisomic fetuses from an Rb(11.16)2H/Rb(16.17)7Bnr×C57BL/6J cross were collected on days 10 to 15 of gestation and examined by scanning electron microscopy and histological serial sectioning. No evidence was found to suggest that atrioventricular septal defect could be explained simply on the basis of “failure of fusion” between the atrioventricular endocardial cushions. Rather, our findings supported two other developmental elements as being important in the genesis of atrioventricular septal defect. The first is an alteration in the configuration of the heart tube, with inadequate remodeling of the inner heart curvature. This resulted in the failure of the atrioventricular junction to expand to the right, with subsequent malalignment of the atrioventricular endocardial cushions with the proximal outflow cushions. The second is a variability in the connection of the primary atrial cardiac segment to the body of the embryo, the so-called dorsal mesocardium, which influences its relationship to the extracardiac mediastinal mesoderm. There appeared little difference in the connection between normal and trisomic embryos at the stage of 20 to 25 somites, but the area subsequently showed marked changes. In most trisomic embryos, the connection with the mediastinal mesoderm of the body was over a larger area than seen in normal embryos. As this area of attachment encloses the pulmonary pit, the entry point of the pulmonary vein, this gives potential for variation in the connection of the pulmonary vein. In addition, in the majority of trisomic embryos, the right pulmonary ridge (the spina vestibuli) did not accumulate extracardiac mesoderm, nor did it undergo the pronounced forward growth seen in normal embryos of equivalent stages. Consequently, the trisomic embryos show incomplete formation of both the atrial and the atrioventricular septal structures. (Circ Res. 1999;84:897-905.)

Key Words: mouse ■ heart ■ trisomy ■ atrioventricular septal defect ■ spina vestibuli

Although it has been suggested by some1 that failure of fusion of the endocardial cushions is the primary reason for formation of the group of congenital cardiac malformations known as atrioventricular canal malformations,2 or atrioventricular septal defects,3 it has become increasingly apparent that other developmental primordia are of importance in formation of the normal atrioventricular septal structures, and by extension, of significance in development of hearts that lack these structures. It was Tasaka et al4 who re-emphasized the significance of the “spina vestibuli” in septal formation, this structure having initially been described by His as long ago as 1880.5 In a recent study of the development of the normal atrioventricular septal structures in the murine heart,6 we demonstrated that a central mesenchymal mass incorporates not only the atrioventricular endocardial cushions but also contributions from mesenchyme on the leading edge of the primary atrial septum and from a cap of mesenchyme on the spina vestibuli. We also highlighted the complex nature of the connection of the primary atrial component of the heart tube to the body of the embryo (often described as the dorsal mesocardium) and defined a pivotal role for the spina vestibuli, along with its associated extracardiac mediastinal tissue, in the formation of both the atrioventricular junction and the associated septal structures.6,7 Another mechanism that we identified as playing a role in separating the atrial from the ventricular chambers was the remodeling of the initial cardiac loop, this being necessary to align appropriately the muscular atrial and ventricular septal structures.
We have also studied the hearts of mice with trisomy 16, a model known to produce severe malformations in development of the atrioventricular septal area. Our initial study concentrated on the hearts obtained from malformed mice close to term. We were able to categorize the atrioventricular septal defects into two types. In one, the common atrioventricular junction was separated into right and left orifices by a tongue of tissue joining two valvar leaflets that bridged, to varying extent, the ventricular septum. In the second, a common atrioventricular junction was connected exclusively to the left ventricle. All hearts had ostium primum atrial and ventricular septal defects, together with abnormal ventriculoarterial connections. Although not typical of the lesions seen most frequently in humans with atrioventricular septal defects and common atrioventricular junction, the lesions identified fitted within the known spectrum of “atrioventricular canal malformations.” We have now extended our investigation to study the earlier stages of development. Here we show that all of the primordia identified in our earlier study as involved in normal septation are abnormal to a greater or lesser extent in the malformed hearts. In this report, we describe these findings and discuss their potential significance for concepts of formation of hearts with deficient atrioventricular septation.

