Cell Lineages and Tissue Boundaries in Cardiac Arterial and Venous Poles
Developmental Patterns, Animal Models, and Implications for Congenital Vascular Diseases

Simonetta Ausoni, Saverio Sartore

Abstract—Multiple cell populations with different embryological histories are involved in the morphogenesis of the cardiac arterial and venous poles as well as in the correct alignment and connection of the developing vessels with the cardiac chambers. Formation of the aorta and the pulmonary trunk is a complicated process orchestrated via a specific sequence of highly integrated spatiotemporal events of cell proliferation, migration, differentiation, and apoptosis. The peculiar susceptibility of this intricate cell network to be altered explains the frequency of congenital cardiovascular diseases of the arterial and venous poles. We review this topic from the “vascular point of view,” putting major emphasis on (1) the existence of different cell lineages from which smooth muscle cells of the aorticopulmonary trunk can be derived, (2) the establishment of cell/tissue boundaries in the cardiovascular connecting regions, and (3) the animal models that can mimic human congenital defects of the arterial and venous poles of the heart. (Arterioscler Thromb Vasc Biol. 2001;21:312-320.)

Key Words: congenital cardiovascular diseases ■ tissue boundaries ■ outflow tract ■ inflow tract ■ animal models

The more we treat the theories of our predecessors as myths, the more inclined we shall be to treat our own theories as dogmas.

J.B. Thornton

A high percentage of cardiovascular congenital malformations arise from an abnormal development of the great vessels and an improper alignment with the heart.1–3 Many of the cardiovascular defects that reach clinical observation are due to an abnormal development of the arterial pole, in particular, aortic arches, aorta, and pulmonary trunk. Abnormalities induced in the venous pole, on the other hand, can mostly be embryonically lethal, as supported by experimental observations in animal models,4 and are likely to be largely underestimated.

Previous reports in the field have dealt mostly with chamber specification, general heart morphogenesis, and cardiac looping. Instead, this report will discuss cell lineages and cell-signaling pathways in normal and abnormal development of the arterial and venous great vessels, inasmuch as this approach can provide more detailed information on the cell fate within the morphogenetic plan.

In the developing cardiovascular system, cell movements and the establishment of boundaries between the heart and the vessels are responsible for casting the outflow tract (OFT) and the inflow tract (IFT) of the heart, and this is why they represent the main topic of the present review. Development of the coronary vessels, derived from the proepicardium by a unique vasculogenetic process,5–7 will not be discussed because it has no primary impact on the arterial pole formation.

Pursuant to the aims mentioned above, we will highlight the following aspects: (1) Which cell lineages contribute to the formation of the arterial and venous poles of the heart? (2) How do vascular cells achieve their final identity and position with respect to cardiac cells? (3) What is the role of different cell lineages in the establishment of connections and boundaries? (4) Which “signals” control cell organization temporally and spatially? (5) Which experimental cardiovascular malformations arise from perturbations of these processes?

In the present review, we will present an updated list of animal models carrying defects of either the arterial or venous pole or both. The rapid generation of these models, thanks to the advances in gene-targeting techniques, are now allowing us to probe deeply into the molecular bases of congenital cardiovascular defects in humans and to underscore unexpected similarities and overlaps in the molecular pathways that control cardiac and vascular development.

A Snapshot of Cardiovascular Development
In the developing embryo, the arterial pole consists of an OFT connected to the aortic arch arteries,8 and the venous pole consists of an IFT connected to the vitelline veins. The OFT has an aortic sac (proximal to the aorta) and a conotruncus (proximal to the ventricle). The IFT has a sinus venosus

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(proximal to the cardinal veins) and a sinusatrial region (proximal to the atria). The OFT and the IFT are transient embryological regions that undergo profound remodeling during development and result in the formation of cardiac as well as vascular distinct structures. This is why, whenever appropriate, we prefer to use the terms arterial pole and venous pole to indicate the outlet and inlet of the heart instead of OFT and IFT. Formation of the arterial and venous poles in the embryo is a complex morphogenetic event whose detailed analysis is beyond the scope of the present review. Nonetheless, we have summarized the main embryological stages in Figure 1 to help the reader visualize the complicated processes. Figure 1 illustrates the initial differentiation of vascular and cardiac lineages in the cardiogenic area (Figure 1A), the progressive septation of the OFT into the aorta and the pulmonary trunk due to migration of neural crest cells (Figure 1B and 1C), the alignment of the great vessels and the cardiac chambers (Figure 1D), and the remodeling of the vitelline veins, umbilical veins, and cardinal veins so that finally all venous blood enters the right atrium via the superior and inferior caval veins (Figure 1C and 1D). For a detailed embryological analysis, we refer to previous articles.3,9–11

