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HUMAN GENETICS '99: THE CARDIOVASCULAR SYSTEM
The Molecular Basis of Vascular Disorders

Jeffrey A. Towbin,[1,2] Brett Casey,[3] and John Belmont[2]

Departments of [1]Pediatrics (Cardiology), [2]Molecular and Human Genetics, and [3]Pathology, Baylor College of Medicine, Texas Children's Hospital, Houston

Cardiovascular malformations occur in a large number of hereditary or sporadic diseases. Here, we review the general pathway by which the vasculature develops, and we discuss the etiology of several groups of diseases in which cardiovascular development or maintenance go awry. Many such abnormalities are also observed in animals, including mice and zebrafish, the latter being an emerging model genetic organism that has already proved useful in the study of the development of the circulatory system. In many respects, morphogenesis in the heart and the vasculature presents challenges that are similar to corresponding events elsewhere in the body. Indeed, many of the diseases discussed here present with a range of effects in other tissues, suggesting that some of the regulatory pathways are shared. Still, blood flow is a unique feature of the heart and the vessels, and we will consider genetic and physiological evidence that blood flow helps shape these organs during development.

Development of the Vascular System

Embryonic blood vessel development (depicted in fig. 1) requires two distinct morphological events: vasculogenesis and angiogenesis. Vasculogenesis occurs by in situ differentiation of mesodermal cells—angioblasts (Cleaver and Krieg 1998)—into endothelial cells, which line the interior wall of vessels. During early embryonic development, angioblasts coalesce to form clusters, which then join to form the vessels, including the dorsal aorta, the endocardium, and the major veins (cardinal and vitelline). Hemangioblasts, also derived from embryonic mesoderm, line the floor of the anterior dorsal aorta and contribute to both the definitive hematopoietic stem cells and the aortic endothelium (Cleaver and Krieg 1998). Angiogenesis, the outgrowth and branching from existing vessels, is responsible for the elaboration of the primitive vascular structures. Remodeling plays a critical role in vessel maturation, particularly in the aortic arches in which the vessels enlarge, join, or regress. After the initial formation of a vessel, vascular walls develop by recruiting other cellular and extracellular components. Mesenchymal cells surrounding the endothelium are recruited and differentiate into vascular smooth muscle cells (Walsh et al. 1998). In the great vessels, the smooth muscle cells are derived, in part, from the neural crest, whereas in other tissues or smaller vessels, these cells derive from the surrounding mesoderm (Walsh et al. 1998).

In vitro studies and, more recently, targeted mutations in mice have helped to define some of the inductive growth factors, receptors, and transcription factors regulating vessel formation. These include fibroblast growth factors, vascular endothelial growth factor and its receptors flk-1 and flt-1, transforming growth factor β (TGF-β) (see Ring and Cho 1999 [in this issue]), and angiopoietin-1 and -2, along with one of their receptors tie-2. The phenotype of mice mutant for *Vegf* and *Flk-1* reveal that these genes are required in embryonic vasculogenesis. Mutation leads to failure of vessel coalescence, with resulting defects in both endothelial and hematopoietic development. *Tie-2* mutants develop a primitive vasculature but die in midgestation with multiple hemorrhages and abnormal cardiac development, presumably because of failure of endothelial proliferation (Vikkula et al. 1996). Endothelins and their receptors are essential for development of subsets of neural crest cell derivatives, and endothelin pathway mutations cause defects in cardiac outflow and great vessel structures derived from cephalic neural crest. These mutants demonstrate abnormal arterial regression, and the consequent disturbance of asymmetrical remodeling of the arch arteries may lead to interrupted aortic arch, hypoplasia of the aortic arch, and ventricular septal defects. Mice with a mutation in the cytosolic component of the transcription factor—nuclear factor of activated T cells (NF-ATc)—fail to develop normal cardiac valves and septa. NF-ATc expression in the early embryo is restricted to the endocardium, a specialized endothelium

