Advances in the knowledge of the molecular and cellular bases of congenital heart diseases. Second of two parts: Congenital heart defects

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Competing interests
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Abbreviations
CHD: congenital heart defect
FGF: fibroblast growth factor
SNP: single nucleotide polymorphism
TF: transcription factors
TGF: transforming growth factor
VSD: ventricular septal defect

ABSTRACT
Congenital heart defect is the most common birth defect in humans. We conducted a review of the medical literature with the aim of identifying the most recent advances in the knowledge of its molecular and cellular bases. The information obtained was divided into two parts: the first one emphasized on genes and cardiac morphogenesis, and this second part complements the previous one, with special focus on congenital heart defects.
Keywords: Congenital heart defects, Morphogenesis, Single nucleotide polymorphism, Transcription factors, DNA methylation, Signal transduction

Avances en el conocimiento de las bases moleculares y celulares de las cardiopatías congénitas. Parte 2 y última: Cardiopatías congénitas

RESUMEN
Las cardiopatías congénitas son los defectos congénitos más frecuentes en los seres humanos. Se realizó una revisión de la literatura médica con el objetivo de identificar los avances más recientes en el conocimiento de las bases moleculares y celulares de las cardiopatías congénitas. La información obtenida se dividió en dos partes: en la primera se dirigió la atención a los genes y a la morfogénesis cardíaca, y esta segunda parte la complementa, haciendo hincapié en las cardiopatías congénitas propiamente dichas.
Palabras clave: *Cardiopatías congénitas, Morfogénesis, Polimorfismos de un simple nucleótido, Factores de transcripción, Metilación de ADN, Vías de transducción de señales*

INTRODUCTION

In the first part we have already commented that there is no exact genotype-phenotype correlation between the molecular mechanisms and the morphological defects of congenital heart defects[1], so it is possible that different pathways and gene mutations may be involved in the same congenital defect, or that due to the pleiotropic effect of mutations in one of the critical genes during the process of cardiogenesis, different types of congenital heart defects (CHD) may eventually arise.

Although CHD are a frequent cause of morbidity and mortality world-

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wide, the basic genetic and molecular mechanisms underlying CHD remain largely unidentified[2,3]. In recent decades the major advances made in molecular genetic technology have begun to be applied to the field of pediatric cardiology, allowing the identification of many genes involved in primary etiology or which are significant risk factors in the development of congenital heart defects[2-7].

To date, there is evidence of mutations in more than 60 genes that are related to the appearance of different types of CHD. Among these, those coding for TF involved in the process of cardiogenesis are the most frequently associated with CHD, which demonstrates the crucial role played by the aforementioned TFs in the process of cardiac morphogenesis and in the origin of this type of heart disease[2,4,8,9].

Researchers from Harbin Medical University in the People's Republic of China have recently identified two single nucleotide polymorphisms (SNP) in the LEFTY2 gene (rs2295418 and rs360057), significantly associated with the risk of presenting CHD. The LEFTY gene is an important transforming growth factor which functions as a negative regulator of the Nodal/TGF signaling pathway that inhibits the proliferation and differentiation of embryonic pluripotent cells into cardiomyocytes, resulting in different types of CHD[10].

MOLECULAR AND CELLULAR BASES OF CONGENITAL HEART DEFECTS

Septal defects

Septal defects are the most common type of CHD and account for 50% of them. They are classified, according to the chambers they divide, into interventricular, interatrial and atrioventricular. The clinical importance lies in the possible consequences of these communications, such as increased pulmonary flow with consequent damage to the vasculature at that level, atrial growth with increased risk of arrhythmias and ventricular growth due to increased blood volumes[11,12].

