Defects were the neural crest ablation model in chick embryogenesis of any heart defects has been difficult to attain because of the lack of models in which the pathogenesis of the defect could be studied prospectively. It is not difficult to induce cardiac malformations experimentally in animals but such malformations are not consistently of the same type, even using the same experimental paradigm. It is difficult to study a particular outflow malformation in a model with only 20% or even 50% incidence of the malformation. There is another problem with outflow development that further confounds our understanding: many but not all of the outflow malformations are related to each other in that they represent more or less severe manifestations of the same developmental pathology.1

The retinoic acid model was one of the earliest teratogenic models of heart defects in the 1940s and 50s.2 A strain of keeshound dog developed by Patterson became the first genetic model to be studied extensively.3 Both of these models provided information about the morphology of some defects at various stages of development, but these models said little about the pathogenesis of the defects because of the problems mentioned above, ie, variability and low penetrance of any particular phenotype.

The first and (possibly) most informative model of heart defects was the neural crest ablation model in chick embryos.4 This model produced an incidence of 90% persistent truncus arteriosus and has become the “gold standard” for this type of defect. Although this model led to a number of misunderstandings about the defects that can be directly attributed versus those that are secondary to neural crest malfunction, it has recently led to the finding that some outflow defects may be caused by failure of addition of the outflow myocardium from a newly identified secondary heart field5 (reviewed in Kelly and Buckingham6). The spectrum of defects associated with failure of the addition of outflow myocardium includes any defect with overriding aorta, ie, tetralogy of Fallot and double outlet right ventricle. It seems pretty clear now that neural crest regulates growth factor availability in the pharynx, which in turn affects addition of myocardium from the secondary heart field to the outflow tract. Thus, it is now believed that absence of neural crest is directly associated with absence of the outflow septum in persistent truncus arteriosus, but only indirectly with malalignment defects that have overriding aorta, because the primary cause of the defects in this case is the failure of the addition of myocardium from the secondary heart field.7

However, neural crest ablation and the process of addition of myocardium to lengthen the outflow tract for proper alignment does not address a major type of outflow tract defect found clinically called transposition of the great arteries. This malformation, which is the most common cyanotic heart defect identified in the first week of life, occurs in approximately 4 in 10 000 infants.7a In transposition of the great arteries, both the aorta and pulmonary trunk are connected to the wrong ventricle. Pediatric cardiologists and pathologists refer to this as ventriculoarterial discordance: the aorta arises from the right ventricle, whereas the pulmonary trunk arises from the left ventricle. In other words, newly oxygenated blood from the lungs returns to the heart only to be sent back to the lungs rather than being delivered to the oxygen starved body. Conversely, oxygen-poor blood returns to the heart from the body and is immediately sent back to the body without being reoxygenated. In babies with this defect who survive birth there is an obligatory associated heart defect that permits the mixing of the systemic and pulmonary circulations to provide some oxygenated blood to the body. Without such a defect, the condition is quickly fatal.

The pathogenesis of transposition of the great arteries was traditionally lumped with other malalignment defects of the outflow tract like double outlet right ventricle and tetralogy of Fallot, many of which were seen after neural crest ablation. It was something of a surprise that transposition wasn’t seen in the neural crest ablation spectrum, and so most recent thought about the pathogenesis of the defect has considered it outside of any known mechanism.

The study by Costell et al8 in this issue of Circulation Research presents the most promising, if not the first, animal model of transposition of the great arteries. They show that the perlecan-null mouse has what is clinically known as “common” or “isolated” SDD transposition of the great arteries (formerly called D-transposition). For anyone who does not thrive on the minute details of heart morphology, the mention of transposition of the great arteries brings apprehension because of the complexity of classifications of the
defects. There are 3 points of reference for transposition: the position of the arterial trunks, the emergence of the arterial trunks from the ventricular chambers, and the relationship of the ventricles to the atrioventricular valves. In common transposition, the form reported by Costell et al,8 the discordance is between the ventricles and the arteries (ventriculoarterial) with no discordance between the atria and the ventricles; in other words, it is “isolated” ventriculoarterial discordance.

Transposition can also occur as congenital, physiologically corrected transposition (designated SLL or IDD transposition). This happens when the heart loops to the wrong side or the atria are reversed. These physiological corrections are mostly associated with abnormal situs, or placement of the asymmetric organs. Several animal models of transposition with altered situs are existent. One is teratogenic, our old friend retinoic acid, and several others are genetically induced. The genetic models harbor null mutations in several different genes: type IIB activin receptor and cryptic.11 However, all of these perturbations alter left-right axis determination and ventricular septation, which are defects associated with physiologically corrected transposition. Importantly, in these models transposition occurs only infrequently in the spectrum of other outflow malformations.