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Address for correspondence and reprints: Dr. Jeffrey A. Towbin, Baylor College of Medicine, Department of Pediatric Cardiology, One Baylor Plaza, Houston, Texas 77030. E-mail: jtowbin@bcm.tmc.edu

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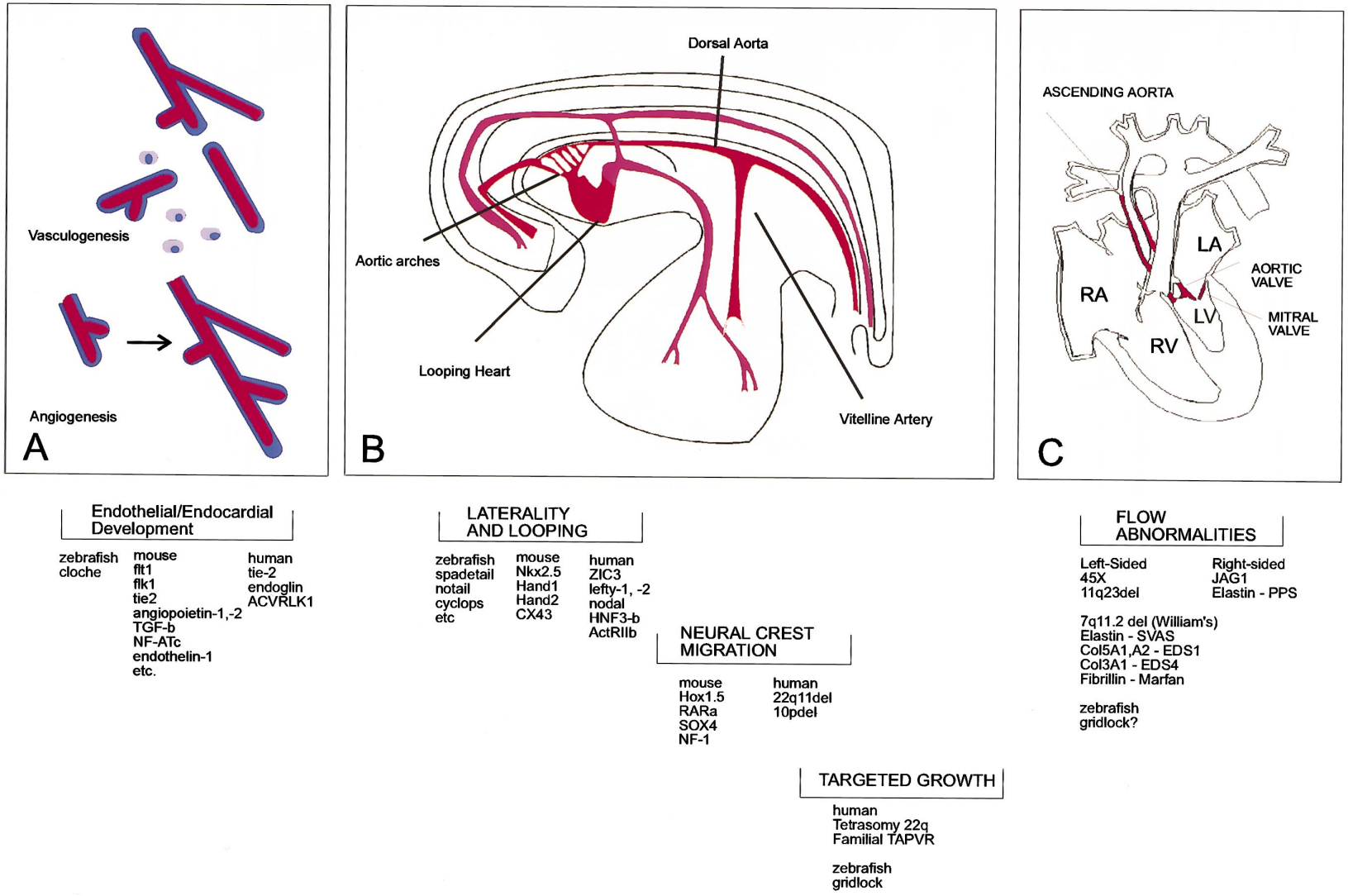