Whether in animal models or in the study of affected families, several molecular aspects in septal defects provide information that coincide in pointing out specific genes. Currently, the knowledge of the importance of the TF NKX2-5, NKX2-6, TBX1, TBX5, TBX20, HAND2, GATA4, GATA5 and GATA6 in this group of CHD is quite remarkable. These TFs, which begin to be expressed early in cardiac lineage cells, also regulate the expression of contractile protein genes in cardiomyocytes. In late stages of cardiac development, mutations in each of these genes result in severe congenital heart defects such as conotruncal heart malformations (NKX2-5, TBX1, TBX20 and GATA6), bicuspid aortic valve (GATA5 and NKX2-5), atrial and ventricular septal defects (NKX2-5, NKX2-6, TBX1, TBX5), TBX20, GATA4, GATA6), conduction defects (NKX2-5), right ventricular hypoplasia (HAND2), tricuspid atresia and Ebstein's anomaly (NKX2-5), and among the syndromic causes the Holt-Oram syndrome (TBX5), which in its clinical phenotype includes -in addition to CHD- reductive limb defects[13-22]. The **table** describes a group of genes and their distribution according to the specific type of CHD in which they are involved[4,9,11,13,21-30].

Different studies have linked CHD to the presence of mutations in genes belonging to the GATA family of TF, with "zinc finger" motifs. Sporadic and family case studies provide further evidence of the role that the GATA4 gene plays in the process of atrial septation by inducing− in a mouse model−the G295S and M310V mutations in the human-like GATA4 gene for atrial septal defects in both cases[7,13-16].

Likewise, in order to validate the possible association of the SMAD3 gene with ventricular septal defects, Chinese researchers analyzed the transcribed region and splice sites of this gene in 176 patients with ventricular septal defect (VSD) and compared it with 456 controls. They found that the polymorphic variant rs2289263, located before the 5′ untranslated region (5′ UTR), was significantly associated with the risk of VSD[10].

Other studies in mouse models have allowed the identification of mutations in genes involved in the Notch signaling pathway (which are part of a gene family that encodes transmembrane and ligand receptors involved in the decisions that mark the fate of a cell) and may play a crucial role in ventricular septation. In the mouse, transgenic inactivation of the basic helix-loop TF gene, Chf1/Hey2, which acts as a nuclear effector of Notch signaling, results in an IVC -type septal defect[31,32].

Specific mutations of many other genes involved in signaling pathways in animal models that produce VSD have so far been described. A partial list of these alterations includes mutations in the retinoic acid receptor X (RXR) gene, which encodes for neurofibromin, the cause of neurofibromatosis type 1,

Table. Genes involved in some specific types of congenital heart defects[4,9,11,13,21-30].

Tipos de cardiopatías congénitas	Genes
Atrial septal defects	NKX2-5, GATA4, TBX20, MYH6, TBX5, CITED2, GATA6
Ventricular septal defects	NKX2.5, GATA4, TBX20, TBX1, TBX5, CITED2, IRX4
Atrioventricular septal defects	TBX5, NKX2-5, CRELD1, PTPN11, KRAS, SOS1, RAF1, GATA4, GATA6
Tetralogy of Fallot	NKX2.5, NOTCH1, TBX1, JAG1, NOTCH2, GATA6, TBX20, CITED2, FOXH1, HAND2, ZFPM2
Persistent ductus arteriosus	TFAP2B, TBX20
Transposition of the great vessels	NKX2.5, THRAP2, ZIC3
Aortic valve stenosis	NOTCH1, PTPN11
Pulmonary valve stenosis	JAG1, NOTCH2, PTPN11
Persistent truncus arteriosus	2TBX1, Crkl2, GATA6, NKX2-6
Double outlet right ventricle	NKX2-5, ZFPM2
Left ventricular hypoplasia	TBX20, NOTCH1
Tricuspid atresia	HEY2, NKX2.5
Ebstein's anomaly	NKX2.5
Interrupted aortic arch	TBX1, NKX2.5
Anomalous pulmonary vein connection	TBX20
Aortic coarctation	NOTCH1, PTPN11

PAX3 and elTGFβ2, all of which lead to VSD, although the etiology is unlikely to be related in each case.

Defects in the RXR gene could be basically related to an epicardial alteration in the trophic signaling required for subsequent proliferation of cardiomyocytes and ventricular morphogenesis. The heart defects that may be part of the clinical phenotype of neurofibromatosis type 1 are thought to be primarily related to the role of neurofibromin in endocardial cells, as indicated by the presence of heart defects in the specific endothelial inactivation of this neurofibromatosis. The PAX3 gene is expressed and involved in the migration of the neural crest. Thus, there are several mechanisms of multiple cell types that can converge to give rise to a phenotype that includes the VSD[23].