**Cell Lineages in the Cardiac Arterial and Venous Pole**

**Endothelial and Endocardial Lineages**

The arterial pole contains endocardial cells of the OFT and endothelial cells of the aortic sac and aortic arches. These distinct cell lineages are, at least in the adult, structurally and functionally distinct in terms of tissue permeability, cell-cell contact, and cell communication with adjacent compartments.12,13 Endothelial precursors of mesodermal origin initiate vasculogenesis and promote the recruitment of surrounding mesenchymal cells to form the definitive smooth muscle cells (SMCs) and fibroblasts of the vascular wall.14,15 Vascular endothelial growth factors (VEGFs) and their cognate receptors (VEGFR-1, VEGFR-2, VEGFR-3, and neuropilins), angiopoietins and their Tie receptors, platelet-derived growth factor, transforming growth factor-β (TGF-β), and the ephrin-Eph receptor system are essential for vasculogenesis and remodeling (see reviews 16,17) and act as carefully orchestrated players in terms of time, space, and dose effect. Unlike the endothelial cells of the aortic sac, endocardial cells of the OFT form 2 endocardial cushions through an epithelial-to-mesenchymal transition.18 These cushions are essential in forming the aortopulmonary septum, as demonstrated by the absence of an aortopulmonary septum in null mice lacking proper endocardial ridges, such as the Sox-4 mutants (see below).19

**SMC Lineage From Different Compartments**

The great vessels connected to the heart contain SMCs with largely diverse embryological origin. Their mesenchymal progenitor cells may be recruited from local and distant sources (Figure 2), among which are the neural crest cells. Neural crest cells migrate from the neural folds to the pharyngeal arches. Here, they separate each arch artery and aortic sac from the pharyngeal ectoderm and condense against the lumen to generate its smooth muscle wall.20–22 A subpopulation of neural crest cells invades the OFT and the base of the heart, thus forming the aortopulmonary septum, pulmonary infundibulum, aortic vestibule, and separation of the great vessels from the right and left ventricles.23 This explains why ablation of cardiac neural crest cells in the chick embryos leads to a wide variety of malformations, including common trunk and ventricular septal defects (VSDs).24,25 There is no significant neural crest cell contribution to the formation of the venous pole, even though neural crest-derived SMCs are present in the tunica media of the anterior.

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**Figure 1.** Morphogenetic events in cardiovascular development. A, The primitive endothelial vascular network and the primitive tubular heart arise from precursor cells (hemangioblast and cardiac precursors) in the cardiogenic area of the embryo. EC indicates endothelial cells; MC, mesenchymal cells; HSC, hemopoietic stem cells; ED, endocardium. B, The heart loops to the right-hand side and connects to the aortic arch arteries (AAA) in the arterial pole and to the vitelline veins (VV) in the venous pole. Neural crest cells migrate from the neural folds to the aortic arches and the aortic sac. C, Neural crest cells migrate into the OFT and contribute to the aortopulmonary septum (ventral view). Also, the endocardial cushions (C), arising from the endocardium, contribute to this process. In the venous pole, the IFT initially receives blood from the vitelline (VV), the umbilical (UV), and the cardinal veins (CV, dorsal view). RA indicates right atrium; RV, right ventricle; and LV, left ventricle. D, OFT septation and remodeling ends with the separation of the aorta and the pulmonary artery, the formation of the interventricular septum, and the formation of aortic and pulmonary valves (ventral view). IFT remodeling occurs through mechanisms of regression, and all venous blood finally enters the right atrium via the superior (SCV) and inferior (ICV) caval veins. PA indicates pulmonary artery; PV, pulmonary vein; LA, left atrium; and Ao, aorta.

