Developmental Cardiology Comes of Age
Jonathan A. Epstein

Over the last 10 years, significant progress has been made in the understanding of molecular and genetic determinants of heart formation. An ever growing number of genes have been identified that are required for cardiogenesis, as evidenced by severe abnormalities in cardiac development produced by inactivation in the mouse or inhibition of gene function in other model organisms.1 In general, scientists have identified these genes because of their expression in early cardiac tissues or because of the severe phenotype produced by mutation or inactivation. Gross abnormalities of cardiac development lead to embryonic demise either during midgestation or in the peripartum period, and the underlying severe defects in cardiac structure or function have been relatively easy to determine and describe.1–3 Similar defects in the human versions of these genes may account for embryonic lethal forms of human congenital heart disease. It has been hypothesized that hypomorphic mutations or reduction in gene dosage may result in less severe forms of congenital heart disease, such as those seen in surviving newborns and adults. Alternatively, clinically relevant cardiovascular developmental defects affecting infants and adults generally may result from mutations in genes entirely unrelated to those critical during early stages of cardiac specification and heart morphogenesis, making these factors less attractive for intensive study as disease-causing genes. Data to support the former hypothesis have recently come to light.

Perhaps the most intriguing example comes from the study of a mammalian homologue of a gene first described in the fruit fly, *Drosophila melanogaster*. This organism has an open circulation in which hemolymph is distributed with the assistance of a contracting dorsal aorta. Although structurally dissimilar from the mammalian heart, the dorsal aorta shares genetic determinants with more complex cardiac structures, and the genetic hierarchy governing development of this organ is homologous to that of its mammalian counterpart.4 In 1993, Ralph Bodmer5 described a gene he named tinman that was required for the formation of the dorsal aorta of the fly. Like the *Wizard of Oz* character, flies without a functional tinman gene had no heart. Quickly, mammalian counterparts of the tinman gene were identified and found to be expressed by early cardiomyogenic precursors and by cardiomyocytes throughout heart development.6 The mammalian gene was named Csx by Komuro and Izumo6 and Nkx2.5 by Lints et al.7 Homologues in multiple invertebrate and vertebrate species, including humans, have been identified.

Nkx2.5 is a nuclear protein with a highly conserved DNA-binding domain, a homeodomain, similar to that found in *Hox* gene products known to be involved in specification of anterior-posterior identity along the body axis.8 The helix-turn-helix motif of the homeodomain is conserved throughout evolution and is characteristic of a class of transcription factors that bind to the major groove of DNA and activate or repress downstream genes. Many cardiac-specific genes, including myosin light chain 2V and atrial natriuretic factor, contain Nkx2.5-binding sites in their promoters,9–11 and it is likely that other genes regulated by this important factor remain unknown.

In the mouse, inactivation of Nkx2.5 by homologous recombination in embryonic stem cells leads to a severe embryonic cardiac phenotype in which the primordial heart tube forms but fails to loop normally, and embryonic lethality results.9,12 Although this result indicates that Nkx2.5 is not required for the specification of cardiac progenitors, it suggests that it is required for early global functions during cardiac morphogenesis. Additional data in mouse and other species suggest that Nkx2.5 is part of a combinatorial network of transcription factors, perhaps including related and redundant Nkx family members, that specifies the cardiomyogenic fate of undifferentiated mesoderm during early embryogenesis.

Hence, it came as a surprise when results from the Seidman groups indicated that mutations in human NKKX2.5 were the cause of atrial septal defects and conduction abnormalities in some families.13 This observation emerged from a genome-wide linkage analysis study using samples from affected families. When the implicated region of the genome on chromosome 5q35 coincided with the known location of NKKX2.5, these investigators turned to a candidate gene approach and identified mutations that segregated with disease in 4 families. These mutations resulted in amino acid substitutions in the highly conserved Nkx2.5 homeodomain or in premature truncations of the protein. Unaffected family members and normal control subjects did not have these mutations. Hence, it is almost certain that abnormal or deficient Nkx2.5 protein expressed from 1 of the 2 chromosomes can result in congenital heart disease that is far more subtle and clinically relevant than the severe defects seen in completely deficient mouse embryos.

Interestingly, in both this study and in a subsequent report,14 the types and severity of congenital heart disease associated with NKKX2.5 mutations seemed quite variable, even within the same family and among individuals with identical mutations. Some patients had atrial septal defects only, whereas others had subaortic stenosis, ventricular septal
defects, Ebstein’s anomaly, or tetralogy of Fallot. Atrioventricular conduction defects were frequent and often progressive. This variability suggests that other unlinked loci or environmental factors modify the disease phenotype in patients with NKX2.5 mutations. Functional analysis of the mutated gene products has identified critical regions of the Nkx2.5 protein, but it is too early to determine if specific mutations are more or less likely to cause particular cardiac disorders.15 Nevertheless, it is hard to understand how this wide range of clinical defects could result from heterozygous NKX2.5 mutations if the encoded factor is solely responsible for early commitment and morphogenetic stages of cardiac development. Rather, it is likely that Nkx2.5 serves reiterated functions at various stages of cardiac development and maturation, perhaps throughout life and during adaptive responses.16

The study in this issue of Circulation Research by Biben et al17 adds another layer to the evolution of this story. Now, the Harvey group, which first inactivated this gene in the mouse,13 has performed a thorough analysis of heterozygous mice carrying 1 mutated copy of the Nkx2.5 gene. Initially, these mice were believed to be normal, because they survived and were able to breed and did not seem to be grossly affected. However, laborious and intensive analysis has revealed relatively mild abnormalities of the atrial septum and subtle prolongation of the PR interval. These defects are homologous to some of those seen in humans with NKX2.5 mutations, although less severe. Thus, these heterozygous Nkx2.5-deficient animals provide a reasonable animal model for the study of the etiology and potential therapy of human congenital defects.

Additional analysis of both affected mice and humans may yield additional relevant information. For instance, are conduction defects related to NKX2.5/Nkx2.5 mutations more or less susceptible to exacerbation by pharmacological agents than those not associated with this genetic disorder? Are NKX2.5/Nkx2.5 mutations associated with other forms of conduction defects, including age-related atrioventricular or bundle branch block? Are patients or animals with NKX2.5/Nkx2.5 mutations at risk for idiopathic atrial arrhythmias or those associated with stress or surgery? Is there a difference in the response of myocardium to injury or hypertrophic stimuli?

The Nkx2.5 story is probably only the tip of the iceberg. A growing number of other transcription factors are being identified as key players in early cardiac development. Complete loss of these factors, such as GATA4, Fog2, dHAND, and others, leads to early embryonic lethality and severe malformations of the developing heart. Heterozygous mutations, however, may turn out to result in various forms of less severe congenital heart disease or may contribute to susceptibility to acquired adult heart disease. The challenge implicit in the Nkx2.5 story is to develop time- and cost-effective methods to screen laboratory animals, such as heterozygous knockout mice, for nonlethal cardiac disorders. Improved methods for detailed analysis of engineered animal models will allow for the identification of additional cardiac disease genes relevant to the study of adult and congenital heart disease. Transcription factors important during early stages of heart development also have activities significant for mature cardiac function, and we can expect significant extensions of this paradigm in the near future. Do not be surprised if the molecules required for forming a heart turn out to be just as important during disease and adaptation in the adult.

References

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