Congenital conotruncal heart defects

Conotruncal CHD, on the other hand, comprise a subgroup of congenital malformations of the outflow tract of the heart and the great arteries, including: persistent ductus arteriosus, interrupted aortic arch, transposition of the great vessels, double outlet of the right ventricle, conoventricular septal defects, tetralogy of Fallot and pulmonary atresia with VSD. All these congenital malformations share a common embryological and structural origin, since they are derived from the heart cells of the neural crest and the SHF (second heart field). Congenital conotruncal defects represent approximately 20-30% of all types of CHD in humans[24,33].

The genetic etiology of some of these CHDs began to be glimpsed when studying the 22q11.2 microdeletion syndrome, which originates a partial monosomy of the long arm of chromosome 22, which includes the DiGeorge/Velocardiofacial/Conotruncalface anomaly syndromes. This is the most common type of deletion and also the second most common cause of CHD associated with syndromes, after trisomy[21]. This syndrome of contiguous genes is phenotypically characterized by malformations associated with defects in the fourth gill arch and the third and fourth pharyngeal sacs, which contribute to the formation of the thymus, parathyroid and heart. Among the CHD, the most common is the persistence of the truncus arteriosus (incomplete or failed septation of the embryonic truncus arteriosus into aorta and pulmonary artery), but it also includes tetralogy of Fallot, interrupted aortic arch and dou-

ble ventricular outflow[25].

The deletion comprises nearly 3 Mb and contains 30 genes, including CRKL, TBX1, TXNRD2 and GP1BB, which are expressed in the pharyngeal arches. This chromosomal microdeletion is responsible for approximately 12% of cases with conotruncal CHD[24-26,33].

Cardiovascular endophenotype in Down's syndrome

The most frequent genetic cause of syndromic CHD is Down's syndrome or trisomy 21. Approximately 50% of cases with Down or trisomy have one or more congenital heart defects, often affecting structures derived from endocardial bearings, such as atrioventricular and valvular septal defects. This aneuploidy is known to cause CHD but the underlying molecular mechanisms are not fully elucidated as yet, although they do appear to be related to deregulation in the expression of multiple genes with loci in HSA21 (Homo Sapiens Autosome 21)[34].

Since all cases of free trisomy Down's syndrome share exactly the same chromosomal abnormality, some additional genetic and epigenetic factors may contribute to the development of CHD in this type of chromosomal aberration, which is the most common aneuploidy in humans. When considering the relevance of epigenetic mechanisms in the regulation of gene expression during embryonic development and the increasing evidence of the link between epigenetic alterations and CHD, Spanish researchers compared the methylation patterns of deoxyribonucleic acid (DNA) in cardiac tissues of syndromic and non-syndromic CHD fetuses with lymphocyte DNA samples from control fetuses and identified a hypermethylation of several intragenic sites of the MSX1 gene, related to ventricular outflow tract morphogenesis, in fetuses with isolated CHD. Similarly, they also found an abnormal DNA methylation of the GATA4 gene in all samples of fetuses with Down's syndrome, with and without CHD. Therefore, the researchers argue that deregulation of GATA4 methylation could be a consequence of trisomy 21 that may contribute to the increased risk of CHD observed in Down's syndrome. However, the levels of methylation and gene expression did not differ between the cases with Down's syndrome with and without CHD, which implies other, hitherto unknown, modifying factors in gene penetrance that would explain the variable expressiveness in the

cardiovascular endophenotype of Down's syndrome[35].

Human patients with 4p33 microdeletion (containing the HAND2 locus) have also been identified as susceptible to presenting CHD, including ventricular septal defects, tetralogy of Fallot, pulmonary atresia and aortic coarctation[20].

