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**SPOTLIGHT REVIEW**

# Developmental aspects of cardiac arrhythmogenesis

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**Abstract** The transcriptional regulation orchestrating the development of the heart is increasingly recognized to play an essential role in the regulation of ion channel and gap junction gene expression and consequently the proper generation and conduction of the cardiac electrical impulse. This has led to the realization that in some instances, abnormal cardiac electrical function and arrhythmias in the postnatal heart may stem from a developmental abnormality causing maintained (epigenetic) changes in gene regulation. The role of developmental genes in the regulation of cardiac electrical function is further underscored by recent genome-wide association studies that provide strong evidence that common genetic variation, at loci harbouring these genes, modulates electrocardiographic indices of conduction and repolarization and susceptibility to arrhythmia. Here we discuss recent findings and provide background insight into these complex mechanisms.

**Keywords** Cardiac development • Ion channels • Transcription factors • Genetic variation

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## 1. Introduction

Rhythmic and synchronized contraction of the atria and ventricles is essential for efficient pumping of blood throughout the body. This process crucially relies on the proper generation and conduction of the cardiac electrical impulse, which leads to a coordinated mechanical response in cardiomyocytes through excitation–contraction coupling. The cardiac electrical impulse generated in the sinus node activates the atria, which propagate it to the atrioventricular node (AVN). Conduction of the electrical impulse through the AVN occurs slowly, allowing the atria to pump blood into the ventricles while the latter are diastolic. Subsequently, the impulse is propagated through the fast-conducting atrioventricular bundle (AVB), the bundle branches, and, ultimately, the Purkinje fibre network, from where it reaches the ventricular working myocardium, allowing for contraction from apex to base. This coordinated process necessitates that cardiomyocytes from the different regions of the heart are specialized and exhibit specific electrophysiological characteristics. Accordingly, considerable heterogeneity exists in action potential characteristics of cardiomyocytes from different cardiac regions (reviewed extensively in Boukens *et al.*[1]). These intrinsic heterogeneities in electrophysiological properties and conductivity between the cardiac compartments are rooted, at least in part, in the regional differences in expression of ion channel genes and gap junction genes mediating intercellular propagation of the action potential.

It is becoming increasingly clear that transcriptional regulation orchestrating the development of the heart and the specification of the different cardiac regions from cardiac precursor cells during embryonic development also plays an essential role in regulation of expression of ion channel and gap junction genes.[2–5] Indeed, several studies have now established a causal relation between these transcription factors and regionalized expression of these genes.[6–10] A hallmark example of this is *Irx5*, a homeobox containing transcription factor, that establishes a transmural gradient of potassium-channel-gene expression across the myocardial wall, ensuring coordinated cardiac repolarization, while also preventing arrhythmias (see below).[8] This has led to the realization that in some instances, abnormal cardiac electrical function and arrhythmias in the postnatal heart may stem from a developmental abnormality causing maintained (epigenetic) changes in gene regulation. The latter may involve structural abnormalities including altered tissue composition. The role of developmental genes in regulation of cardiac electrical function has recently been brought further into focus as genome-wide association studies have provided strong

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evidence that common genetic variation, at loci harbouring these genes, modulates electrocardiographic indices of conduction and repolarization and susceptibility to arrhythmia.[11–15]

## 2. Development of the cardiac chambers and conduction system

The heart of mammals contains four chambers and it develops from a single tube that initially only contains the precursors for the left ventricle. During development, the precursors needed for the rest of the heart are added at both the arterial and venous poles.[16,17] The heart tube continues to elongate as a result of the recruitment of myocytes and bends ventrally and rightwards in a process called looping, which occurs around mouse embryonic day 8.5–9 (E8.5–9 *Figure 1*), similar to 23 days of human development. At this time, the heart tube has started to form a distinct ventricular chamber at the original ventral side, while the future atrial appendages differentiate slightly later at the dorsal and caudal end.[3,18,19]

The myocardium of this heart tube and the myocardium that is subsequently added to its poles display slow conduction and contraction, together with the ability to spontaneously depolarize (automaticity), which is mainly Cx45 dependent,[20] resulting in peristaltic like, rhythmic contractions. Dominant pacemaker activity is always located in the newly formed myocardium at the venous pole.[21,22] This generates a unidirectional blood flow from the venous to the arterial pole. While the ventricular and atrial chamber myocardium differentiates and rapidly proliferates at specific sites within the heart tube, the remainder of the myocardium maintains the primary phenotype. Once chambers develop, this primary myocardium can be recognized as the sinus venosus, the atrioventricular canal (AVC), and outflow tract (OFT). The sinus node develops from a small component of the sinus venosus[22,23] and the AVN and AV junction myocardium develop from the AVC.[24,25] Both nodal components still display phenotypic features of the primary myocardium they develop from, such as automaticity, slow conduction, and poorly developed sarcomeres and SR.[5] The maintenance of the primary phenotype of the myocardium is fundamental and regulated by the transcriptional repressor genes *Tbx2* and *Tbx3*,[9,26] which are selectively expressed in the primary myocardium and developing and mature conduction system. A deficiency of Tbx3 in the myocardium results in expansion of the expression of working myocardial gens *Cx40*, *Cx43*, *Nppa*, and *Scn5a* into the sinus node domain. In contrast, forced expression of Tbx3 leads to development of ectopic functional pacemaker tissue.[9] Both Tbx2 and Tbx3 are required to pattern and form the AVC in a redundant fashion (our unpublished observations). Thus, Tbx2 and Tbx3 inhibit differentiation into working myocardium, which allows for the development of components of the cardiac conduction system. In conclusion, the primary myocardium lies at the basis of the pacemaker tissues of the cardiac conduction system.