Materials and Methods
Mice with trisomy 16 were generated by mating Rb(11.16)2H/Rb(16.17)7Bnr males overnight with C57BL/6J females, a breeding regime that produces normal and trisomic embryos within the same litter. The presence of a copulation plug the next morning was taken as evidence of successful mating, and this was designated day 1 of gestation. A temporal series of 47 trisomic embryos, at least 6 for each stage, were collected on days 10 to 15 of gestation. All embryos were examined under a stereomicroscope to determine their gross morphology. Embryos up to, and including, 11 days gestation were staged by counting the number of somite pairs. The yolk sacs of embryos collected on days 10 to 12 of gestation were reserved for karyotyping by the acetic acid disaggregation method, whereas the embryos themselves were immediately fixed by immersion in 2% glutaraldehyde and 1% formaldehyde and buffered with 0.05 mol/L sodium cacodylate, pH 7.4 (adjusted to 330 mOsm with sodium chloride) for a minimum of 2 hours at room temperature. Trisomic embryos collected on day 13 of gestation or above were identified on the basis of their phenotypic characteristics. These embryos were fixed by perfusion, followed by overnight immersion in the same fixative. The abnormal embryos were compared and contrasted with a similar series of normal embryos previously described and reported.

Serial Sections
After fixation, embryos were rinsed, dehydrated, embedded in paraffin wax, and serially sectioned on a nominal thickness of 5 μm. The smallest embryos (<50 somites) were embedded in agarose before processing to facilitate subsequent orientation. Once cut and mounted, sections were dewaxed, rehydrated, stained with Masson stain, dehydrated, and coverslipped using dibutyl, polystyrene, xylene mounting medium. Micrographs of the sections were taken using a Zeiss D-7082 transmitted-light photomicroscope.

Scanning Electron Microscopy
Selected embryos, at least 3 for each stage, were prepared for examination by scanning electron microscopy. After fixation, microdissection was done by hand under a stereomicroscope, using iridectomy scissors. All samples were postfixed in 1% osmium tetroxide dehydrated through a graded series of alcohols, critical point dried using liquid carbon dioxide, mounted on stubs, and then gold sputter coated. Samples were viewed on a Zeiss SM940 scanning electron microscope at an accelerating voltage of 25 kV.

Results
Connection of the Atrial Cardiac Segment to the Body
At the stage of 23 somites, on day 10 of gestation, the trisomic embryos appear remarkably similar to their normal counterparts in terms of the attachment of the venous pole of the heart to the body of the embryo (Figure 1). The venous pole is attached by reflections of the atrial wall that are continuous with the epithelium lining the coelomic space. When viewed from inside the heart, the myocardial reflections of the atrial wall form the pulmonary ridges that bound the presumptive pulmonary portal, which we call the pulmonary pit (Figure 1A and 1C, solid arrow). The pit is positioned in the midline at this stage. More caudally, the right and left sinus horns enter the atrial segment, their respective mouths embedded in mediastinal mesoderm, with their point of bifurcation also being in the midline of the embryo (Figure 1B and 1D, open arrow). The horns of the systemic venous sinus are, therefore, symmetrical at this stage, with the so-called sinus septum separating them.

With further growth, three significant differences in this connection become apparent between trisomic and normal embryos. The first is the difference in the extent of the myocardial-mediastinal connection. The second reflects the amount of extracardiac mediastinal mesoderm that infiltrates the heart. The third is the relative difference in the placement of this extracardiac mesoderm in trisomic as opposed to normal embryos. Sagittal sections taken through embryos of 40 to 43 somites demonstrate these differences. In a normal embryo (Figure 2A), the primary atrial septum (septum primum) is seen extending well into the atrial cavity, with a cap of mesenchyme on its leading edge. The upper part of the primary atrial septum has broken down to form the secondary foramen (ostium secundum). The contiguity with the mediastinal mesoderm is limited to the region of the pulmonary ridges, which are positioned immediately caudal to the primary atrial septum. In contrast, the area of contiguity between the posterior wall of the atrium and the developing mediastinum around the pulmonary pit is more extensive in trisomic than in normal embryos (Figure 2B and 2C). The primary septum is less well formed in the trisomic embryos, although a mesenchymal cap is still recognizable on its leading edge (Figure 2B and 2C). Moreover, the axial mesodermal cells appear to infiltrate the posterior wall of the atrial septum in some trisomic samples (Figure 2C), instead of the right pulmonary ridge, as is normally seen (Figure 2A).