Embryonic stages in panels A, B, C, and D are day 7–8, day 9.5, day 12.5, and adult, respectively, and refer to mouse development.
Recent reports indicate the endocardium, the mesothelium, and the myocardium as other possible sources of vascular SMCs, but these contributions, if any, remain speculative and will require further investigation. The fourth possible SMC origin is the endothelium. Endothelial cells transdifferentiate into SMCs and migrate into the media and adventitia in the chick dorsal aorta. Whether endothelial cell transdifferentiation contributes to form the tunica media of other vessels has yet to be investigated. SMC origin from endothelial cells can also be explained differently. Endothelial precursors share a common progenitor, the hemangioblast, with the hematopoietic stem cells (see Figure 1A). The intimate relationship between hematopoietic and vascular cells is exemplified by the common expression of the CD34 cell surface glycoproteins, by the presence of CD34+ cells in the mouse para-aortic mesenchyme, and by the absence of hematopoietic and endothelial cells in the zebrafish mutant cloche. Intra-aortic hematopoietic cells can be derived from endothelial cells and play a role in postnatal angiogenesis. Whether hematopoietic stem cells also participate in prenatal vasculogenesis is a tempting speculation, but this is still under debate.

Cardiac Lineage

Septation of the OFT ends with the formation of an outlet septum that separates the 2 great arteries and allows the aorta and the pulmonary artery to drain into the left and right ventricle, respectively. Initially, this septum is a mesenchymal structure originating from the OFT endocardial ridges, but later in development, it becomes muscular through an ingrowth of a newly formed myocardium into the mesenchymal endocardial cushions. Impaired myocardialization results in the persistence of an embryonic outlet septum and can lead to a variety of congenital heart diseases, ranging from VSD to double-outlet right ventricle (DORV). There is much evidence to indicate that myocardialization is under multiple control signals from the aortic sac mesenchyme and from the neural crest cells.

Morphological, Cellular, and Molecular Boundaries in the Arterial and Venous Pole

Formation of the great vessels at the arterial and venous pole involves multiple cell types that rearrange themselves at the correct space in the body to realize a precise morphogenetic plan. The existence of a prepattern on which the heart and the
## Animal Mutants With Defects in Cardiac Arterial and Venous Poles

<table>
<thead>
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<th>Gene</th>
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<td>74, 75</td>
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<td>9.5</td>
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<td>Tran factor</td>
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<td>Myocardium, SMCs, endothelium</td>
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<td>Tran factor</td>
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<td>Perinatal</td>
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<td>19</td>
<td>Tran factor</td>
<td>KO</td>
<td>14</td>
<td>Endocardial cushions</td>
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<td>13.5–17.5</td>
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<td>No semilunar valve formation, VSD</td>
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<tr>
<td>RAR α/γ</td>
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<td>KO double mutation</td>
<td>Viable*</td>
<td>ND</td>
<td>Aortic arch abnormalities, common trunk, VSD</td>
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<tr>
<td>ROR α</td>
<td>86</td>
<td>RA receptor</td>
<td>KO</td>
<td>13.5–14.5</td>
<td>ND</td>
<td>Common trunk or incomplete aorticpulmonary septum, DORV, pulmonary artery stenosis, VSD</td>
</tr>
<tr>
<td>ET-1</td>
<td>61</td>
<td>Cytokine</td>
<td>KO</td>
<td>Perinatal</td>
<td>Endothelium, endocardium</td>
<td>VSD, common trunk, DORV, pulmonary stenosis, aortic arch abnormalities</td>
</tr>
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<td>ETα receptor</td>
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<td>G-protein receptor</td>
<td>KO</td>
<td>Perinatal</td>
<td>NCC derivatives, myocardium</td>
<td>Aortic arch abnormalities, interrupted aortic arch</td>
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<tr>
<td>ECE-1</td>
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<td>Gap junction protein</td>
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<td>TGF-β2</td>
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<td>Perinatal, postnatal</td>
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<td>Deletion</td>
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<td>Neurofibrin-1</td>
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<td>GAP</td>
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<td>14.5</td>
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<tr>
<td>Versican (hdf)</td>
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<td>Matrix protein</td>
<td>Insertional mutation</td>
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<td>Endocardial cushions, myocardium</td>
<td>No cardiac jelly, no cushions</td>
</tr>
<tr>
<td>Hyaluronan synthase-2</td>
<td>73</td>
<td>Matrix protein</td>
<td>KO</td>
<td>9.5–10.0</td>
<td>Endocardial cushions, myocardium</td>
<td>Reduction of OFT, no cardiac jelly, no cushions</td>
</tr>
<tr>
<td>ActRIIB receptor</td>
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<td>Serine/threonine receptor</td>
<td>KO</td>
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<tr>
<td>NCC ablation (chick)</td>
<td>25, 27</td>
<td>. . .</td>
<td>. . .</td>
<td>Viable</td>
<td>. . .</td>
<td>Common trunks, TGA, DORV, VSD</td>
</tr>
<tr>
<td>Syrian hamster</td>
<td>95</td>
<td>. . .</td>
<td>Spontaneous</td>
<td>Viable</td>
<td>. . .</td>
<td>Bicuspid aortic valve, abnormal origin of the coronary arteries</td>
</tr>
<tr>
<td>iv/iv</td>
<td>96</td>
<td>. . .</td>
<td>Spontaneous</td>
<td>Viable</td>
<td>. . .</td>
<td>DORV, Fallot</td>
</tr>
</tbody>
</table>