In contrast, formation of the chambers is marked by the differentiation of localized regions of primary myocardium of the embryonic heart tube into the fast-conducting working myocardium of the chambers. A hallmark feature of this working myocardium is the expression of a chamber-specific gene program (*Figure 1*).[4] This includes genes for rapid propagation of the action potential such as *Cx40* and *Cx43* and the secreted factor Nppa (Natriuretic precursor peptide type A, also known as Anf). Moreover, working myocardium expresses the α-subunit of the sodium channel Nav1.5, encoded by *SCN5a*, and develops a functional sarcoplasmatic reticulum.[27] At the cellular level, a local increase in cell size followed by local re-initiation of proliferation marks this area.[28] At the morphological level, initiation of chamber formation is marked by the appearance of trabecules, a sponge-like myocardial structure developed on the luminal side of the outer curvatures of the developing heart. This series of events has been dubbed the ballooning model of chamber formation.[4] Both the chamber-specific gene program and gene expression in the maintained primary myocardium are regulated among others by cardiac transcription factors such as Nkx2–5, Gata4, and a number of T-box transcription factors (Tbx), such as Tbx5 and Tbx20. Disruption in any of these crucial processes leads to misspecification of working myocardium or primary myocardium which eventually can result in local heterogeneity and arrhythmia.

## 3. Nkx2–5, AV conduction and atrial fibrillation

One of the most intensely studied transcription factors involved in cardiac development is Nkx2–5. This homeobox transcription factor is expressed in the heart and other tissues and is part of the core cardiac transcriptional network essential for cardiac development. Targeted disruption of *Nkx2–5* in mice causes early embryonic lethality, with cardiac development arrested at the linear heart tube stage, prior to looping.[29] Cardiac expression of Nkx2–5 continues throughout development and into adult life (*Figure 2*). In humans, dominant mutations in *NKX2–5* cause a variety of cardiac anomalies as well as atrioventricular abnormalities. Atrioventricular conduction abnormalities and atrial septal defects (ASD) are, in fact, the most common clinical presentations. However, other abnormalities such as ventricular septal defects, double-outlet right ventricle, tetralogy of Fallot, and tricuspid valve abnormalities have also been noted.[7,30] Cardiac dysfunction and sudden death have also been reported in mutation carriers. Atrioventricular conduction disease can occur in the absence of associated congenital heart defects.[7,30] It is progressive in nature, and electrophysiological studies have indicated that the conduction abnormality affects specifically the AVN. The fact that conduction defects can occur in the absence of cardiac structural malformations suggests that NKX2–5 has a function in conduction system development that is independent of its role in cardiac morphogenesis, and the fact that conduction disease is progressive with increasing age underscores the importance of normal *NKX2–5* function in maintenance of AV conduction in adult life. Recent findings that genetic variation in the region of the *NKX2.5* gene modulates the PR interval in the general population seem to support such a role. The association signal detected at this locus, however, is located at some distance from the *NKX2–5* gene and further research will be required to establish an unequivocal link between the genetic variation underlying this signal, NKX2–5 expression levels, and the link to the PR interval.[13] This is further underlined by studies using transgenic mice that carry a loss of function allele (DNA-non binding mutant) for *Nkx2–5*. These transgenic mice were born with structurally normal hearts but displayed progressive atrioventricular conduction defects and heart failure. PR prolongation was observed at 2 weeks of age, rapidly progressing into complete AV block by 4 weeks of age. A dramatic decrease in expression of gap junctional channels, Cx50 and 43, probably contributed to the conduction phenotype.[31] Geno-phenotype correlations on the various *Nkx2–5*