Formation of the Interatrial Structures
When the heart loop is removed to give an internal view of the primary atrium, differences in the developing interatrial structures also become apparent. In normal embryos, by 34 somites, the systemic venous sinus (the sinus venosus) is situated exclusively within the right atrium, although atrial septation is not yet complete. Its margins are clearly demar-
cated from the remainder of the atrium by the venous valves (Figure 3A). The anlage of the primary atrial septum is seen as a broad ridge immediately cranial to the pulmonary ridges that bound the prospective pulmonary portal. The right pulmonary ridge (ie, the spina vestibuli) has increased in size because of an accumulation of extracardiac mesoderm. It protrudes ventrally, toward the atrioventricular junction, to abut the inferior endocardial cushion. A lateral view of a 36-somite normal embryo shows the primary atrial septum growing toward the atrioventricular canal, whereas, more caudally, the spina vestibuli is in continuity with the primary atrial septum and the leftward margin of the left venous valve (Figure 4A).

The differences between normal and trisomic embryos are most marked in the region of the pulmonary pit. By the stage of 34 somites in normal hearts (Figure 3A), the pulmonary portal is slitlike, being positioned to the left of the spina vestibuli at the dorsocaudal margin of the primary atrial septum. In trisomic embryos of similar stage, the size and position of the pit were variable, but typically the pit was round and broad (Figure 3B). By the time the pit had become luminized, the opening of the pulmonary vein was often located more cranially in the posterior atrial wall (Figure 5D). Development of the left venous valve was also delayed (compare Figure 3A and 3B). Crucially, few trisomic embryos showed any development of the right pulmonary ridge, ie, the spina vestibuli (Figure 3B), even at later stages (Figure 4B and 4D).

By the stage of 48 somites, changes are increasingly evident in the arrangement of the venous valves. In normal embryos, the mesenchymal cap on the spina vestibuli fuses with the inferior endocardial cushion, forming the leftward commissure of the venous valves (Figure 4C). The left venous valve has become more evident in the trisomic embryos by the same stage (Figure 4D), but unlike the normal arrangement, the valves are widely separate at their leftward caudal margin (compare Figure 4C and 4D). Again, there is markedly diminished formation of the primary atrial septum, and apparent absence of the spina vestibuli.

The differences in atrial structure seen in embryos of 45 to 50 somites set the foundation for the final structure of the heart. In normal embryos, the primary atrial foramen is almost closed (Figure 5A), with the upper margin of the primary septum fenestrated to form the secondary interatrial foramen (Figure 5B). The entrance of the pulmonary vein is seen as a slitlike channel at the caudal margin of the atrial septum, adjacent to the atrioventricular junction (Figure 5B). In contrast, atrial septation in the trisomic embryos is rudimentary (Figures 5C and 5D), with little forward growth of the primary atrial septum and no appearance of a secondary interatrial foramen. The entrance of the pulmonary vein is positioned more cranially in the posterior wall of the left side of the atrial septum, adjacent to the primary septum (Figure 5D).

**Alignment of the Cardiac Chambers**

Although it can be seen that abnormalities in the development of the spina vestibuli play a major role in the genesis of atrioventricular septal defect in the trisomic mice, changes are also evident because of the abnormal config-
uration of the heart loop. The heart tube of the trisomic embryos differs in two ways. One is the lack of remodeling of the inner heart curvature. This alters the position of the atrioventricular junction relative to the muscular ventricular septum. Consequently, the atrioventricular junction is either exclusively contained within the left ventricle (Figure 6C) or else extends only marginally toward the right ventricle. In normal hearts, by the stage of 45 somites, remodeling of the inner heart curvature results in the right atrioventricular channel being positioned to the right of the developing ventricular septal crest, thus dividing the ventricular inlets. The inferior atrioventricular cushion straddles the developing ventricular septum, whereas the superior endocardial cushion bridges the right atrioventricular channel, having continuity with mesenchyme that lines the inner heart curvature (Figure 6A). This mesenchymal continuity (Figure 6A, white arrow) forms a crucial component of the developing subaortic outflow tract.