Figure 1 Genetic defects affect various stages of cardiovascular development. Mutants from zebrafish, mouse, and human relating to primary developmental processes or maintenance of the vascular system. *A*, Vasculogenesis and angiogenesis. *B*, Embryonic development of the vascular system. *C*, Obstruction of the left ventricular outflow tract leading to hypoplastic left heart syndrome. Note the small left ventricle (LV), atretic aortic and/or mitral valves, with aortic hypoplasia.

that gives rise to the valves and septum. Several zebrafish mutants also affect the vascular development. Of these, the best studied may be the *cloche* mutant, in which endocardial and endothelial development and hematopoiesis are disrupted. Animal model studies should allow the eventual definition of the most important developmental pathways and help in the identification of human homologues that contribute to cardiovascular disease, including many of those discussed in this report.

Abnormalities of the Aorta and the Left Side of the Heart

The left side of the heart and its outflow into the aorta may be affected because of obstruction and vascular diminution or dilatation of the great artery and left ventricular chamber. In most, but not all, cases, these vascular defects occur as part of a syndrome. The best-described obstructive disorder is supraventricular aortic stenosis (SVAS), which may occur as an isolated condition or as part of Williams syndrome (WS). Marfan syndrome (MFS) is the best known of the disorders associated with aortic dilatation. Other disorders, such as hypoplastic left heart syndrome (HLHS), coarctation of the aorta (COA), and Ehlers-Danlos syndrome (EDS) have significant aortic lesions.

Obstruction of the Aorta in HLHS

HLHS occurs typically as a diminutive left ventricular chamber with aortic atresia and mitral atresia. The ascending aorta is usually quite small, and the systemic circulation is dependent on a patent ductus arteriosus (fig. 1C). The most widely accepted hypothesis is that HLHS develops because of embryonic alterations in blood flow (Clark 1986; Ferencz et al. 1997).

Cardiac morphogenesis apparently requires both intrinsic processes of pattern formation and blood-flow-directed remodeling. Intracardiac streaming of the systemic venous (future right ventricle) and pulmonary arterial (future left ventricle) blood begins before ventricular septation is complete, and flow may play a role in directing the process of chamber formation (Clark 1986; Ferencz et al. 1997). The left ventricular outflow tract (LVOT) obstruction defects account for 15%–20% of congenital heart defects (CHD). LVOT obstruction lesions include aortic valve stenosis (AS), SVAS, COA, and HLHS; bicuspid aortic valve may occur in asymptomatic parents and first-degree relatives of probands with AS, COA, and HLHS. These defects appear to arise from defective flow in the LVOT during early embryogenesis. Available relative recurrence risk data for LVOT obstructive defects would be consistent with 70% of the variance on the inferred underlying liability distribution accounted for by genetic factors.

Normal blood flow is apparently required for modeling and growth of myocardium and valve, as well as the vascular structures. This hypothesis is supported by the observation in animal models that imposition of outflow tract obstruction leads to structural defects (Lyons et al. 1995). Prenatal ultrasound has given additional insight into the evolution of left ventricular growth in HLHS. The observation of worsening of left ventricular disproportion as fetal growth proceeds possibly indicates a role of obstructed flow in suppressing left ventricular growth but might also be compatible with a more complex mechanism in which an intrinsic defect in myocardial growth interacts with an increasingly severe obstruction. Several mouse mutations (Fishman and Chien 1997) affect myocardial growth and produce thin ventricular walls that may be prone to septal defects. Mouse genes implicated include *CXC, NT-3, TEF-1, WT-1, Neuregulin, ERB2,* and *ERB4,* and it was recently shown that the transcription factor NF-ATc is required for normal valvulogenesis in mice. Mutations in *endothelin 1, endothelin-A receptor, Hand2, MFH-1,* and the retinoid receptor genes can all lead to aortic arch malformations, as can *gridlock* mutations in zebrafish.