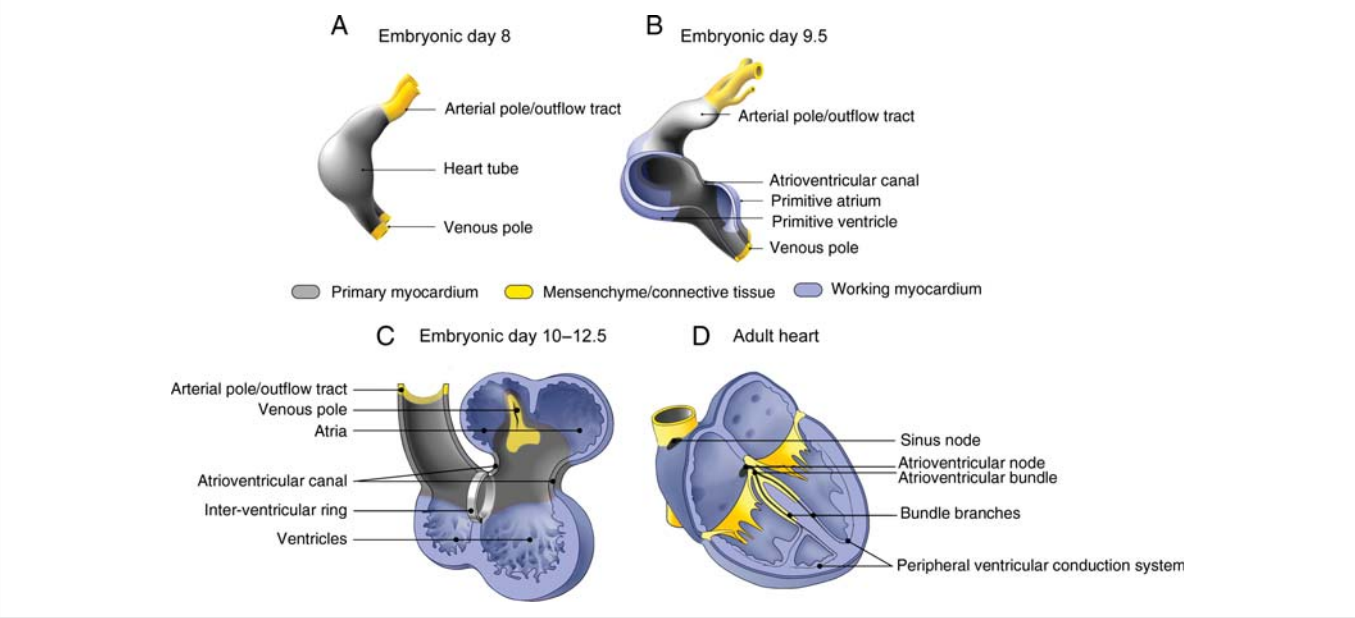


**Figure 1** Overview of the formation of the four-chamber heart (ballooning model). (*A*) Lateral view of the linear heart tube, composed entirely of primary myocardium (expressing connexin 30.2 and 45). (*B*) Lateral view of the looping chamber-forming heart. The heart tube expands ventrally and gives rise to the ventricles and laterally to give rise to the atria. (*C*) Side view of embryonic day 10–12.5, the outflow tract has been moved to the side for easier viewing. (*D*) Adult heart, four chamber view, all the components of the conduction system arose from the primary myocardium. Adapted from Ref. 36; used with permission from Elsevier.

mutants *in vitro* suggested that the principle determinant of the two most common phenotypes (AV block, ASD) was the total dose of Nkx2–5 capable of binding to DNA.[32] It turned out that *Nkx2–5* haploinsufficiency in the conduction system results in a significantly reduced number of normal cells, and that the specific functional defects observed in the knockout mice could be attributed to hypoplastic development of the conduction system. Thus, the postnatal conduction defects arising from *Nkx2–5* mutations may results from a defect in the development of the embryonic conduction system. This conclusion was in line with experiments on heterozygous *Nkx2–5* knockout mice with eGFP expression, knocked in the *Cx40* locus, permitting the visualization of the conduction system. Indeed, hypoplasia and disorganization of the Purkinje fibres were observed in the heterozygous *Nkx2–5* mice and these were associated with abnormal ventricular electrical activation.[33] Analysis showed that maximal Nkx2–5 levels are required cell-autonomously and that a reduction in Nkx2–5 levels is associated with a delay in cell cycle withdrawal from surrounding Cx40-negative myocytes. This suggests that the formation of the peripheral conduction system is time- and dose-dependent on the transcription factor Nkx2–5, which is cell-autonomously required for the postnatal differentiation of Purkinje fibres. Experiments on Id2, a member of the Id gene family of transcriptional repressors, showing AVB-specific expression, proved that a critical transcriptional network, including Tbx5, Nkx2–5, and Id2, is required for the differentiation of ventricular myocytes into specialized cells of the conduction system.[34] Taken together, Nkx2–5 is required for development and homeostasis of atrioventricular conduction system and Purkinje fibres. In the case of the AVB, some details of the molecular mechanism have been elucidated (Id2, Tbx5). It is noteworthy that Tbx3 also cooperates with Nkx2–5[9] providing a link between this factor and Tbx3 in the AVB and AVN.

Not all mutations in *Nkx2–5* are linked to AV block, but to atrial fibrillation,[35] an arrhythmia long thought to have a possible origin in development.[36] In the majority of cases of paroxysmal atrial fibrillation, the myocardial sleeves surrounding the pulmonary veins at the orifice to the left atrium carry the triggers and possibly the substrate for the arrhythmia. Indeed, cells with presumed pacemaker activity have been found in the pulmonary veins of rat hearts[37] and in the pulmonary veins of patients with atrial fibrillation.[38] However, these observations are controversial, as data show that the pulmonary myocardium is created from a distinct lineage of precursor cells, distinct from the lineage that forms the muscle encompassing the sinus venosus (myocardium around caval veins, including the sinus node and around coronary sinus).[23,39–41] In contrast to the sinus muscle, the transcription factor Nkx2–5 and its target gap-junction gene Cx40 are expressed in the pulmonary myocardium from the outset, suggesting it has an atrial working phenotype from the outset. Intriguingly, when Nkx2–5 protein levels are lowered experimentally, a Cx40-negative, Hcn4-positive phenotype can be observed in the pulmonary myocardium, similar to sinus nodal-like cells. Thus, a decrease in the expression level of a single transcription factor, Nkx2–5, activates a gene program sufficient to generate automaticity in the pulmonary myocardium. This observation suggests that the inter-individual variation in Nkx2–5 dosage could be an important contributing trigger to the development of atrial fibrillation. Intriguingly, as mentioned above, genetic variation in the region of *NKX2.5* has been associated with the PR interval in the general population, which when prolonged, is in turn associated with risk of AF.[42] Taken together, variations in the (regulatory) sequence of the *Nkx2–5* gene (and/or its interacting partners) are prime candidates for further research into the AV conduction and atrial fibrillation.