The other difference in the configuration of the heart tube in trisomic embryos is due to the caudal deflection of the atrioventricular canal, which was clearly seen by the stage of 40 to 43 somites (compare Figure 2A with Figure 2B and 2C). This alters the position of the atrioventricular endocardial cushions relative to the proximal portion of the outflow cushions. In normal mice, as already mentioned, there is a mesenchymal continuity (Figure 6A, white arrow) between the superior atrioventricular endocardial cushion and the mesenchyme that lines the inner heart curvature (Figure 6A, asterisk). This mesenchyme appears to be an extension of the dextrodorsal outflow cushion, being separated from it by a furrow. In contrast, few trisomic embryos attain correct mesenchymal continuity between the superior atrioventricular endocardial cushion and the mesenchymal extension of the dextrodorsal outflow cushion (Figure 6C).

**Discussion**

The embryos examined within our temporal series were of diverse morphology, as would be expected, given the variability in the morphology of the atrioventricular junction seen in the trisomic fetuses examined close to term. Significantly, no evidence was found to suggest that the variation in the atrioventricular junctional arrangement could be explained simply on the basis of “failure of fusion” between the atrioventricular endocardial cushions. Instead, this series

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**Figure 2.** Parasagittal sections demonstrate the connection of the atrial segment of the heart to the body of the embryo. A, Section through a 43-somite normal embryo demonstrates the extent of this connection in the normal situation (between arrowheads). Micrographs in panels B and C show sections through a 40-somite trisomic embryo and a 42-somite trisomic embryo, respectively. Note the extensive area of connection between the atrium and the body in the embryos (between arrowheads). In both, the primary atrial septum is underdeveloped, and there is no secondary atrial foramen. In both embryos the ventricular loop is displaced caudally. The embryo depicted in panel B shows an accumulation of extracardiac mesoderm within the fabric of the primary atrial septum (MC, arrow). Note also that the plane of section no longer transects the atrioventricular junction in the trisomic embryos, reflecting the altered architecture of the ventricular loop. IEC indicates inferior atrioventricular endocardial cushion; MC, mesenchymal cap; SEC, superior atrioventricular endocardial cushion; OP, ostium primum; and OS, ostium secundum. Scale bar = 100 μm.
highlighted in particular a role for at least two additional developmental elements in the cardiac dysmorphogenesis associated with trisomy 16. One is a variability in the connection of the atrial cardiac segment to the body, which influences its relationship to the extracardiac mesoderm of the body stalk. Another is the consequence of an alteration in the configuration of the heart tube.

**Connection of the Atrial Cardiac Segment to the Body**

The atrial segment of the heart tube is initially connected to the body of the embryo by reflections of the atrial wall, the so-called dorsal mesocardium. This area of attachment encloses the pulmonary pit, the entry point of the pulmonary vein, which is positioned immediately cranial to the orifices of the systemic venous tributaries (the sinus horns). There was little difference in the systemic venous connection between normal and trisomic embryos at the stage of 20 to 25 somites, but the area subsequently showed marked changes. In most of the trisomic embryos, the connection with the mediastinal mesoderm was over a larger area than seen in normal embryos. This often resulted in a more cranial point of entry for the pulmonary vein, where its portal of entry could be found within the cranial margin of the primary atrial septum. In addition, the right pulmonary ridge (the spina vestibuli) did not accumulate extracardiac mesoderm, nor did

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**Figure 3.** Scanning electron micrographs in which the heart tube has been removed at the level of the atrioventricular canal to reveal the atrial structures. A, 34-somite normal embryo. Venous valves are evident, distinguishing the systemic venous sinus from the primary atrium. The right venous valve is longer than the left. The right pulmonary ridge, or spina vestibuli (*), forms a marked elevation to the right of the slitlike pulmonary pit. B, In contrast, both the spina vestibuli and the left venous valve are lacking in this 33-somite trisomic embryo. The pulmonary pit forms a large, round depression in the dorsal wall of the atrium. PAS indicates primary atrial septum; LVV and RVV, left and right venous valves, respectively; and LA and RA, left and right atria, respectively. Scale bars = 50 µm.