In contrast to the other models of transposition, which are physiologically corrected, that of Costell et al8 presents common or uncorrected transposition. The high incidence of common transposition (11 of 15 perlecan-null embryos) with intact ventricular septum (10 of 11) is not found in any other animal models. Intact septum is found with some frequency in humans with transposition.

So, what is perlecan and how does it cause transposition of the great arteries? Perlecan is a heparan sulfate proteoglycan (HSPG2) that is expressed in all basement membranes, in cartilage, and several other mesenchymal tissues during development including, according to new data presented by Costell et al,8 the cardiac neural crest. Perlecan binds growth factors and interacts with various extracellular matrix proteins and cell adhesion molecules. Because the heparan sulfate side chains bind fibroblast growth factors (FGFs), perlecan may even serve as a low-affinity receptor. If so, perlecan could modulate a number of other FGF-controlled processes.12,13

The other aspects of the phenotype in perlecan-null mice are associated with deterioration of well-formed basement membranes, suggesting that perlecan is needed for stabilization of basement membranes.14 There are two existent functional knockouts of perlecan, which involve deletion of one exon: exon 6 in the mouse reported by Costell et al8 in this issue, and exon 7 from a separate group.15 In both, either the transcript or the protein is missing, so both appear to be functionally null mutations. The phenotype of the mouse with exon 7 deletion is similar to the one with the exon 6 deletion but transposition has not been analyzed in the exon 7 deleted line.

Most of the perlecan-null embryos die by embryonic day 13.5. All of these embryos are normal in size and generally well-formed. The embryos die because the compact myocardium leaks blood into the pericardial space because it lacks stable basement membranes. A second smaller population of embryos survives the second half of gestation, but these develop osteochondrodysplasia characterized by disrupted collagen synthesis and assembly because perlecan protects cartilage extracellular matrix from degradation.15 This type of chondrodysplasia is seen in a human disease called Silverman-Handmaker type dyssegmental dysplasia, which is a lethal autosomal recessive form of dwarfism. The phenotype of the perlecan-null mouse led to the linkage of perlecan gene mutations in patients with Silverman-Handmaker dwarfism.16 Thus, the perlecan mouse has already served us well in the quest to find genetic linkages with human diseases. A cardiovascular phenotype has never been reported in patients with Silverman-Handmaker syndrome, some of whom are effectively null for perlecan. Although there is no empirical explanation for this discrepancy between the mouse and human phenotypes, it is possibly the result of functional redundancy in the human genome.

Although myocardial problems were identified in the original articles describing the phenotype of the perlecan-null mouse, cardiac malformations were not noted. The present finding of common transposition in perlecan-null mice represents a second look at the heart morphology of these embryos. Perlecan-null embryos showed no abnormalities in the morphology or placement of the lungs, liver, stomach, and spleen, which are sentinel organs for assessing normal left-right axis determination. The significance of this should now be obvious as the perlecan-null mouse represents the first animal model of common or isolated transposition.

Costell et al8 describe alterations in the mesenchyme of the outflow tract that disrupted the formation of the outflow tract cushions. These cushions are ridges that serve as a template for septation of the outflow tract. In normal development they spiral in a counterclockwise manner when viewed from above. The mesenchyme of the outflow tract has two well-described origins: endocardial cell–derived mesenchyme that arises by epithelial-to-mesenchymal transformation,17 and cells migrating from the pharyngeal arches.18 The cells that migrate from the pharyngeal arches are mostly derived from neural crest but may also be from other sources.19 Disruption of the ridge pattern in the lumen of the outflow tract results in formation of a straight outlet septum rather than a spiraling septum. The spiraling septum ensures connection of the left ventricle with the systemic circulation via the aorta and the right ventricle with the pulmonary circulation via the pulmonary trunk.

One limitation of the study by Costell et al8 is the lack of definitive demonstration that the overpopulating mesenchymal cells of the outflow tract are derived from neural crest.
and that these cells express perlecan. A major problem in studying cardiac neural crest cells in the mouse has been our lack of markers to specifically identify them. This problem was solved in the last few years with the availability of several genetically altered mouse lines with marked neural crest cells. In the absence of data from crossbreeding, one of these lines of mice with marked neural crest with the perlecan-null mutant, the authors have done the best analysis one of these lines of mice with marked neural crest with the manuscript and helpful discussions.

Resolution of the neural crest issue using appropriate lineage tracing techniques in the perlecan-null mutant mouse should yield a wealth of new information about the pathogenesis of common transposition. Regardless of the outcome, this mouse has my admiration as it has already provided more than its share of insight into human disease.

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References

1. Deleted in proof.
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