The CHD observed in the TBX1 gene defects correspond to outflow tract anomalies. The SHF gene is known to be involved in the pathogenesis, since it regulates the proliferation of these cells, which are destined to participate in the formation of the outflow tract. In addition, this gene is necessary for the cells that express NKX2-5 in order to form the aortopulmonary septum, which is responsible for dividing the aorta from the pulmonary artery in the embryonic outflow tract. Another candidate for the phenotype is the Crkl gene, which encodes for an adaptive protein involved in signaling processes, and which has been implicated in CHD in mice, with the same phenotype as the mutations in TBX110[18].

The absence of NKX2-5 prevents the formation of the loop and the differentiation of the ventricles. The heterozygous mouse model show defects in the atrial and ventricular septa, which is compatible with the human phenotype of mutations in this gene. More than 30 mutations have been identified in the NKX2-5 gene. Heterozygous mutations of NKX2-5 are explanatory for nearly 4% of all CHD. Although interatrial septal defects are the most common, it is also related to interventricular septal defects, tricuspid valve malformations, tetralogy of Fallot, Ebstein's anomaly, among others. The different phenotypical manifestations related to this TF ratify its multifunctionality during cardiac development[11,23,36].

Other types and causes

The MYH6 gene is activated by the TFs encoded by the GATA4 and TBX5 genes, and it has been related to interventricular septal defects. The gene TBX20 was linked to CHDs for the first time in 2007. This FT interacts with the genes NKX2-5, GATA4 and TBX5, which had previously been associated with CHD. Mutations in the T-box of this gene are associated with different CHDs, including septal defects, valve diseases and dilated cardiomyopathy in adults[11,13,14,18,21].

Recently, a link was found between GATA6 gene mutations with defects in the outflow tract, specifically with the persistent ductus arteriosus and te-

tralogy of Fallot. GATA6 is a member of the GATA-binding protein family. Its expression and function regularly overlaps that of GATA4. The latter has already been related to different CHD; however, the role of GATA6 in these CHD is just beginning to be explained; it is known so far that this FT regulates the expression of the genes encoding the neurovascular guide protein Semaphorin 3C and its receptor Plexin A2[16].

The ductus arteriosus is an extremely important structure for fetal circulation that must be occluded and disappear shortly after birth. The molecular study of Char syndrome, an autosomal dominant disorder characterized by persistent ductus arteriosus, dysmorphic facies and digital abnormalities, allowed for the identification of the TFAP2B gene at the molecular basis of this syndrome. This TF is expressed mainly in neural crest cells, which play an important role in the septation that originates between the aorta and the pulmonary artery in the common primordial tract; which actually highlights the role of these cells in the closure of the ductus arteriosus. In addition, gene mutations have also been identified that produce non-syndromic isolated ductus arteriosus persistence. The TFAP2B cofactor, CITED-2, has also been associated with CHD, mainly with tetralogy of Fallot[23,36].

Obstructive defects, whether in the aorta or the pulmonary artery, vary in intensity and may lead, at their worst, to ventricular hypoplasia. Like other CHD, the first clues about the genetic etiology of this group of congenital defects were obtained from the study of genetic syndromes that had the desired phenotype. Williams syndrome is characterized, from the cardiovascular point of view, by supravalvular aortic stenosis and peripheral stenosis of the pulmonary arteries, and also has several extravascular characteristics, such as intellectual disability and neonatal hypercalcemia, among others. Microdeletion in this syndrome, 7q11, leads to haploinsufficiency in the elastin gene (ELN), which causes vascular congenital defects. Furthermore, mutations in the ALN gene have been identified in some sporadic cases with supravalvular aortic stenosis[5,9,23].

Another essential mechanism that leads to obstructed blood outflow is the thickening of the semilunar, aortic and pulmonary valves; which can also be associated with bicuspid valves. The dysmorphic pattern of Noonan syndrome is characterized by short stature, facial dysmorphia and CHD, mainly pulmonary stenosis associated with a dysplastic pulmonary valve; however, it has also been associ-

ated with dilated cardiomyopathy and atrioventricular canal defect. Point mutations with gain of function in PTPN11 have been found in at least 50% of patients with this monogenic syndrome. This gene, with 12q24.1 locus, encodes for a non-tyrosine phosphatase receptor protein. The relevance of this gene and the mechanisms that lead to heart disease have been tested in mouse models, in which its deletion leads to dysplastic and bivalve valves. The mechanism seems to be the hyperproliferation of the outflow tract cushions, structures from which arterial valves derive. The protein product of PTPN11 (SPH 2) is essential for the activation of the Ras-Erk cascade in most tyrosine kinase (RTK) receptors[9,23].