SVAS and WS

SVAS is an obstructive vascular disease with an estimated incidence of 1 in 20,000 births and autosomal dominant inheritance (Schmidt et al. 1989) or sporadic onset. Any artery of the body may be affected in inherited SVAS; it is the aortic involvement that is most often responsible for clinical outcome. Arterial lesions are variable. Pathological findings (O'Connor et al. 1985) include loss of normal parallel orientation of elastic fibers, resulting in a disordered array of elastic fibers and hypertrophied smooth muscle cells. Hyperplasia of elastic tissue in the media of the arteries usually occurs. In many cases of SVAS, the initial arterial lesion is peripheral pulmonic stenosis, which in most cases improves with time (Giddins et al. 1989). SVAS is a common finding in WS, a contiguous gene–deletion syndrome that is occasionally inherited. In addition to the cardiac malformation, individuals with WS (Morris 1998) exhibit mild mental retardation, dysmorphic facial features, hypercalcemia of infancy, and a unique personality described as a "cocktail party personality." The cardiovascular abnormalities are identical to those found in familial SVAS.

Both SVAS and WS are linked to chromosome 7q, where the elastin gene (7q11.23) maps (Morris 1998). *ELN* rearrangements, point mutations, deletions, splice site mutations, and nonsense mutations have been found in families and sporadic cases of SVAS or WS. Elastin is an extracellular matrix protein that comprises 90% of the elastic matrix that restores a vessel's shape after it has been stretched. The precise mechanism by which

haploinsufficiency for *ELN* leads to human arterial pathology is unknown. Since *Eln* knockout mice show evidence of structural abnormality of the arterial wall, the animal model could elucidate the pathogenesis of SVAS. The region surrounding *ELN* includes multiple genes that may account for the complex set of WS symptoms.

gridlock *and Aortic Coarctation*

Coarctation of the aorta is defined as an abrupt constriction situated in the aortic isthmus between the origin of the left subclavian artery proximally and the aortoductus arteriosus distally, resulting in an obliterative or obstructive "curtain lesion" (Ho and Anderson 1979). This congenital lesion is the most common congenital anomaly of the aortic arch in humans, occurring in 5%–8% of children with congenital heart defects, and can have devastating consequences in infants. Familial inheritance has been described, usually with autosomal dominant transmission. The etiology of this arterial lesion continues to remain elusive. However, insight into the potential molecular causes of this disorder was suggested by Weinstein et al. (1995), who described a genetic mutation called *gridlock* in the zebrafish.

The *gridlock* mutation is an autosomal recessive mutation brought about by saturation mutagenesis studies of the zebrafish genome. This mutation generates abnormal vascular development, specifically a failure of vascular channel formation at the site where the bilaterally paired dorsal aortae join together and continue posteriorly as a single dorsal midline channel (the medial dorsal aorta). In some genetic backgrounds, Weinstein et al. (1995) found the anomaly to be associated with development of a major arteriovenous (A-V) shunt, whereas in other backgrounds, A-V shunting failed to develop, but extensive collateral vessels were recruited to connect the anterior circulation (including the heart) with the posterior medial dorsal aorta. Blood flow to the tail was impeded by the localized vascular defect from blockage in the anterior trunk, where the paired lateral dorsal aortae normally merge to form the single midline aorta. Although not exactly the same as coarctation of the aorta (Towbin and McQuinn 1995), this mutant certainly represents a genetic locus that specifically affects a localized aspect of vascular development and provides a model system for the study of disorders of the major vasculature of humans. This model could provide supportive evidence for the most likely hypothesis of obstruction of the left side of the heart—abnormalities of blood flow.