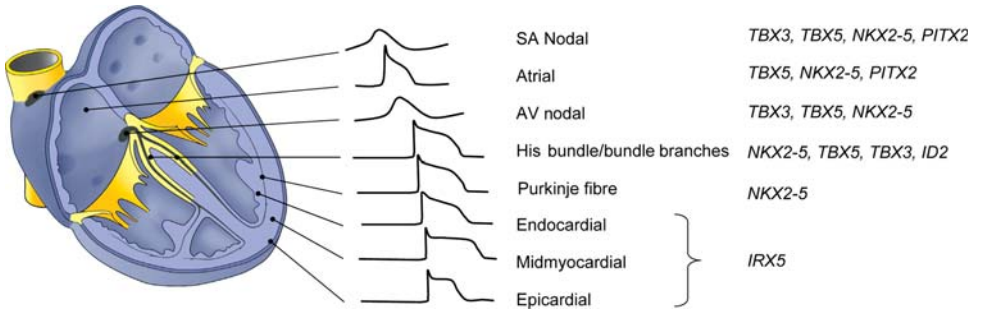


**Figure 2** Four-chamber view, adult heart. Schematic drawing of the action potential of the various parts of the conduction system. Adapted from Nerbonne *et al.*[100] Transcription factors that are involved in the development of regions of the heart and influence the electrical properties have been marked.

## 4. TBX5, atrial fibrillation and left–right chamber differences

Another crucial factor in cardiac development is TBX5, which belongs to the evolutionarily conserved T-box family of transcription factors that bind DNA through a highly conserved DNA-binding domain called the T-box. The consensus DNA sequences of the target genes where T-box factors bind are called T-box-binding elements.[43] Tbx5 expression displays a posterior–anterior gradient along the heart tube with the most intense expression at the inflow tract of the heart. After chamber development has been initiated, Tbx5 expression is found in the sinus venosus, atria, AVC, left ventricle, including the left aspect of the ventricular septum, and to a lesser extent in the right ventricular trabecules (*Figure 2*).[44,45] In humans, *TBX5* haploinsufficiency has been shown to cause Holt–Oram syndrome (HOS), which includes congenital heart defects, conduction system abnormalities, and upper limb deformities.[6,46] Heterozygous *Tbx5* knockout mice recapitulate many of the phenotypic abnormalities observed in HOS patients.[47] Expression of both *Nppa* and *Cx40*, targets of Tbx5, was also reduced in these mice during development. Complete deletion of *Tbx5* in mice leads to embryonic death (around E9.5) with failure of heart tube looping and an underdeveloped caudal part.[47] Furthermore, Tbx5 overexpression in an embryonic cell line results in significant upregulation of *Nppa* and *Cx40*, and, counter intuitively, in cells displaying a spontaneous beating phenotype.[48,49] Moreover, as mentioned earlier, Tbx5 is required for the differentiation of the AVB in the crest of the ventricular septum.[50]

Recent work further links TBX5 to arrhythmogenic mechanisms and atrial fibrillation in particular. Atrial fibrillation is sometimes observed in sporadic patients with Holt–Oram,[51] though mostly in the setting of congenital heart disease and the resultant hemodynamic effects, i.e. atrial enlargement. We recently reported on a family in which affected patients have mild skeletal deformations (a hallmark feature of HOS) and almost none has congenital heart disease. However, the great majority exhibited paroxysmal atrial fibrillation at an unusually young age.[52] Sequencing of *TBX5* revealed a novel mutation, p.G125R, co-segregating with the disease. This mutant protein results in significantly enhanced DNA-binding properties, which augment expression of *Nppa*, *Cx40*, *Kcnj2*, and *Tbx3* genes in comparison to wild-type TBX5, i.e. functioned as a gain-of-function factor. These findings are in contrast to all-known Holt–Oram mutations, as these result in a loss-of-function phenotype. This TBX5 gain-of-function mechanism probably underlies the paroxysmal atrial fibrillation and mild HOS phenotype. The mechanism underlying atrial fibrillation in TBX5 gain-of-function mutants may involve direct stimulation of TBX5 target genes involved in familial atrial fibrillation (*NPPA*, *KCNJ2, CX40*). Alternatively, it may involve TBX5-stimulated *TBX3*, as it was recently shown that *TBX3* is highly sensitive to *TBX5* dosage,[53] and TBX3 controls the sinus node gene program and induces pacemaker activity in atrial muscle.[9] There is thus evidence in support of a role of *TBX5* in the development of (paroxysmal) atrial fibrillation, a proposition that was recently further substantiated by the fact that genetic variation within the *TBX5* gene was found to be associated with AF.[15]