Receptors with RTK activity mediate the actions of multiple growth factors. Mutations in the genes that code for these receptors can result in a proliferative signal in the absence of a growth factor and trigger alterations in embryonic development and cell differentiation, resulting in CHD. Deregulation of cell proliferation and differentiation processes may also be caused by changes in the expression or activity of cytosolic adaptive proteins that carry the RTK signal. Alterations in the Src family of cytosolic tyrosine kinases, generally due to the loss of some of their self-regulatory mechanisms, are also far-reaching due to their repercussions on cell cycle control, cell adhesion and survival, as well as angiogenesis, and are therefore related to congenital cardiovascular defects[12].

Epigenetic mechanisms contribute to the regulation of several physiological processes during embryonic development. Among the different epigenetic mechanisms, alterations in the patterns of DNA methylation have been associated with the presence of congenital defects in humans. Folic acid plays a crucial role in monocarbon metabolism for the synthesis of nucleotides and amino acids, as well as for DNA methylation, which is essential for the dynamics of conformational changes in chromatin and the subsequent gene expression. Decreased levels of this acid produce reduced levels of S adenosyl methionine leading to insufficient DNA methylation, which is an important epigenetic mechanism regulating genomic programming during embryogenesis[31,36].

The possible contribution of alterations in DNA methylation to the origin of CHD has been explored through the study of genes related to the folate pathway. Thus, several SNPs have been identified in the folate transporter gene SLC19A1 (Solute Carrier Family 19 Member 1) that are significantly associat-

ed with the presence of CHD in patients with Down's syndrome. This gene is an activator of the enzyme DNA polymerase and is essential for the synthesis and repair of DNA[31-33,36].

Since periconceptional supplementation with folate has a protective effect during the development of SHF, resulting in a decrease in conotruncal CHD, The US conducted the largest case-control study of genetic variants in this type of CHD[33]. The researchers studied the association between conotrachial heart defects and 921 maternal and fetal SNPs in 60 genes involved in the folate, homocysteine and transulphuration pathways, as well as the effect of folic acid supplementation treatment on SNPs. The results obtained were consistent with previous studies suggesting that SNPs in these three pathways related to folic acid metabolism are associated with the risk of contruncal DCs; this research also concluded that the consumption of supplements containing folic acid could produce changes in the impact of SNPs on cardiac development[33].

In 2014, Elsayed *et al*[37] studied the genotype of 61 Egyptian mothers with offspring affected by septal type CHD (25 with Down's syndrome and 36 with isolated CHD) and an equal number of controls. The polymorphism studied was MTHFR C677T, which proved to be significantly more frequent in mothers of children with Down's syndrome and atrioventricular septal defect, compared with mothers in the control group (OR 1.21, 95% CI 1.02-1.43). The results of this research, according to this same author[37], coincide with another where the same and other MTHFR polymorphisms were studied, in suggesting a possible contribution of folic acid metabolism in the development of CHD.

Non-syndromic CHDs are known to have a multifactorial etiology which has led to the study of multiple "candidate" genes; however, little research has explored the expression of the pattern of DNA methylation in the fetal heart. A comparison of this overall pattern of methylation in 18 syndromic and non-syndromic CHD-affected fetuses and the leukocyte DNA of 656 individuals as a control group showed an absolute correlation with tissue type, with significant differential methylation enrichment in genes related to muscle contraction and in cardiomyocytes of the developing heart, and an abnormal pattern of cardiac tissue DNA methylation with syndromic and non-syndromic CHDs.

An average of three regions with aberrant methylation patterns per sample and 18 regions with differentiated methylation between groups were identified. Similarly, they were able to confirm hypermethylation of several intragenic sites at the MSX1 and GATA4 genes, related to the morphogenesis of the outflow tract, indicating that epigenetic alterations in relevant genes are present in the developing heart, both in syndromic and isolated CHD. These epimutations possibly contribute to the pathogenesis of such congenital defects through the effect of *cis*-action on gene expression[35].

