Advances in Molecular Genetics of Congenital Heart Disease

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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.[1,2] 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

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(TBX5) underscoring the critical role played by the disruption of early heart development and morphogenesis in the genesis of CHD.[3-6]

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).[7] *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.[8] Mutations in *PTPN11* encoding a protein tyrosine-phosphatase (SHP-2) have been proposed to play a role in the pathogenesis of Noonan syndrome characterized by conduction defects, pulmonary stenosis, and hypertrophic cardiomyopathy,[9] and have been also recently implicated in the pathogenesis of LEOPARD syndrome, which likely represents an allelic disorder.[10]

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

TABLE. Incidence of CHD in Human and Gene Defects

Cardiac Phenotype	Frequency	Genes Affected (Loci)
VSD	1:280	TBX5, TBX1, NKX2-5
ASD	1:1062	TBX5, NKX2-5
AVC	1:1372	TBX5, NKX2-5
Tetralogy of Fallot	1:2375	JAG1, TBX1, NKX2-5
Pulmonary stenosis	1:2645	JAG1
Transposition of great arteries	1:3175	
Hypoplastic left heart syndrome	1:3759	NKX2-5
Double-outlet right ventricle	1:6369	
Laterality/looping	1:6944	ZIC3, LEFTYA
Coarction of aorta	1:7142	
Pulmonary atresia	1:7576	
Ebstein's anomaly	1:8772	NKX2-5
Truncus arteriosus	1:9346	
PDA	1:11 111	TFAB2B
Aortic valve stenosis	1:12 395	
Tricuspid atresia	1:12 658	
Bicuspid aortic valve	1:13 513	
Interrupted aortic arch	1:17 291	

ASD indicates atrial septal defect; AVC, atrioventricular canal; PDA, patent ductus arteriosus; VSD, ventricular septal defect.

conal cushions) and are critical to its development. Conal structure position can also be a critical factor in the development of TOF and interrupted aortic arch. Also, cell populations extrinsic to the developing heart, including the neural crest, appear to influence the process of ventricular septation through inductive interactions with neighboring tissues. Furthermore, a variety of hereditary syndromes are associated with VSD. Individuals with chromosomal abnormalities such as trisomy 21 (Down syndrome), the most common genetic cause of CHD, display VSD. Similarly, VSD is commonly present in hearts of mice with trisomy 16, the murine model of human trisomy 21.

Chromosomal deletions have been reported in association with CHD and some may have been previously overlooked due to smaller size and chromosomal location. Newer molecular cytogenetics techniques with high resolution such as fluorescence in situ hybridization (FISH), microsatellites (ie, repeated DNA units of 1-6 base pairs in length, neutral and co-dominant, present in both nucleus and organelles, they are used as molecular markers to study gene dosage and identification of duplications or deletions of a particular genetic region) genotyping and bioinformatics (ie, information technology applied to the field of molecular biology) are some of the techniques capable to facilitate the clinical diagnoses of chromosomal damage such as chromosomal microdeletions and small translocations.

In this issue of the *Revista Española de Cardiología*, Lee et al[11] analyzed DNA from 85 VSD patients and 176 sibling and parents for loss 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.[11] Interestingly, in several cases no LOH in the HSA22q11 regions was found, including a family with 2 children with VSD. To explain these results it is suggested that some other chromosomal regions might be affected. In addition, LOH in the HSA22q11 was detected in several children without apparent CHD (from 2 families with other affected children). This is an important finding that underlines the importance of screening siblings of each proband.

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 *GNEB1L* 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 re-arrangement 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 *Chf1/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 1 neurofibromatosis gene (Nf1)[16], Pax3,[17] and TGFβ-2[18] 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 velo-cardiofacial 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 (del22q1) 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]

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