The left ventricle and right ventricle are distinct as they have a different morphology, tissue architecture, geometry, and function. Moreover, myocytes of the left and right ventricles differentiate to similar, but not identical phenotypes.[1] It is likely that differences in the regulatory programs of progenitors of the left and right ventricles underlie the differences in these phenotypes. Tbx5 likely contributes to this, as it is expressed in an antero-posterior gradient in the heart tube, which is regulated by retinoic acid.[54] Both the left and right ventricles are specified along the antero-posterior axis, and, as a consequence, the developing left ventricle robustly expresses *Tbx5*, in contrast to the right ventricle.[44] Taken together, this suggests that *Tbx5* is necessary for the left ventricular identity, and that it provides the boundary between the left and right ventricles.[55] Consequently, it is likely that target genes of Tbx5 are differentially regulated between the two ventricles. In fact, target gene *Cx40* mimics the pattern of Tbx5[47] and is differentially expressed between both ventricles in the developing and adult heart. As the right ventricle is especially sensitive to the development of various arrhythmias, such as those seen in Brugada syndrome[56] and arrhythmogenic right ventricular dysplasia,[57] and given the fact that *Tbx5* is actively involved in segregating between both ventricles,[55] it is possible that *Tbx5* target genes might oppose or contribute to arrhythmias. Indeed, recent work shows numerous genes that are influenced by differences in *Tbx5* dosage, such as those expressed during heart development like transcriptions factors (*Tbx3*, *Irx2*), cell–cell signalling molecules, and ion channels (*Cx40*, *KCNA5*).[53] This, together with the link between *TBX5* and atrial fibrillation, warrants further investigation into *TBX5*

and its downstream genes in relation to arrhythmias and ion channel genes.

## 5. Pitx2 prevents atrial fibrillation

Another gene linked to both cardiac development and atrial fibrillation is Pitx2. The pituitary homeobox (Pitx) family contains multiple genes. The *Pitx2* gene encodes three isoforms (Pitx2a,b,c), but *Pitx2c* is the major isoform expressed in the heart and drives asymmetrical cardiac morphogenesis.[58] Mutations in *PITX2* are associated with Rieger syndrome I, a disorder that includes ocular, tooth, and anterior body wall defects as primary characteristics.[59] *Pitx2c*-isoform specific knockout mice die before birth and display right atrial isomerism, septal defects, persistent truncus arteriosus and abnormal aortic arch remodelling.[60] Normally, *Pitx2c* is expressed in the embryonic and postnatal left atrium and pulmonary vein (*Figure 2*). *Pitx2c*-null embryos develop bilateral functional sinus nodes. Moreover, pulmonary vein myocardium does not develop in mutants.[40] Interestingly, adult mice that are haploinsufficient for *Pitx2* are susceptible to atrial arrythmias and atrial tachycardia.[60,61] *In situ* and expression studies demonstrated that Pitx2 suppresses sinus node-specific gene expression in the left atrium of both embryos and young adults. Furthermore, *in vivo* chromatin immuno-precipitation and transfection experiments demonstrated that Pitx2 inhibits the SAN-specific genetic program in left atrium directly. These findings implicate that Pitx2 and Pitx2-mediated left–right asymmetry signalling pathways are involved in prevention of atrial arrhythmias by suppressing abnormal pacemaking.[60] Furthermore, previous work indicated that a gain-of-function mutation in *Kcnq1* results in familial atrial fibrillation[62] and *Kcnq1* is also under control of Pitx2c. Taken together, these findings suggest that the cardiac transcription factor Pitx2c is actively involved in the prevention of atrial arrhythmias and supports *Pitx2c* as a genuine atrial arrhythmia susceptibility gene. This is supported by recent genome-wide association studies that identified sequence variants on chromosome 4q25, located in a region 150 kb distal to the *Pitx2* homeobox gene. These variants were associated with an increased risk for atrial fibrillation in various human populations.[11,12] These variants were all strongly associated with atrial fibrillation cases diagnosed before the age of 60, suggesting that modulation of the expression (through genomic variants) of Pitx2 might underlie the susceptibility to atrial fibrillation.

## 6. Irx5 establishes the cardiac repolarization gradient

The *Iroquois* homeobox (*Irx*) genes were first identified as pattern genes that control the expression of neural genes in *Drosophila*.[63] In mammals, six *Irx* genes exist organized in two clusters, the IrxA cluster containing *Irx1*, *Irx2*, and *Irx4*, and IrxB cluster containing *Irx3*, *Irx5*, and *Irx6*.[63] They encode proteins with a conserved homeodomain of the three-amino acid length extension superclass and a conserved 13 amino acid-residue motif, the Iro box, which is unique to the family.[64] Mammalian *Irx* genes show overlapping expression patterns, suggesting that they possess functionally redundant roles during development. Moreover, all six *Irx* genes display specific expression patterns in the developing heart.[65,66] In 2005, two publications shed light on the role of Irx5 in mice by use of knockouts.[8,67] Heterozygous Irx5 mutants were indistinguishable from wild-type littermates, but homozygous mutants were on average 25% smaller and had a defect in the differentiation of retinal cone bipolar cells. Intercrosses of heterozygous mutant mice demonstrated skewed mendelian ratios, suggesting that Irx5-deficient mice have a reduced viability. It was established that mice lacking Irx5 have a flattened cardiac repolarization gradient due to an increase in Kv4.2 potassium-channel expression in endocardial myocardium. This leads to a selective increase in the major cardiac repolarization current, I(to,f), and consequently an increased susceptibility to arrhythmias. Normally, Irx5 is expressed in an endocardial-to-epicardial gradient, while the Kv4.2 expression gradient is the exact opposite (*Figure 2*). It was shown that Irx5 actually represses expression of *Kcnd2* encoding Kv4.2 via recruitment of the cardiac transcriptional repressor mBop (*SMYD1*). In conclusion, an Irx5 repressor gradient negatively regulates potassium-channel-gene expression in the heart, forming an inverse I(to,f) gradient that ensures coordinated cardiac repolarization, while also preventing arrhythmias. Thus far no mutations in either Irx5 or Kv4.2[68] have been identified in patients with arrhythmias.