### Defects of venous pole

| Coup-TFII | 97 | Tran factor | KO | 10 | Myocardium, vascular mesoderm | Sinus venosus, atrial malformation, cardinal vein malformations                           |
| NT-3      | 87 | Growth factor | KO | Perinatal | Endocardial cushions | Sinus venous malformation, reduction of SMCs in the pulmonary veins                      |
| iv/iv      | 96        | . . . | Spontaneous | . . . | . . . | Common sinus venosus                                                                   |

List shows animal mutants with cardiovascular defects either in the arterial or in the venous pole or in both. Defects of the arterial pole include abnormalities of the aortic arches, aortic sac, and OFT. Defects of the venous pole include abnormalities of the IFT and caval veins. Animal mutants were obtained by targeted disruption of single genes (knockout [KO]). Exceptions are the ablation of neural crest cells in the chick, spontaneous mutants iv/iv, Syrian hamster, Sploch mice and the Df1 mouse, derived by deletion of the chromosomal region homologue to human 22q11, and the hdf mouse, derived by insertional mutation. RAR α/γ is a double mutant obtained by crossing heterozygous mice for the single mutations. Survival (time) indicates time of death in the embryos, unless differently specified. Tissue expression, restricted to the cardiovascular system, may indicate either protein or mRNA distribution or both. MFH-1 indicates mesenchyme forkhead-1 gene; RAR and RXR, retinoic acid receptors; ET-1, endothelin-1; ETα, endothelin receptor A; ECE-1, endothelin-converting enzyme-1; NT-3, neurotrophin-3; Cx43, connexin43; PDGF, platelet-derived growth factor; NCC, neural crest cell; iv/iv, inversus viscerum; tran, transcription; GAP, GTPase-activating protein; ND, not precisely determined at the cell lineage level; DGS, DiGeorge syndrome; Fallot, tetralogy of Fallot; DILV, double-inlet left ventricle; and TGA, transposition of great arteries.
great vessels are built is suggested by much evidence, most of which pertains to the heart more than to vessel formation. Strict boundaries for Hox gene expression exist in the pharyngeal arches, but nothing similar has ever been demonstrated in other vessels. So far, the Hairy-related family members HRT1, HRT2, and HRT3 are the only genes that exhibit distinct expression patterns in the vascular system, with strict boundaries along the anterior-posterior axis. In zebra fish, the grid-lock mutation for the glr gene, which encodes a Hairy-type basic helix-loop-helix protein, selectively perturbs assembly of the aorta, suggesting that identity of this vessel is determined before the onset of circulation. This is in agreement with the idea that the embryonic circulatory plan is genetically established.

