Mouse Models of Congenital Heart Defects
What’s Missing?
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One of the puzzling things about genetic disruptions in mice that affect heart development is the overwhelming number of conotruncal anomalies and the infrequency with which certain other malformations are reported. For instance, venous malformations such as total anomalous pulmonary venous return (TAPVR) or partial anomalous pulmonary venous return (PAPVR) have not been reported in a mutant mouse. Coronary artery anomalies are reported infrequently, and when they are reported it is because they are obvious. Conotruncal anomalies such as common trunk, double-outlet right ventricle, and pulmonary stenosis with overriding aorta are frequently associated with mouse germ line and conditional gene deletions. Although conotruncal malformations represent the highest percentage of congenital heart defects in the human population, TAPVR and PAPVR occur in a significant number of babies. Further, coronary anomalies affecting the stems of the coronary arteries frequently are seen in conotruncal malformations in babies but are rarely reported in mouse models.

Isolated TAPVR is a rare lesion and occurs in only about 1 in 17,000 live births.1 PAPVR is much more common and has been reported in 1 in 160 individuals (0.6%) and 0.4% to 0.7% of postmortem examinations, with 90% of these associated with an atrial septal defect.2 The majority of patients with TAPVR have symptoms of cyanosis and congestion within the first year of life. If left untreated, about 80% would die. In contrast, PAPVR frequently is not discovered until adulthood and is not usually a cause of death. Although the overall incidence of PAPVR in autopsy cases has been estimated to be 0.7%,3 the true prevalence may be higher,4 as several reports have appeared with PAPVR as an incidental finding that in most of these cases is asymptomatic. PAPVR can present as an isolated structural abnormality, but it is a common finding with other cardiac abnormalities. Patients with Turner syndrome are at an increased risk for PAPVR.5

The incidence of anomalous systemic venous return is more difficult to assess. Persistent left superior vena cava (SVC) is found in 0.5% of the normal population and in 3% to 4.3% of patients with congenital heart defects. Because the cardiac pacemaker is derived from musculature of the developing SVC, anomalies of systemic venous drainage are frequently associated with cardiac rhythm abnormalities. In a study of 300 patients with rhythm abnormalities but no congenital heart defects, 4% had an associated persisting left SVC.6 Systemic venous return abnormalities also are associated with complex congenital disease. Van Mierop et al7 estimated that about 90% of patients with heterotaxy have abnormal systemic venous connections. Bilateral SVC with unroofed coronary sinus occurs in 67% patients with heterotaxy/asplenia and 13% with polysplenia on autopsy.8 Other rare variations include absent right SVC in situs solitus, right SVC to left atrium, and retroaortic innominate vein.

Coronary anomalies are understudied in the human population too; thus, we have a poor understanding of the functional consequences of coronary variabilities. It is an important problem because coronary anomalies cause 12% to 19% of deaths in athletes.9 Coronary anomalies have a high probability (26.7%) of accompanying an aortic valve defect. Interestingly, Angelini et al9 believed that coronary anomalies are underreported and understudied in the human population and that MRI surpasses conventional angiography, which fails to detect coronary anomalies, especially in patients with congenital defects.

Although coronary anomalies are usually compatible with normal prenatal myocardial development and postnatal growth and function, a coronary anomaly can lead to a suddenly occurring pathological state with disastrous consequences. In a study published in 1992, an incidence rate of 80 in 13,010 adults (0.6%) undergoing coronary arteriography showed coronary anomalies.10 In a more recent study of 12,059 individuals, 100 (0.8%) were diagnosed with primary congenital coronary anomalies.11 Ninety-five percent had anomalies of origin and distribution, whereas 5% had coronary artery fistulae. The most common of the anomalous vessels (48%) was left main coronary artery. Anomalous origination of the left coronary artery from the right sinus is consistently related to sudden death.9 Anomalous right coronary artery was the second most common (22%) anomaly, with anomalous circumflex artery (17%) and coronary artery fistulae (5%) following.11

Few animal models of coronary anomalies exist. The inbred Syrian hamster is the best studied, but the anomalies have not been tested to determine the effect of the abnormality on myocardial function because of the low incidence of coronary anomalies in this model (130 detected in 1202 animals).12
An important factor in constructing animal models of human disease is that the model reflects the spectrum of features found in the human disease. Although the genetic basis of congenital heart defects has been expanding rapidly because of our ability to manipulate the mouse genome, the models generated have not always reflected their human counterparts. Therefore, any techniques that enhance our ability to visualize defects that might otherwise go unnoticed are welcome.