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**Figure 4.** A, Right lateral atrial view of a 36-somite normal embryo. The anlage of the primary atrial septum is seen growing toward the atrioventricular canal. The venous valves demarcate the opening of the systemic venous sinus, and the left venous valve is seen in continuity with the elevation that forms the spina vestibuli (*). The spina vestibuli is situated at the caudal margin of the developing primary atrial septum. B, 42-somite trisomic embryo in which the right venous valve has been removed to reveal the venous "pouch" or saccus reuniens that forms at the confluence of the caval veins. The primary atrial septum remains underdeveloped, and the spina vestibuli is lacking. C, Frontal view of a normal 48-somite embryo in which the heart tube has been removed at the level of the atrioventricular junction. The spina vestibuli has fused with the inferior endocardial cushion to form the leftward commissure of the venous valves. The point of fusion, indicated, was cut during dissection. Note the cap of mesenchyme visible on the leading edge of the primary atrial septum. The dorsocranial margin of the primary atrial septum has broken down to form the secondary atrial foramen. D, In contrast, the primary atrial septum is underdeveloped in a 48-somite trisomic embryo. The spina vestibuli is not apparent, and there is no secondary foramen present within the fabric of the primary atrial septum. Both venous valves are evident, but they have not come together at their caudal margin to form a leftward commissure. PAS indicates primary atrial septum; VV, venous valves; SR, saccus reuniens; LVV and RVV, left and right venous valves, respectively; and RA and LA, right and left atria, respectively. Scale bars = 50 µm.
it undergo the pronounced forward growth seen in normal embryos of equivalent stages.

**Venous Valves**

The mice in our trisomic series showed delayed formation of the venous valves and failure of formation of the leftward valvar commissure. As development progressed, the course of the left superior caval vein in trisomic embryos was more oblique than normal, entering the so-called saccus reuniens caudal to the atrioventricular junction. This resulted in an abnormal positioning of the venous valves and the sinus septum, with the sinus septum retaining a craniocaudal position, more akin to the position it occupied in normal embryos before the rotation of the sinus horns (data not shown). This altered connection also gave rise to anomalous venous valves. The right venous valve tended to be shorter in the trisomic embryos than in their normal counterparts. In contrast, the left venous valve was consistently found to be longer than normal. As a consequence, the left and right venous valves were of equal length in the trisomic embryos.

**Primary Atrial Septum**

The atrial septum may be considered to be formed from 3 separate elements in the mouse, although in reality they form in concert. One element is the muscular primary atrial septum (septum primum), which grows forward toward the atrioventricular canal from the dorsalcranial wall of the primary atrium. The second is the right pulmonary ridge (spina vestibuli), which forms the caudal margin of the primary atrial septum and fuses with the inferior endocardial cushion. The third element is the so-called septum secundum, in reality a muscular infolding of the atrial wall to the right of the primary atrial septum, which plays a role in the closure of the ostium secundum at the end of the fetal period. It appears that it is the contribution of extracardiac mesenchyme to the spina vestibuli that is underdeveloped or lacking in the trisomic mice, this being a major contributing factor in the “ostium primum” defects seen in these animals. This confirms the preliminary observations of Tasaka cited by Markwald et al.12

Markwald et al,12 however, hypothesized that extracardiac mesenchyme enters the posterior atrial wall at the base of the primary septum and then migrates along the leading edge of the septum to contact the atrioventricular endocardial cushions, with the atrial myocardium trailing behind. In this scenario, the mesenchymal cap seen on the leading edge of the primary atrial septum would have an extracardiac origin. In the trisomic mice we examined, all had a substantial cap of mesenchyme on the leading edge of the primary atrial septum, although they lacked their spina vestibuli. The mesenchyme on the leading edge of the atrial septum, nonetheless, showed the same continuity with the mesenchyme of the superior endocardial cushion as was seen in the normal embryos. Its continuity was lost only at the caudal margin of the septum because of the absence of the spina vestibuli. In normal embryos, the spina vestibuli projects forward toward the atrioventricular canal, where it fuses with the inferior endocardial cushion. The left margin of the pulmonary pit does not develop further but retains a more dorsal position within the atrium. This would suggest that the mesenchyme on the leading