Likewise, an abnormal pattern of methylation in the NKX2-5 and HAND1 genes was identified in patients with tetralogy of Fallot and it was observed that this abnormal methylation correlated negatively with RNA expression of both genes in the cardiac tissue. This evidence suggests that changes in DNA methylation patterns may contribute to negative transcriptional dysregulation of the aforementioned genes throughout the process of cardiogenesis[36].

The Notch cell signaling pathway has been shown to further regulate cell differentiation of the proepicardium and the adjacent pericardial mesoderm, so that inhibition of their expression in the epicardial lineage inhibits coronary artery formation and reduces cardiomyocyte proliferation and myocardial wall thickness. Mutations in the JAG1 gene (also known as JAGGED1) or the inhibition of Notch signaling in the SHF brings about different CHD, mainly in the aorta and ventricular outflow tract. The JAG1 gene encodes for a ligand that binds to the Notch receptor which is involved in specifying the fate of each cell. Recently, mutations in the Notch1 signaling regulator have been implicated in aortic valve disease, while mutations in JAG1 and Notch 2 are associated with the occurrence of tetralogy of Fallot[31,38].

A wide variety of malformative syndromes in humans results from a disruption of the Notch signaling pathway, for example Alagille syndrome, which is an autosomal dominant disorder that in its clinical phenotype includes right heart CHD, ranging from moderate pulmonary artery stenosis to tetralogy of Fallot, in addition to extracardiac disorders such as biliary stenosis. In its molecular bases, point mutations or deletions comprising the JAG1 gene locus have been identified in up to 94% of cases. Mutations in JAG1 have also been identified in patients with pulmonary stenosis and tetralogy of Fallot, without other phenotypic alterations of the syndrome. The keys to interpreting congenital defects secondary to JAG1 mutations have only recently begun to be understood and involve SHF[23,31,32,38].

A team of American researchers[27] proved,

through experiments in mice models, that the absence of the JAG1 gene causes different types of CHD, mainly from the aorta and the output tract. Mouse embryos in which the signaling pathway was interrupted showed decreased expression of FGF8 and BMP4 that ultimately resulted in a defective development of tissues neighbouring the SHF, for example, defects in the migration of neural crest cells and in the endothelial-mesenchymal transition within the endocardial cushions of the outflow tract. The latter defect was reversed in vitro with exogenous FGF8 enhancement. Thus, a model is proposed that relates the function of JAG1 within SHF and its impact on neural crest cell migration and endocardial cushion development[27].

The importance of neural crest cells in the formation of the semilunar valves and the smooth muscle of the ascending aorta has been proven, along with the repercussions associated with their alteration: valve defects, aortic stenosis, aneurysms and dissections. In this way, the interaction of different cellular and molecular factors, especially the Notch cell signaling pathway, all converge to adequately integrate the outflow tract. This one is a fresh and ongoing research issue[23]. At the same time, some researchers have found that another signaling pathway, the Nodal/TGF, also plays a pivotal role in cardiac embryogenesis, in angiogenesis and in the organization of the aortic wall, since in animal models, when mutations in genes involved in this pathway will therefore trigger cardiovascular morphological defects[39].

Point mutations in the FBN1 gene, which codes for fibrillin 1, with gene locus on 15q15-21, carry all the pleiotropic manifestations of Marfan syndrome, including valve defects and aneurysmal dilations of the aorta. Fibrillin is a glycoprotein, formerly described as a structural protein, which is part of extracellular microfibrils that are the main component of elastic fibers, however, it is now known that they are also an important negative regulator of the TGFB signaling pathway, and precisely the overexpression of this intercellular signaling pathway is partially responsible for the cardiovascular alterations that make up the cardiovascular phenotype of this connective tissue disorder, transmitted with an autosomal dominant inheritance pattern[5,9,23].