Dilation of the Aorta: MFS and EDS

MFS, a heritable disorder of connective tissue, is caused by a defect in fibrillin protein encoded by the *FBN1* gene on chromosome 15 (15q15-q20) (Milewicz 1998). It has been estimated that MFS occurs in ~1 in 10,000 individuals, but its clinical expression is variable and may include overlap with nonpathological features (such as tall stature) that can be observed in the general population. MFS affects the skin and the skeletal, ocular, pulmonary, and central nervous systems and commonly leads to cardiovascular abnormalities, including those of the mitral apparatus and aorta. Other disorders share some of the same phenotypes; congenital contractural arachnodactyly has sometimes been described as a subtype of MFS or a separate disorder, but recent data suggest that it arises through defects in the fibrillin-2 gene on 5q23-q31 (Milewicz 1998). Fibrillin-1 is a large glycoprotein with a molecular weight of 350 kD and is a component of the microfibrils that are ubiquitous in the connective tissue space. *FBN1* defects have also been found in patients with severe neonatal MFS, and in individuals with atypical symptoms such as autosomal dominant ectopia lentis, the MASS phenotype (mitral valve, aorta, skeleton, and skin), or isolated ascending aortic aneurysm with dissection.

EDS comprises a group of conditions that present with markedly loose joints and with easy bruising of the skin. Of the four most common forms of EDS (types I–IV), type I and type IV are discussed here. The features of EDS type I include hyperextensible and fragile skin, hyperextensibility of the joints, and cardiovascular disease (De Paepe et al. 1997), particularly mitral valve prolapse, tricuspid valve prolapse, and aortic root and/or sinus of Valsalva dilation. As in MFS, in EDS the most significant cardiovascular defect is the increased susceptibility to dissecting aortic aneurysm, which can lead to death. EDS type I is inherited as an autosomal dominant disorder with variability in expression. The defect in this disorder involves synthesis of normal collagen, and mutations in the type V collagen genes *COL5A1* (De Paepe et al. 1997) and *COL5A2* genes (Michalickova et al. 1998) have been reported. In addition, mutant mice with *Col5a2* deletions have been created with phenotypic resemblance to that of EDS type I (Andrikopoulos et al. 1995). The type V collagen is qualitatively a minor component of connective tissues that are rich in type I collagen (bone, tendon, ligament, cornea, and dermis), but it appears to play a critical role in determining the diameter of the heterotypic collagen fibrils in these tissues. Abnormal collagen fibrillogenesis is responsible for the severe vessel and dermal fragility and joint laxity seen in patients because of the reduction in the amount of normal type V collagen available for collagen fibrillogenesis.

The autosomal dominant disorder EDS type IV is sometimes referred to as the malignant form of EDS, since there is marked susceptibility to spontaneous rupture of the bowel or of any of the large blood vessels. The hyperelasticity and hyperextensibility tend to be less

obvious than in EDS type I. However, easy bruising and bleeding are very prominent. The type III collagen gene (*COL3A1*) appears to underlie EDS type IV (Milewicz 1998); defects in this gene have been reported and include point mutations altering glycine residues within the triple helical domain of type III collagen, exon splicing errors deleting a single exon but usually maintaining the reading frame of the protein, or multiexon deletions.

The molecular mechanism in which *COL3A1* mutations lead to disease is felt to be a dominant negative pathogenesis, similar to that of *FBN1* mutations (Milewicz 1998). Mutations in one *COL3A1* allele lead to the production of a mutant proa1 polypeptide. Because type III collagen is a homotrimer of proa1, when abnormal proa1 is incorporated into type III collagen, the great majority of the type III collagen molecules produced will be abnormal. The abnormal type III collagen is inefficiently secreted, leading to diminished amounts of type III collagen in the matrix.

