Congenital Heart Defects
Trapping the Genetic Culprits

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More children die from congenital heart disease (CHD) each year than are diagnosed with cancer. Although CHD is the most common human birth defect, the etiology of the vast majority of CHDs remains unknown. The difficulty in elucidating the molecular and genetic bases for CHD reflects the complex nature of this and other birth defects. Most CHD occurs sporadically, but the recurrence risk in subsequent pregnancies of parents with one affected child is ≈5%, or 5-fold higher than in other pregnancies. For some types of CHD, the recurrence risk can be as high as 10% to 15%.[1] Thus, a genetic predisposition to CHD is likely present in affected individuals, but a second or third insult may occur during embryogenesis to contribute to the cardiac defects observed clinically. This possibility is similar to the multiple-hit theory in the pathogenesis of cancer and could come in the form of environmental or other genetic factors that accentuate effects of predisposing genetic mutations. Evidence for this theory in CHD comes from patients with similar genetic mutations who display widely varying forms of CHD or no CHD at all.[2,3] This complexity and the often lethal nature of CHD have resulted in a paucity of large human families amenable to genetic linkage analysis,[4,5] limiting the usefulness of classical genetic analyses.

The study by Lee et al[6] in this issue of *Circulation Research* provides evidence that a more systematic approach directed at identifying genes important in cardiac formation may yield insight into the bases of CHD. Lyons and his colleagues have harnessed the powerful genetics available in mice and performed a large-scale screen to search for genes that are activated during the critical periods of cardiogenesis. By combining embryonic stem (ES) cell technology and a retroviral-based gene trap approach, *lacZ* was randomly incorporated into intronic regions of individual genes in ES cells.[7,8] As a result, cells that normally transcribed the gene in which *lacZ* had been trapped were marked by the color blue. Subsequent differentiation of ES cells into embryoid bodies containing beating cardiomyocytes allowed for rapid screening of targeted genes expressed in the heart. Sequencing genes that had trapped *lacZ* could lead to identification of novel genes active during cardiac development or demonstrate cardiac expression of previously known genes. As an added bonus, genes containing *lacZ* were usually disrupted by insertion of the *lacZ* transgene, resulting in ES cells containing heterozygous mutations of individual genes. Such ES cell clones could be used to generate mice heterozygous for disrupted genes with *lacZ* "knocked in" to specific loci.

Through this approach, Lee et al[6] demonstrated that the previously known gene implicated in neural tube formation, *jumonji* (*jmj*),[9] was expressed at high levels in the developing heart. Taking advantage of the insertion of *lacZ* into the *jmj* gene through the screen described above, these investigators generated mouse mutants homozygous for the *jmj* gene disruption ($jmj^{-/-}$) to determine its functional role during cardiogenesis. $jmj^{-/-}$ animals died soon after birth from cardiac and respiratory defects.[6] The cardiac defects shared some similarity to the types of CHD seen in humans. All animals had ventricular septal defects (VSDs), the most common cardiac defect in humans. Most also had an abnormality of alignment of the outflow tract, known as double-outlet right ventricle (DORV), in which the aorta and pulmonary artery arise from the right ventricle. Malalignment of the outflow tract results in an obligatory VSD because of displacement of the conal part of the ventricular septum at the level of the great vessels. This, too, is a common defect seen in tertiary care centers and is often associated with other defects of alignment of the inflow and outflow tracts. Demonstration that the gene trap method was effective in identifying a gene necessary for normal cardiac development provides hope that this approach may be useful in a systematic search for genes involved in cardiogenesis.

Like many mouse knockout strategies, the gene trap method falls short of elucidating the functional pathways involving *jmj*. Although Lee et al[6] show that *jmj* is a nuclear protein, its cellular role remains unclear. Whether it functions in regulating transcription, as suggested, remains to be determined and will require alternative approaches. Similarly, defects in cell proliferation are suggested but are yet to be documented in $jmj^{-/-}$ mice.

Beyond a cellular role, the precise role of *jmj* in cardiogenesis remains unknown. DORV has been documented in numerous mouse mutants and likely results from abnormalities affecting one of two broad aspects of cardiogenesis: cardiac looping and cardiac neural crest development. The process of cardiac looping is a critical event in the development of a 4-chambered heart and is necessary for proper alignment of atrioventricular and ventriculoarterial connections.[10] Abnormalities of looping often result in DORV, as evidenced by the high incidence of DORV in patients with heterotaxy syndrome in which left-right asymmetry and

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cardiac looping are affected.[11] In contrast, defects in neural crest development can also result in DORV, but through a distinct mechanism. Early in development, a single arterial vessel arises from the right ventricle (truncus arteriosus). This vessel is invaded by migrating neural crest cells, which somehow contribute to septation of the truncus arteriosus into an aorta and pulmonary artery; subsequent rotation and leftward movement of the aorta allow it to overlie the left ventricle. Disruption of neural crest cells[12] can result in a spectrum of outflow tract defects, including persistent truncus arteriosus, DORV, and tetralogy of Fallot where the aorta overrides the ventricular septum and communicates with the right and left ventricles. Thus, it is necessary to trace the fate of neural crest cells in *jmj* mutants and to determine if *jmj* affects expression of the many genes known to play a role in neural crest development. Alternatively, *jmj* may play a role in proper alignment of the looping heart. Differentiating between the 2 processes in the *jmj* mutant and other mutants with DORV is important in extrapolating information gained from mouse mutants to human CHD.

As the human genome project nears completion, the estimated 100 000 human genes will soon be known. The major challenge then will be to determine which genes play essential roles and, in CHD, which genes contribute to cardiac defects when mutated. The approaches described above and other gene targeting strategies will undoubtedly provide a large list of genes capable of affecting cardiogenesis. It will be necessary to assemble these genes into vertical and horizontal genetic pathways that control specific steps of cardiac morphogenesis. Because single nucleotide polymorphisms (SNPs) between individuals may form the basis for susceptibility to complex disease traits[13] such as CHD, it will be important to identify SNPs in the numerous genes found to be critical for cardiac development. A profile of SNPs associated with CHD may finally provide information about the genetic predisposition to CHD. Careful dissection of the pathways in which critical factors function may lead to an understanding of the "second" hits that ultimately result in children being born with CHD. Thanks to the recent explosion in genetics and genomics, this type of knowledge may provide the information necessary to formulate preventive public health measures aimed at reducing the incidence of CHD.

We are beginning an exciting and revolutionary era in biology and medicine that should transform our approach to treatment and prevention of human disease. It is tempting to declare that the future is now, but many challenges lie ahead.

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