Organic symmetry

Situs is the term that defines the position of the or-gans in the chest and abdomen. *Situs inversus* or *visceral heterotaxy* is one of the most complex congenital defects. It is a syndrome characterized by a severe alteration of the right-left symmetry pattern and the spatial relationship of the organs (the chest and abdomen are positioned in a mirror image from their normal positions). During development, the heart is the first organ to break symmetry in the developing embryo[12].

Studies in several species have led to the discovery of more than 80 genes that regulate right-left symmetry and provide a basis for accounting for lateral symmetry defects. In chicken embryos asymmetric expression of the Shh protein leads to the expression of two members of the Nodal/TGF signaling pathway, Nodal and LEFTY in the left mesodermal plate. The expression of the Nodal gene on the left side of the developing embryo induces right-sided torsion and rotation of the heart tube. In the right lateral mesoderm an activin receptor-mediated signaling pathway inhibits Nodal and Shh expression. The activin and Nodal signaling pathways result in the final expression of Pitx2 FT, on the left side of the visceral organs, which is sufficient for the establishment of right-left asymmetry in the developing heart, lungs and bowel[23].

Congenital long QT syndrome

Finally, reference will be made to the molecular bases of one of the congenital cardiac channelopathies: the long QT syndrome (LQTS)[40]. This primary disorder of cardiac repolarization is characterized by a severe alteration in ventricular repolarization, electrocardiographically recorded as a prolongation of the corrected QT (cQT) by Bazzet's formula (cQT= $QT/\sqrt{\bar{R}}$)≥460 ms, which predisposes to sudden death by torsades de pointes arrhythmia[41]. Several types of LQTS are recognized; However, the congenital arrhythmogenic channelopathies of this type that have been better studied to date are Jervell and Lange-Nielsen syndrome (which is transmitted with an autosomal recessive inheritance pattern and includes in its clinical phenotype the presence of congenital neurosensorial deafness, due to homozygous mutations) and Romano-Ward syndrome, which is the most common form, with autosomal dominant inheritance, variable expressivity and reduced penetrance, due to generally heterozygous mutations[40-42].

LQTS presents a great heterogeneity of allelic and non-allelic genes, because more than 500 mutations

in at least 17 genes with different loci have been identified at molecular level, among them are described: KCNQ1 (11p15.5), HERG (7q35-36), SCN5A (3p21-24), KCNE1 (21q22.1), KCNE2 (21q22.1), ANKB (4q25-27), KCNJ2 (17p23), CACNA1 (12p13.3), CAV3 (3p25), SCN4B (11p23), among others, which mostly encode for the different pore-forming subunits of the ion channels that generate the transmembrane action potential. While the ANKB gene encodes for ankyrina-β, a structural protein that links proteins of the myocardial membrane with proteins of the cytoskeleton and whose mutations result in an increase in the concentration of intracellular calcium that leads to early and late post-depolarization[28-30].

Hundreds of SNPs have been currently described in these genes, such as K897T in HERG, which has not only been associated with an increase in individual susceptibility to develop arrhythmias when using certain drugs, but also favors the pathogenic effect of mutations in this same gene. The polymorphic variant R1193Q in the SCN5A gene is considered a common polymorphism in Asian populations and has been related to both LQTS and Brugada syndrome. Another polymorphism in the same gene, S1103Y, has been associated with an increased risk of sudden death in childhood[29,30].

Knowledge of the molecular bases of these channelopathies has made it possible to optimize treatment and improve the survival of affected people, thus generating an important genotype-phenotype-drug treatment correlation and a higher level of information that favors the genetic counseling process in these cases.

CONCLUSIONS

Cardiogenesis is a complex and dynamic process that requires precise spatial-temporal cooperation of multiple genes encoding specific transcription and growth factors, morphogens, intercellular signaling pathways, structural proteins and ion channels. Different genetic and epigenetic factors may disrupt these molecular and cellular mechanisms and generate a broad phenotypic spectrum of congenital heart defects. Knowledge of the molecular and cellular bases of these heart diseases allows for a more effective classification of these congenital defects and a future optimization of the individual treatment for each patient, in addition to offering possible specific targets that could be intervened on to prevent some of the most frequent congenital defects in humans.

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