Abnormalities of the Pulmonary Artery and Right Side of the Heart

Similar to obstructive lesions in the left side of the heart, obstruction of the right side of the heart and pulmonary arteries leads to cardiovascular compromise. In addition, these abnormalities are most commonly associated with complex syndromes such as the CATCH-22 syndromes (DiGeorge syndrome (DGS)–velocardiofacial (VCF) syndrome phenotype) and Alagille syndrome.

CATCH-22 Syndromes

The combination of dysmorphic features, palatal abnormalities, thymic hypoplasia, parathyroid hypoplasia, and cardiac defects has been termed DGS (Scambler 1993). The thymic, parathyroid, and cardiac defects all result from developmental abnormalities of the third and fourth branchial arches and neural crest. The estimated frequency of this disorder is 1 in 4,000 live births. Similar disorders—VCF and conotruncal face anomaly (CTFA) syndrome—have similar findings and are clinically grouped with DGS as the so-called CATCH-22 disorders.

Defects in neural crest–derived cardiac structures are an important feature comprising ~15% of all CHD. According to the classification system of Clark (1986), the two types of defects associated with DGS (and with VCF and CTFA) are conotruncal defects and branchial arch mesenchymal tissue defects. Among conotruncal defects, truncus arteriosus is the most common type. Among the branchial arch mesenchymal tissue defects, interrupted aortic arch type b and right aortic arch are the most common. Other lesions include tetralogy of Fallot (defined as pulmonary stenosis with ventricular septal defect, overriding aorta, and right ventricular hypertrophy), double-outlet right ventricle, transposition of the great arteries, and absent pulmonary valve syndrome.

Multiple etiologies for DGS have been found, including chromosome abnormalities, single-gene defects, and teratogenic exposures. Approximately 15% of infants with DGS can be found to have obvious chromosome abnormalities, of which about two-thirds involve a monosomy 22q11. This usually results from an unbalanced translocation involving chromosome 22 and another chromosome. FISH analysis with probes from the critical region have shown that a total of ~85% of DGS patients carry deletions in 22q11 (Scambler 1993). A critical region appears to encompass at least nine genes that are found over a 480-kb interval (Funke et al. 1998). It is likely that haploinsufficiency of one or more of these genes in this interval contributes to the etiology of this disease in most patients. However, two nonoverlapping regions of chromosome 10p have also been found to be deleted in some patients with DGS, raising the possibility of genetic heterogeneity (Gottlieb et al. 1998).

No detailed pathway is yet known for cardiac neural crest migration, but several genes have been identified that may be relevant to the etiology of the CATCH-22 syndromes. Mice bearing a mutation for the homeobox transcription factor *Hoxa3* have aortic arch defects reminiscent of DGS, and a subset of *Pax3*-deficient mice have similar defects (Belmont 1998). Additional mutations in the transcription factors *Rxrα* and *Sox4* and in the *Nf1* gene give conotruncal and ventricular septation abnormalities typical of altered neural crest integration. Interestingly, various CHD have recently been noted to occur in ~1.6% (threefold relative risk) of individuals with NF1.

Alagille Syndrome

Alagille syndrome primarily affects the liver, heart, eye, and spine (Krantz et al. 1997), but 10%–20% of affected infants also exhibit cardiac defects, primarily pulmonic stenosis. This disorder appears to be inherited with autosomal dominant transmission and variable expression.

The general map location for Alagille syndrome was provided by the observation of rearrangements and deletions in 20p12. However,<10% of patients with Alagille syndrome carry such lesions, and point mutations in a human homologue of *Drosophila jagged* gene (*JAG1*) are sufficient to cause the condition (Li et al. 1997; Oda et al. 1997). *JAG1* encodes a ligand for *Notch*, a cell surface receptor that was originally defined in studies of fly wing morphogenesis. Both *jagged* and *Notch* homologues have been identified in humans and rodents. Mice deficient in NOTCH1 or Delta (another

Notch ligand) show defects in somite development that may correlate with the vertebral anomalies in observed Alagille syndrome. *JAG1* mutations have recently been identified in children with tetralogy of Fallot but with no evidence of Alagille syndrome.