## 7. Right ventricular outflow tract (RVOT) and arrhythmia

Both the Brugada syndrome and ARVC/D are diseases in which the right ventricular free wall and the right ventricular outflow tract (RVOT) are affected. It is suggested that activation of the RVOT is delayed in both diseases and that the cause of this may be either conduction slowing or structural block.[69] In both these diseases, the ventricular arrhythmias are mostly initiated in the RVOT.[70] It is possible that the right ventricular embryonic origin partly underlies the right ventricular susceptibility to arrhythmias. As described earlier, the cardiomyocytes of the embryonic OFT retain much longer the original slowly conducting and poorly contracting properties.[71] This causes long-lasting contractions that are possibly involved in preventing the regurgitation of blood before the formation of the semilunar valves. *In vivo* labelling studies demonstrate that the proximal as well as the distal embryonic OFT give rise to both the trabeculated free wall, and the smooth-walled conus of the RVOT. Some molecular pathways controlling the OFT development have been identified,[72] for instance the OFT is devoid of Tbx5 and consequently of *Cx40* expression.[47] Moreover, the OFT expresses Tbx2, a repressor that competes with Tbx5. Like Tbx3, Tbx2 suppresses working myocardial differentiation of the AVC,[73–75] suggesting it may do so for the OFT as well. Interestingly, *Cx43* expression is not found in the foetal OFT, but it is detectable in the RVOT of adults, though to a lesser extent than the free wall of the right ventricle.[76] This could indicate that inadequate upregulation of *Cx43* and other genes in the RVOT results in heterogeneous expression in the right ventricular wall. One clue might be the gradual downregulation of *Tbx2* towards the end of development, which in turn probably contributes to the gradual upregulation of *Cx43* in the RVOT myocardium. In summary, remnants of the embryonic OFT phenotype and expression profile in the adult RVOT could determine the structural and electrophysiological characteristics that underlie the increased vulnerability for arrhythmias in the right ventricle. Moreover, perturbations by genetics variations in transcription networks and signalling pathways that interfere with this process might actually further aggravate the propensity for arrhythmias.

## 8. Accessory conduction pathways are associated with defective patterning and gene regulation within the atrioventricular canal myocardium

Wolff–Parkinson–White (WPW) syndrome is an arrhythmogenic defect characterized by a normal conduction system and one or more accessory AV connections, bypassing the AV node and His bundle, which cause ventricular pre-excitation and predispose to re-entrant supraventricular tachycardia.[77] These abnormal accessory pathways may conduct faster than the AV node and this leads to ventricular pre-excitation, evidenced by a short PR interval and a slurred upstroke of the QRS complex on the ECG. The majority of WPW occurs isolated and sporadic, affecting 1–3 persons per 1000,[77] most patients with WPW syndrome have structurally normal hearts, though in a small portion of WPW patients, accessory pathways occur in association with congenital heart defects, such as Ebstein anomaly.[78] Nonetheless, autosomal dominant WPW families have been described.[79,80] *PRKAG2* and *LAMP2* gene mutations were found in familial left ventricular hypertrophy and pre-excitation, while a *PRKAG2* mutation was detected in a single family with isolated WPW.[81,82] Patients with mutations on the *PRKAG2* gene have a variable combination of glycogen storage cardiomyopathy, progressive conduction system disease including sinus bradycardia and atrioventricular block, ventricular pre-excitation, arrhythmias, and sudden death.[81] Ventricular pre-excitation is presumably caused by a disruption in the annulus fibrosis, which electrically insulates the atrial and ventricular muscle masses, distinct from the muscular-appearing bypass tracts observed in typical WPW syndrome.[83] However, using a cardiomyocyte-specific overexpression model, accessory pathways developed only when the mutant PRKAG was overexpressed during development. If the overexpression was started in adulthood, the glycogen storage disease and conduction system degeneration still occurred, but no accessory pathways developed, suggesting that a developmental defect may underlie pre-excitation in PRKAG mutants.[84] In addition, various *NKX2–5* gene mutations in patients with CHDs are also associated with WPW, and point to a role of this critical cardiac transcription factor gene in WPW pathogenesis.[30,34]

A milder form of arrhythmias involving accessory pathways is the atrioventricular reentrant tachycardia (AVRT). This is the most common type of supraventricular tachycardia in both the foetus and the newborn.[85] Although AVRT can be potentially life-threatening and are sometimes difficult to control with antiarrhythmic drug therapy, in general these tachycardias resolve spontaneously within the first months of life, and >60% of patients require no antiarrhythmic drug therapy and remain free of symptoms after the age of 1 year.[86] This self-resolving character of most perinatal AVRTs suggests that the majority of the accessory pathways eventually disappear after birth. Studies in animal models have shown that accessory AV myocardial connections are present until the late stages of cardiac development.[87] Moreover, a recent study on human hearts from neonates without episodes of supraventricular tachycardia found that isolation of the AV junction is a gradual and ongoing process.[88] The right lateral accessory myocardial AV connections in particular are commonly found at later stages of normal human cardiac development. These transitory accessory connections may therefore act as a substrate for AV reentrant tachycardias in foetuses or neonates.

