While congenital heart diseases (CHD) are common causes of mortality and morbidity in infants and children, the basic underlying genetic and molecular mechanisms have remained largely undetermined. Breakthroughs in molecular genetic technology have just begun to be applied in pediatric cardiology stemming from the use of chromosomal mapping and the identification of genes involved in both the primary etiology and as significant risk factors in the development of cardiac and vascular abnormalities. These advances have been applied to study families with several affected individuals, providing new insights into the genetic basis of a number of CHD, including ventricular septal defect (VSD). Moreover, developing new technology may offer a great opportunity for further advancement in genetic diagnostics and for the future of gene therapy.

Increasing evidence suggests that single gene mutations are present in a broad spectrum of genes involved in cardiac structure and function. Pleiotropic cardiac malformations can result from discrete mutations in specific nuclear transcription factors, proteins recognized as playing key regulatory roles during cardiovascular development and morphogenesis. Factors such as GATA4, Nkx2.5, dHAND, TFAP2, and Tbx5 are among the earliest transcription factors expressed in the developing heart and are crucial in the activation of cardiac-specific genes. Mutations in each of these genes result in severe cardiac abnormalities including septal defects (GATA4), conduction defects (Nkx2.5), right ventricular hypoplasia (HAND2), patent ductus arteriosus (PDA) in Char syndrome (TFAP2B), and Holt-Oram syndrome (TBX5) underscoring the critical role played by the disruption of early heart development and morphogenesis in the genesis of CHD.

Genetic defects in proteins involved in the multiple signaling pathways that modulate cell proliferation, migration and differentiation in early cardiovascular development have also been identified. Mutations in JAG1 have been found in kindred studies in association with Alagille syndrome, a complex autosomal-dominant disorder presenting with CHD including pulmonary artery stenosis and tetralogy of Fallot (TOF). JAG1 encodes a ligand that binds the Notch receptor, an evolutionarily conserved signaling pathway involved in cell fate specification. Mutations in the signaling regulator Notch1 have recently been implicated in aortic valve disease.

Specific cardiac malformations have been shown to have a genetic basis as predicted by findings of similar isolated cardiac malformations in other species, and many with heritable components. Table illustrates the most common cardiac malformations present in human subjects and provides information concerning their incidence, and genetic etiology where known. Nearly one third of the congenital heart abnormalities are VSD, but atrial septal defect (ASD), atrioventricular canal (AVC), and TOF are not uncommon. It is of interest that clinically distinct malformations can arise from single genetic defects, suggesting that unrelated cardiac structures likely share similar developmental pathways.

The heterogeneous structural composition of the ventricular septum suggests a variety of possible developmental mechanisms leading to VSD ontogeny. Although not present in the final anatomy, transitory structures are important in cardiac septation (eg, proximal portions of the...
of heterozygosity (LOH) in the 22q11 region using a combination of microsatellite genotyping, dosage analysis of several candidate genes employing polymerase chain reaction (PCR), and bioinformatics strategies. The authors believe that this type of evaluation on siblings of the proband will be valuable in early diagnosis and treatment.

It is known that FISH accuracy in the diagnosis of Homo Sapiens (HSA)22q11 varies with the type of probe used as well as with the number of combined probes. In syndromic CHD (mainly conotruncal defects) the incidence of HSA22q11 deletions detected by FISH with a combination of several probes may reach as high as 50%. On the other hand, microsatellite genotyping detected 48% of a group of 21 cases with TOF and 100% of cases of DiGeorge syndrome. Notwithstanding, using microsatellite genotyping, Lee et al were able to identify a higher number of deletions compared to FISH methodology, probably because the resolution was higher with microsatellite and likely because in some cases a syndromic VSD was present. Interestingly, in several cases no LOH in the 22q11 region using a combination of microsatellite genotyping, dosage analysis of several candidate genes employing polymerase chain reaction (PCR), and bioinformatics strategies. The authors believe that this type of evaluation on siblings of the proband will be valuable in early diagnosis and treatment.