Abnormalities of Laterality

In some cases, the great vessels and systemic veins are malpositioned with or without malposition of the cardiac chambers of abdominal organs. The causes of these complex abnormalities of laterality have recently begun to unfold.

Rightward looping of the midline heart tube is the first overt manifestation of anatomic left-right (LR) differences, which eventually come to include the asymmetry of the lungs and most of the unpaired organs of the abdomen (Casey 1998). Heart malformations associated with abnormal looping, therefore, usually occur as one manifestation of a more global abnormality of LR-axis development. The typical patient presents with complex structural heart anomalies, abnormalities of spleen position and/or number, and some degree of gut malrotation. The overall LR anatomy of this individual might be described as situs ambiguus, or indeterminate sidedness, in contrast to situs solitus (normal sidedness) and situs inversus (complete LR reversal). Manifestations are quite variable, however, even among affected individuals from the same family.

Heart malformations attributable to abnormal laterality represent 3.4% of all cardiac defects (1.44 in 10,000 live births) (Ferencz et al. 1997, pp. 165–225). The familial clustering of situs shows that genetics contributes significantly to these abnormalities, and several mutant mouse lines with similar defects are known (Casey 1998). Molecular genetic studies have yielded some support for this hypothesis. One locus for human situs defects was mapped to Xq26.2 (Casey 1998), where the gene for the zinc-finger transcription factor *ZIC3* resides. *ZIC3* mutations occur among sporadic and familial cases (Casey 1998), but they are relatively rare (K. Kosaki and B. Casey, unpublished data), which is not surprising given the similar incidence of disease among males and females and the variety of inheritance patterns seen among familial cases.

Studies in other vertebrates are yielding additional candidate genes for human LR abnormalities. For example, several genes are asymmetrically expressed along the LR axis in chick prior to the development of anatomic LR asymmetry (Levin 1997). Some of these genes are also asymmetrically expressed in mouse, including *nodal* and *pitx2,* as well as *lefty-1* and *lefty-2,* two TGF-β family members (see Ring and Cho 1999) whose chick orthologues have yet to be identified (Casey 1998). Genetic studies in mice have also implicated several additional genes in LR axis development in which asymmetric expression has not been detected, including *HNF3β, ActrIIb,* and *Smad2* (Casey 1998). Mutation analysis has been carried out on a large panel of situs ambiguus cases for human homologues of nodal, *HNF3β, ActRIIb,* and *lefty-1* and *lefty-2*. A small number of mutations have been detected in each (Casey 1998; Kosaki et al. 1999).

Other Vascular Growth Defects

Connection of the pulmonary veins to the left atrium involves fusion of the common pulmonary vein to the primitive left atrium. Failure of connection has been attributed to abnormal targeted growth, although direct evidence is lacking. The resulting defects (total anomalous pulmonary venous return [TAPVR] or partial anomalous pulmonary venous return, cor triatriatum, etc.) reflect establishment of abnormal pulmonary vein connections or incomplete connection. The repeated occurrence of these defects in at least two genetic abnormalities—tetrasomy 22q (cat eye syndrome) and familial TAPVR (i.e., pulmonary venous return entering into the right side of the heart instead of the left atrium in multiple family members)—strongly suggests that they form a distinct mechanistic class. Dominant mutations in the growth factor endoglin (McAllister et al. 1994) and an activin-like receptor (Marchuk 1998) lead to the hereditary hemorrhagic telangiectasias (see Ring and Cho 1999). Individuals with these disorders have multiple small vascular anomalies but may have very significant pulmonary or cerebral arterial-venous malformations. Two families with this rare disorder have been reported showing vascular repair defects associated with the growth factor receptor Tie-2 mutations (Vikkula et al. 1996).

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