The molecular mechanisms by which these accessory bypasses are formed are, however, poorly understood. The atrioventricular (AV) node and AV bundle are the only muscular connections that cross the annulus fibrosus. However, during heart development, in the absence of an annulus fibrosus, the AV canal myocardium still maintains adequate AV delay, allowing for the coordinated alternating contraction of the atria and ventricles.[89] Remnant strands of AV myocardium should disappear when the annulus fibrosus is fully formed;[88] however, sometimes these are observed in normal hearts around and after birth (see above). These strands are likely to maintain the slow conduction properties of the AV myocardium. Moreover, in the adult heart, slow-conducting AV canal-type myocardium is detected around the orifices of the mitral and tricuspid valve.[25,90] Together, this suggests that AV canal myocardium has an essential role in the AV delay and that defects in AV canal myocardium could lead to formation of functional accessory pathways. The AV canal myocardium is actually specified early in cardiac development by the expression of bone morphogenetic protein 2 (Bmp2) in the AV canal myocardium progenitors in the early heart tube stimulating the expression of Tbx2.[91] This transcription factor is required for the development of the AV canal.[75] Indeed, in a recent study by Aanhaanen *et al.*,[10] it was shown that if *Tbx2* was specifically inactivated in the myocardium, this resulted in the formation of fast-conducting accessory pathways, malformation of the annulus fibrosus, and ventricular pre-excitation in mice. Several other genes and pathways have been implicated in AV canal development such as the Bmp and Notch pathways. Interestingly, microdeletions of both BMP2 and JAGGED1, a Notch ligand, are associated with ventricular pre-excitation in human.[92,93] Moreover, deletion of the *Alk3* receptor (activated by Bmp2) in the AV canal myocardium results in AV nodal defects and accessory pathways in mice.[94] The fact that Notch signalling is involved in maturation of the AV canal-derived myocardium was underscored by another recent paper in which it was shown that activation of Notch signalling in the developing myocardium of mice produces fully penetrant accessory pathways and ventricular pre-excitation.[95] Conversely, inhibition of Notch signalling in the developing myocardium resulted in a hypoplastic AV node, with specific loss of slow-conducting cells expressing Cx30.2 and a loss of physiologic AV conduction delay. In conclusion, these results indicate that defective gene regulation and patterning within the AV canal myocardium, via disruption of the AV canal regulatory network, leads to malformation of the annulus fibrosus, formation of accessory AV connections, and ventricular pre-excitation.

## 9. Ion channels have a possible non-electrogenic role in heart development

Recent work has demonstrated that heart development does not only rely on interactions of transcription factors and target genes, but, surprisingly, that ion channels themselves play a novel and possibly non-electrogenic role in heart development. The first evidence for this emerged from a study in which a strain of zebrafish called island beat was identified, in which the ventricle failed to grow and did not contract, while the atrium exhibited rapid, isolated, and

discoordinated contractions. Positional cloning led to the identification of mutations in the pore-forming α1C subunit of the L-type calcium current.[96] Thus, abolishment of this current caused the ventricle to fail to grow and remain electrically silent, which demonstrated that the L-type calcium current is essential for normal cardiac form and function during embryonic development. This was followed by the discovery that knockouts for *Scn5a*, the cardiac sodium channel, led to early embryonic lethality. Hearts of knockout mice showed uncoordinated contractions, and developmental defects were seen such as a common ventricular chamber, with reduced chamber size, reduced trabeculation of the ventricular wall, and a reduced number of thin, spindle-like cardiomyocytes.[97] This ventricular defect in *Scn5a* knockout embryos is unlikely to reflect a generalized failure of cardiac development, as the endocardial cushions of the AVC, the common atrial chamber, and the truncus arteriosus appeared normal. This suggested the possibility that ion channels have an important influence on ventricular development. Indeed, antisense knockdown of zebrafish homologue *Scn5a* results in marked cardiac chamber dysmorphogenesis and perturbed looping.[98] Moreover, these abnormalities were associated with decreased expression of the myocardial precursor genes *Nkx2.5*, *Gata4*, and *Hand2* and significant deficits in the production of cardiomyocyte progenitors. Interestingly, these early defects did not appear to result from altered membrane electrophysiology, as prolonged pharmacological blockade of sodium current failed to phenocopy the ion channel knockdown. Moreover, embryos grown in calcium channel blocker-containing medium (similar to the island beat experiments described earlier) had hearts that did not beat but developed normally. This established that voltage-gated ion channels have important roles in vertebrate heart development and supports the hypothesis that such roles might be mediated by non-electrogenic functions of the channel complex. Additional evidence for the role of ion channels in heart development came from studies on mice lacking HCN4 (carrier of the If pacemaker current), either globally or specifically in the heart. Such mice die during embryonic development between E9.5 and E11.5.[99] The functional correlation between the early embryonic death and the inactivation of *Hcn4* was not obvious. A possible cause might have been the deficiency to develop functional or mature pacemaker cells. In summary, recent results argue that ion channels are required for heart development in addition to their canonical role as regulators of heart rhythm. Moreover, the data suggest that this developmental role is mediated by a non-electrogenic function of the ion channel.

## 10. Summary

It has become increasingly clear that transcriptional regulation which orchestrates both the development of the heart, and the specification of the different cardiac regions from cardiac precursor cells during embryonic development, plays an essential role in regulation of expression of ion channel and gap junction genes. A causal relation between transcription factors and regionalized expression of genes involved in cardiac electrical activity has been established (*Table 1*). In some instances, abnormal cardiac electrical function and arrhythmias in the postnatal heart may stem from a developmental abnormality causing maintained (epigenetic) changes in gene regulation.