**Figure 5.** A, Rightward view of a 49-somite normal embryo in which the primary atrial septum can be seen as a broad ribbonlike structure. The secondary foramen is patent, and the leading edge of the primary atrial septum is approaching the atrioventricular canal to close the ostium primum. B, Left atrial view of a 48-somite normal embryo, showing the primary atrial septum with the entrance of the pulmonary vein at its caudal connection with the floor of the atrium. C, Right atrial view of a 48-somite trisomic embryo showing an underdeveloped primary atrial septum (its leading edge denoted by white arrows) with no secondary foramen visible within its dorsal cranial margin. D, Left atrial view of a 47-somite trisomic embryo also showing an underdeveloped primary atrial septum. Note the more cranial position of the entrance of the pulmonary vein. OP indicates ostium primum; OS, ostium secundum; PAS, primary atrial septum; RV, right ventricle; VV, venous valve; and PV, pulmonary vein. Scale bar=100 μm.
edge of the atrial septum forms, in situ, as the result of local transformation of endocardial cells, rather than having its origin in the extracardiac mediastinal mesoderm of the body of the embryo.

As yet, there are no specific markers for the extracardiac tissues of the spina vestibuli. The cellular morphology of this tissue is distinguishable from that of the cardiac mesenchymal tissue when stained with Masson trichrome, the former being denser and darker staining. Examination of serial sections showed that, in the normal embryos, the extracardiac mesenchyme appeared to be restricted to the level of the pulmonary ridges at which the myocardium had connection to the mediastinal mesoderm. In the trisomic embryos, in contrast, the more extensive area of connection of the atrial segment to the body resulted in ectopic positioning of the extracardiac mesoderm within the primary atrial septum itself, rather than being restricted to the area bounding the pulmonary pit.

It appears, therefore, that incorrect formation of the spina vestibuli has a number of consequences. Not only does the primary foramen not close, but the primary atrial septum itself remains underdeveloped, with the secondary foramen often absent. In normal mice, the ingrowth of the spina vestibuli induces a conformational change in the atrial aspect of the superior endocardial cushion at the point at which it has continuity with the primary atrial septum. In trisomic mice, the ectopic position of the extracardiac mesoderm, coupled with the underdevelopment of the spina vestibuli, often precludes a comparable conformational change. Consequently, the atrioventricular septum, and the leftward commissure of the venous valves, fail to form. In these cases, the atrioventricular junction appears “sprung,” paralleling the common atrioventricular junction as seen in the setting of human atrioventricular septal defect.

Alignment of the Cardiac Chambers and Expansion of the Right Atrioventricular Junction

All of the trisomic embryos examined exhibited a dextrally looped heart, although, as we have described, by day 10 of gestation there was significant alteration in its configuration when compared with that of normal embryos of the same developmental stage. In normal embryos, there appears to be an important phase of development in which the inner heart curvature is remodeled, thereby bringing the outflow tract ventral to the atrioventricular junction. This occurs concomitant with the expansion of the right atrioventricular junction and formation of the right ventricle. In trisomic embryos, it appears that remodeling of the inner heart curvature does not occur to the same extent, giving rise to an accentuated inner heart curvature, or a more “relaxed” heart loop, although we have not monitored directly the processes involved. The degree of abnormality seen in the configuration of the loop appears to be reflected in the extent to which the atrioventricular junction gains access to the right ventricle. It seems that those embryos with the most relaxed heart loop retain an atrioventricular junction that is exclusively committed to the left ventricle, along with either double-outlet right ventricle or an aorta that overrides the crest of the ventricular septum. This remodeling of the inner heart curvature is independent of the direction of the initial heart loop, as, in
other strains, one can see perfectly formed mirror-imaged hearts.