Why are venous and coronary malformations less commonly seen in mouse models? One significant factor is the ease with which a malformation can be assessed first in whole mount and later in sections. Conotruncal malformations are easily seen on the front of the heart without much dissection. Classifying them takes a little more effort, but the dissection still requires little skill, and getting to know them is relatively easy. As such, conotruncal malformations are “low-hanging fruit.” By contrast, identifying venous anomalies in mutant mice, many of which do not live much past E13.5 when the heart is relatively small, is hardly possible in whole mount, the easiest way to understand this complicated anatomy. The atria and both the systemic and pulmonary veins are extremely fragile and easily ripped during the dissection behind the heart. Thus, the only reliable method has been to section and reconstruct the embryos. Most laboratories do not do reconstructions because they are time consuming and require expensive software that has a steep learning curve.

Does the mouse get venous pole malformations, or is this a particularly human problem? In this issue, Degenhardt et al.13 report that the technique, they use the PlexinD1 mutant mouse. PlexinD1 is expressed in endothelial cells and colocalizes with the panendothelial marker PECAM (platelet endothelial cell adhesion molecule). The phenotype of the PlexinD1 mouse reported originally was common trunk. The atria were enlarged and thin walled, and an ectopic coronary artery was noted arising from the aorta above the coronary sinuses. In addition, the ductus arteriosus was missing.14 The surprise in this new study is that the authors were able to identify an aberrant vein connected to the left SVC in the PlexinD1 null mouse. The ability to identify abnormal venous patterns near the great veins opens a new era in our ability to create mouse models of congenital cardiovascular malformations.

Coronary anomalies also are hard to see, and this method makes it relatively easy. Interestingly, abnormal coronary artery anatomy was reported in the original PlexinD1 phenotype,14 but this is an exception. Another example that has only recently been reported is in the Tbx1 null mouse,15 which would be expected because Tbx1 is one of the genes thought to underlie the DiGeorge phenotype, which is associated with coronary anomalies. One of the early mouse models showing coronary patterning defects was connexin43 null.16 However, these few reports do not reflect the human incidence of coronary artery defects.

Obviously, the microcomputer tomography technique reported in this issue has several advantages. It appears to provide nice resolution without disturbing the anatomy. Shrinkage is always a problem in fixed and processed embryos, and this technique minimizes shrinkage, giving a much more realistic view of the heart that is particularly prone to shrinkage. Several malformations that have not been accessible before now, except to a few laboratories doing 3D reconstructions, can now be studied. However, the technique comes with its own drawbacks. These include availability and expense of the equipment as well as the expertise to run the equipment and interpret the images. The technique is limited at the early end of development to E11.5, which is 2 to 3 days before embryogenesis is over. It would be good if the technology could be extended to earlier embryogenesis (eg, E7–8). Degenhardt et al.13 report embedding the embryos in paraffin to make them immobile for imaging, which takes 2 hours. However, a low-melt agarose would be a quick-setting, clear embedding medium that would be less likely to shrink the tissue than paraffin.

Other methods such as high-resolution ultrasound17 can provide structural detail and show dynamics of cardiac activity so as to elucidate cardiac mechanics in knockout models but only at later stages of development. Additionally, episcopic fluorescent sectioning and reconstruction,18 while damaging the embryos by sectioning, can provide similar resolution to the technique demonstrated by Degenhardt et al. Finally, MRI has been used but is not as high in resolution, especially for detailing coronary anatomy as does the technique reported here.19

With the rapid advances in imaging typified by the new technique reported by Degenhardt et al.13 we can hope that animal models of human congenital heart defects, both simple and complex, will begin to reflect the variety of lesions seen in the human population. Such advances will allow us to understand the embryogenesis of the defects and to study functional significance of the defects in the long term.

Disclosures

None.

References


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