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Taking advantage of available bioinformatics methodology Lee et al were able to establish the
identity of a number of genes within the HSA22q11 regions, and genomic dosages were measured using quantitative PCR. Heterozygous (ie, exhibiting 2 different alleles for a single trait) deletion of several genes, including HIRA, TUBAS8, and GNEBIL could be responsible for the presence of VSD in a number of patients with HSA22q11 LOH; on the other hand, no hemizygous (ie, when in diploid species one part of the genome is present in only 1 copy, as the single X chromosome in the male) or homozygous (ie, having identical alleles for a single trait) deletion of TBX1 gene was identified in 16 VSD patients. Previously, TBX1 mutations have been found in patients with HSA22q11 deletion but without 22q11 microdeletion or apparent rearrangement within this region.12

In animal models, mutations in a large number of genes have been associated with VSD, usually in combination with other complex heart defects. Human syndromic and sporadic cases of VSD have been related to NKX2-5, TBX5, and GATA4 mutations,1,2,13 and generally display an autosomal dominant pattern of inheritance. Furthermore, the signaling function of the Notch receptors, members of a gene family encoding transmembrane receptors and ligands involved in cell fate decisions, may be critical for ventricular septation. In mice, transgenic inactivation of the basic helix-loop-helix transcription factor gene Chy1/Hey2, which acts as a nuclear effector of Notch signaling, results in VSD.14 Targeted disruption of many other genes participating in signaling pathways have been implicated in animal models that produce VSD. A partial list includes mutations in the retinoic acid X receptor gene (RXR),15 the Type I neurofibromatosis gene (Nf1)16, Pax3,17 and TGFβ-218 all result in VSD, although the etiology is unlikely to be related in each case. RXR defects may primarily relate to an epicardial abnormality in trophic signaling required for cardiomyocyte proliferation and ventricular morphogenesis.15 Nf1 cardiac defects are thought to be primarily due to the role for neurofibromin in endocardial cells as shown by the presence of cardiac defects in endothelial-specific inactivation of Nf1.16 Pax3 is expressed and functions in neural crest migration. Hence, diverse mechanisms in multiple cell types can converge to result in a phenotype that includes VSD. Large chromosomal deletions have also been implicated in developmental and structural malformations of the heart, which include conotruncal abnormalities, AVC, VSD, and ASD.19,20 Cardiac outflow tract defects are a manifestation of the complex genetic disorder velocardiofacial syndrome/DiGeorge syndrome, also termed CATCH-22. Most patients are hemizygous for a 1.5- to 3.0-Mb deleted region of chromosome 22 (22q11), suspected to be critical for normal pharyngeal arch development, which contains over 30 genes; deletion 22q11 (del22q11) is a relatively common event occurring in approximately 1 in 4000 live births. A gene TBX1 derived from the central area of the deleted region has been identified as the critical factor in the development of this congenital defect. TBX1, a member of a phylogenetically conserved family of genes that share a common DNA-binding domain (ie, the T-box)21 encodes a transcription factor involved in the regulation of cardiac development; reduction in TBX1 expression, which occurs in the deleted hemizygous state, often referred to as haploinsufficiency impacts greatly on the early gene expression involved in cardiac morphogenesis.

In summary, recent progress in molecular genetics technology have just begun to be applied in studies of CHD by allowing chromosomal mapping, and the identification of many genes involved in both the primary etiology and also as significant risk factors in the development of these anomalies. Identification of novel genes involved in cardiac organogenesis and vascular development will serve as an important foundation for our understanding how specific congenital gene defects generates their cardiac phenotypes. Furthermore, new methods, including bioinformatics can be employed to search existing databases with the use of reverse genetics techniques (ie, techniques that try to identify possible phenotypes that may derive from a specific genetic sequence versus forward genetics techniques that try to identify the genetic basis of a phenotype or trait), with subsequent cloning of novel genes/cDNAs of interest, followed by the characterization of spatial-temporal patterns of specific gene expression in the developing embryo (using in situ hybridization).

Although not yet precisely known, the mechanisms governing the early specification of cardiac chambers in the developing heart tube appear to involve novel cell-to-cell signaling amongst migrating cells, as well as the triggering of chamber-specific gene expression programs, mediated by specific transcription factors and growth factors. Forthcoming research will focus on elucidating the role of network modules of signaling molecules using conditional gene knock-outs (in a variety of genetic backgrounds), and accessing their interaction with critical transcription factors. These approaches may become important tools in the early diagnosis of cardiac defects during embryogenesis increasing the possibilities of treatment (eg, gene delivery), prior to the forming of the heart. Finally, as pointed out by Lee et al, evaluation of CHD at the genomic level may allow a more effective stratification of patient subclasses,
as well as the targeting and optimization of patient-specific therapy.\(^{11}\)

**REFERENCES**