In trisomic embryos, the abnormal configuration of the heart tube alters both the relative orientation of the endocardial cushions within the atrioventricular canal and their relationship to other cardiac structures. In particular, the relationship of the atrioventricular endocardial cushions with respect to the proximal portion of the outflow cushions is altered. Moreover, the superior and inferior valvar leaflets of the common junction, which originate from the superior and inferior endocardial cushions, respectively, do not bridge the ventricular septum but originate from the septal crest and remain predominantly or exclusively within the left ventricle.9

Examination of the series of trisomic embryos suggests that the greater the reduction in the inner heart curvature, the closer the endocardial cushions are to attaining their normal orientation within the atrioventricular canal. Of course, this is an oversimplification, as cardiac morphology is influenced by other factors, such as the flow of blood through the heart. In this respect, the relationship of the crest of the developing muscular ventricular septum to the right-hand atrioventricular channel is crucial. All of the trisomic hearts examined displayed hypoplasia of the right ventricle, with a variable degree of severity. A more extensive commitment of the atrioventricular junction to the right ventricle appeared to result in a less hypoplastic right ventricle. This may be a reflection of the altered patterns of flow of blood in these embryos. Although we have not studied these patterns experimentally, it is clear that, unless the crest of the developing ventricular septum is to the left of the right atrioventricular channel, as it is in normal mice, flow will be severely impaired, perhaps leading to hypoplasia of the right ventricle.

Do the Endocardial Cushions Play a Role in the Cardiac Defects Seen in Trisomy 16?

In those hearts in which the atrioventricular junction has connection to the right ventricle, the bridging leaflets of the common atrioventricular valve are fused to each other. This would suggest that the defect seen in these hearts is not caused by failure of fusion of the atrioventricular endocardial cushions per se. Previous studies by Hilgen et al17 suggest that 1 of the reasons for failure of fusion of the endocardial cushions in the trisomy 16 mouse is their elongated shape, a feature that was reported as “hypoplastic cushions” by Miyabara.18 We have seen no evidence that the atrioventricular endocardial cushions are hypoplastic at any stage in the mouse with trisomy 16;11 although they do become dysplastic. The elongated shape of the cushions may, in part, result from the caudal displacement of the heart loop. We have shown that there is fusion between the atrioventricular endocardial cushions in those trisomic mice in which the right atrioventricular junction has a degree of rightward expansion.7 It is, therefore, questionable whether the trisomic mice exhibit a primary defect in the process of mesenchymal fusion.

The common atrioventricular junction in mice with trisomy 16 has been demonstrated by Miyabara et al.8,18,19,20 but they did not describe the more complex range of defects we have presented here. Study of the trisomic mice has served to highlight the complexity of cardiac septation and to indicate the temporal sequence of some of the processes involved. For correct septation, the primary septal structures of the ventricles and atria have first to be correctly aligned, an alignment that depends heavily on the remodeling of the inner heart curvature and ingrowth of the spina vestibuli. Isolation of the cardiac chambers, division of the outflow tract, and formation of a central mesenchymal mass that initially divides the atrioventricular junction are brought about by fusion of the mesenchymal structures. In the case of the inlet portion of the heart, these mesenchymal structures are the atrioventricular endocardial cushions, the cap of mesenchyme on the spina vestibuli, and the mesenchyme on the crest of the primary atrial septum. The septation of the outlet portion of the heart is achieved by fusion of the proximal outflow cushions with the rightward ventricular tubercles of the atrioventricular endocardial cushions. If one or more of the elements involved is wrongly positioned, defective, or lacking, then cardiac septation may be disrupted and incomplete.

We have already shown two early events that contribute to the cardiac abnormalities found in trisomy 16 hearts, which are altered shape and volume of the endocardial cushions and malalignment of the heart tube, both apparent by the stage of 20 somites. In this study we have shown an additional, apparently independent and later change, namely the altered connection of the atrial segment of the heart to the extracardiac mesoderm. The relationship between the three types of change is not clear and requires further study.

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