To create strict boundaries between the heart and specific vascular segments, integrated cellular and molecular events are required; these include the following: specific cell-cell and cell-matrix adhesion and cell migration, proliferation, differentiation, and apoptosis. In this respect, neural crest cells may be fundamental because their migration follows a precise colonization territory. In the chick, there is a strict boundary between the aortic arches, ascending aorta, and pulmonary trunk invested by neural crest cells on one side and descending aorta and pulmonary arteries that are completely devoid of neural crest cells on the other side.

What is the significance of boundaries in the embryo, and how are they formed? Tissue boundaries and, hence, morphogenetic patterning are likely to be the result of a combined effect of endogenous and exogenous “driving cues” and “positional cues” (Figure 3). The former impose the correct positional cues acting on the establishment of boundaries between the heart and the great vessels. ECM indicates extracellular matrix.

connection network, is paradigmatic. It is noteworthy that some of the molecular cues that guide neural crest cell migration and stabilization of neural patterns play a role in vasculogenesis and angiogenesis, too. Three major groups of molecules can be involved in these processes: (1) diffusible molecules, such as semaphorins and netrins, (2) membrane-bound proteins, such as the ephrins-Eph receptor system, and (3) extracellular matrix proteins. The semaphorin Sema3A is able to inhibit endothelial cell motility, capillary formation, and sprouting by competing with VEGF for the neuropilin-1 coreceptor. Other molecules, such as netrins, act as either a chemoattractant or chemorepellent in the nervous system. 

Figure 3. Schematic representation of putative driving cues and positional cues acting on the establishment of boundaries between the heart and the great vessels. ECM indicates extracellular matrix.
lap those of the fate mapping. This may occur in the establishment of cardiovascular boundaries, too. Ephrins also regulate surface density of integrins α5β1 and α6β1, which are known to have a role in early vasculogenesis.

Cell adhesion and extracellular matrix are likely to be involved in setting boundaries between 2 neighboring tissues. For example, in the OFT extracellular matrix components, such as fibronectin, elastin, and laminin, collagens I and VI have a specific temporal and spatial distribution, and versican, a cell adhesion molecule, seems to play a nonpermissive role in cell movements. Lack of versican in the mouse mutants and lack of hyaluronan synthase-2 in the embryo results in severe cardiovascular defects for impaired endocardial cushion formation. Possible consequences in septation of the OFT cannot be observed in these mutants because of early death in the littermate.

**Cell Lineage and Tissue Boundary Defects in Animal Models**

Many mouse mutants that reproduce aspects of human congenital cardiovascular defects are currently being generated. These animal models provide an exciting instrument for identifying the factors involved in cardiac morphogenesis and are extremely powerful in establishing the relationship between a genetic defect and its functional consequences in vivo. Animal models with defects in the cardiac arterial and venous pole are listed in the Table, where associated references can be found.

In this section, we will mainly focus on animal models with abnormal development of the arterial pole. Abnormalities of the venous pole will not be reviewed extensively because there are just a few examples of animal mutants with defects in this region. In addition, the origin of these defects is still a matter of discussion for clinicians and embryologists. The only animal model with an exclusive defect in the venous pole is the knockout mouse for the steroid receptor chicken ovalbumin upstream promoter transcription factor II (COUP-TFII). This mutant shows sinoatrial malformations and either ovalbumin upstream promoter transcription factor II (COUP-TFII). This mutant shows sinoatrial malformations and either.

A second observation in the interpretation of complex knockout phenotypes is that we need to distinguish, whenever possible, between primary defects and secondary defects. Primary developmental defects originating in the heart can have dramatic consequences for the vessels and vice versa. For example, DORV, characterized by the persistence of an embryonic configuration in which septated aorta and pulmonary trunk drain into the pulmonary ventricle, is associated with VSD. In other cases, malformations of the great vessels can be the consequence of cardiac defects. For example, an abnormal cardiac looping, such as in the iliv mice, can lead to transposition of the great arteries, DORV, and VSD. The interdependence between cardiac and vascular development is well illustrated in the MEF2C knockout mouse, which exhibits myocardial and endocardial defects as well as abnormal vessels and atresia of the great vessels connected to the heart. Early lethality in the littermate occurs because of congestive heart failure, which is presumably due to deficient blood circulation in the rapidly growing embryo.

Analysis of the Table suggests some general comments. The first is related to survival times. Animal mutants with abnormalities in the arterial pole die at around 3 specific periods: embryonic day 10, embryonic days 14 to 15, and perinatally. It may be that the vascular system maturation proceeds according to a spatiotemporal sequence of events, whose completion ensures the correct progression along the developmental pathway. The establishment of a morphogenetic abnormality during this process could not be tolerated if it did not guarantee a successful outcome of embryogenesis. The first decision to be made by the forming organism is related to OFT colonization by neural crest cells. The second concerns the completion of OFT septation and the muscularization of the septum. The third is the activation of pulmonary circulation. Nothing is known about the ways in which such decisions are made, and the use of the term heart failure to indicate the cause of embryonic death may be not always adequate. Some data highlight the role played by altered hemodynamic factors on inducing cardiovascular malformations. The physiological increases in blood volume, pressure, and flow with their inherent increases of shear stress and wall stretching may have a profound impact on the onset of cardiovascular abnormalities. The establishment of an abnormal pulmonary circulation is also a crucial event causing sudden death. For instance, mice lacking connexin43 die neonatally as a consequence of an obstruction of the subpulmonary OFT.

A third observation is that the same defect can be generated by mutations in different genes. For example, common trunk can be due to genes that act either on the neural crest cells or on the endocardial cushions, suggesting a functional cooperation among different cells in OFT septation. Conversely, one gene mutation can lead to a broad spectrum of abnormalities either because a gene controls the same function in multiple tissues or, more frequently, because a cellular compartment is involved in multiple functions. For example, mutations in genes of the endothelin-mediated pathway lead to aortic arch abnormalities, common trunk, and DORV, presumably because endothelin-1 released by the endothelium and the endocardium controls neural crest differentiation, endocardial cushion formation, and myocardialization, 3 closely related events.

Among the human pathologies that best reflect the spectrum of abnormalities observed in these animal mutants are the DiGeorge syndrome and velocardiofacial syndrome. The common features of these syndromes are interrupted aortic arch, OFT malformations, hypoplastic thymus, and parathyroids. Both diseases are due to haploinsufficiency of ≥1 gene in the q11.2 region of chromosome 22. The minimal critical region that is deleted in most DiGeorge patients has been mapped, but the identification of the genes involved is complicated by the fact that some patients show deletions in distinct nonoverlapping regions. Using the Cre-LoxP system, Lindsay et al generated a mouse that carries a deletion (Dfl)
homologous to the human deleted region in DiGeorge and velocardiofacial syndrome. The mouse mutant lacks 14 of the almost 30 genes of the DiGeorge critical region and recapitulates most of the human cardiovascular defects but lacks thymic, parathyroid, and craniofacial abnormalities. Thus, it is likely that the DiGeorge syndrome requires the deletion of a whole group of genes and/or regulatory elements that control multiple genes in a cluster.

**Future Directions**

Our present knowledge about the biology and embryology of OFT and IFT does not allow for the assembling of data in a definitive picture. However, the discovery of new genes and the generation of models for gene function studies in vivo are making progress in this field faster. Identification of genes that regulate commitment and differentiation of SMCs will be essential in understanding how the vascular network arises and is organized. Unfortunately, this search is still in its infancy compared with analogous studies in cardiac and skeletal muscle, but it will certainly profit from the fact that genes involved in cardiac development can also have a profound impact on vascular development. In addition, it will be important to identify (1) the genes that control arterial and venous SMC diversification and (2) the genes involved in induction and maintenance of tissue boundaries in the connecting cardiovascular regions. The lesson derived from the discovery of the ephrin-Eph receptor system for vascular endothelium clearly indicates the direction.

On the other hand, a great effort has to be devoted to the generation of new animal models. The new inducible and conditional knockouts will be extremely useful in this respect. They will help to overcome problems of genes that control some morphogenetic events but cause a premature death. In addition, they will contribute in the dissection of a function whenever a single gene controls multiple morphogenetic events. Although some caution must be used when a direct correlation between mouse mutants (eg, the DiGeorge mouse models) and human diseases is made,9,10 the availability of more models for studies in vivo is certainly the strategy of choice for disclosing the mechanisms of cardiovascular abnormalities.

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**References**


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