**Table 1 Genes involved in developmental aspects of cardiac arrhythmogenesis**

| Gene | Main function | Developmental role in heart | Associated GWAS phenotype | Associated human disease |
|---|---|---|---|---|
| TBX2 | Transcription factor | Regulates AV canal development | | None |
| TBX3 | Transcription factor | Regulates conduction system development | QRS[13,14,15] | Ulnar-mammary syndrome |
| TBX5 | Transcription factor | Promotes chamber and conduction development | [a]QRS[13,14,15] PR-interval, atrial fibrillaton[15] | Holt–Oram syndrome |
| IRX5 | Transcription factor | Establishes transmural gradient of potassium-channel-gene expression | | None |
| NKX2–5 | Transcription factor | Core cardiac transcription factor, multifunctional co-factor | [a]PR-interval, atrial fibrillation[13] | ASD with AV conduction defect, Tetralogy of Fallot |
| GATA4 | Transcription factor | Core cardiac transcription factor, multifunctional co-factor | | ASD |
| PITX2 | Transcription factor | Asymmetrical cardiac morphogenesis, prevention of atrial arrhythmias | Atrial fibrillation[11] | Axenfeld–Rieger syndrome |
| GJC3 (Cx30.2) | Gap junction | Establishes slow-conduction | | None |
| SCN5A | Ion channel, sodium | Activation, non-electric physiological function | QRS[14] | Long QT 3, Brugada syndrome, progressive conduction disease, sick sinus syndrome, atrial fibrillation, atrial standstill, dilated cardiomyopathy |
| CACNA1C | Ion channel, calcium | Activation, non-electric physiological function | | Timothy syndrome, Brugada syndrome, Brugada syndrome with short QT |
| HCN4 | Ion channel, potassium | Activation, non-electric physiological function | | Sick sinus syndrome 2 |

[a]Not at genome-wide statistical significance.

Surprisingly, a developmental role of ion channels themselves might be mediated by a non-electrogenic function of the ion channel. The role of developmental genes in regulation of cardiac electrical function was corroborated by recent genome-wide association studies providing strong evidence that common genetic variation, at loci harbouring these genes, modulates electrocardiographic indices of conduction and repolarization and susceptibility to arrhythmia.

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## References

1. Boukens BJ, Christoffels VM, Coronel R, Moorman AF. Developmental basis for electrophysiological heterogeneity in the ventricular and outflow tract myocardium as a substrate for life-threatening ventricular arrhythmias. *Circ Res* 2009;**104**:19–31.
2. Bruneau BG. Transcriptional regulation of vertebrate cardiac morphogenesis. *Circ Res* 2002;**90**:509–519.
3. Habets PE, Moorman AF, Christoffels VM. Regulatory modules in the developing heart. *Cardiovasc Res* 2003;**58**:246–263.
4. Moorman AF, Christoffels VM. Cardiac chamber formation: development, genes, and evolution. *Physiol Rev* 2003;**83**:1223–1267.
5. Christoffels VM, Smits GJ, Kispert A, Moorman AF. Development of the pacemaker tissues of the heart. *Circ Res* 2010;**106**:240–254.
6. Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, Soults J *et al*. Mutations in human TBX5 [corrected] cause limb and cardiac malformation in Holt-Oram syndrome. *Nat Genet* 1997;**15**:30–35.
7. Schott JJ, Benson DW, Basson CT, Pease W, Silberbach GM, Moak JP *et al*. Congenital heart disease caused by mutations in the transcription factor NKX2–5. *Science* 1998;**281**:108–111.
8. Costantini DL, Arruda EP, Agarwal P, Kim KH, Zhu Y, Zhu W *et al*. The homeodomain transcription factor Irx5 establishes the mouse cardiac ventricular repolarization gradient. *Cell* 2005;**123**:347–358.
9. Hoogaars WM, Engel A, Brons JF, Verkerk AO, de Lange FJ, Wong LY *et al*. Tbx3 controls the sinoatrial node gene program and imposes pacemaker function on the atria. *Genes Dev* 2007;**21**:1098–1112.
10. Aanhaanen WT, Boukens BJ, Sizarov A, Wakker V, de Gier-de Vries C, van Ginneken AC *et al*. Defective Tbx2-dependent patterning of the atrioventricular canal myocardium causes accessory pathway formation in mice. *J Clin Invest* 2011;**121**:534–544.
11. Gudbjartsson DF, Arnar DO, Helgadottir A, Gretarsdottir S, Holm H, Sigurdsson A *et al*. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature* 2007;**448**:353–357.
12. Kaab S, Darbar D, van Noord C, Dupuis J, Pfeufer A, Newton-Cheh C *et al*. Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. *Eur Heart J* 2009;**30**:813–819.
13. Pfeufer A, van Noord C, Marciante KD, Arking DE, Larson MG, Smith AV *et al*. Genome-wide association study of PR interval. *Nat Genet* 2010;**42**:153–159.
14. Sotoodehnia N, Isaacs A, de Bakker PI, Dorr M, Newton-Cheh C, Nolte IM *et al*. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat Genet* 2010;**42**:1068–1076.
15. Holm H, Gudbjartsson DF, Arnar DO, Thorleifsson G, Thorgeirsson G, Stefansdottir H *et al*. Several common variants modulate heart rate, PR interval and QRS duration. *Nat Genet* 2010;**42**:117–122.
16. Buckingham M, Meilhac S, Zaffran S. Building the mammalian heart from two sources of myocardial cells. *Nat Rev Genet* 2005;**6**:826–835.
17. van den Berg G, Abu-Issa R, de Boer BA, Hutson MR, de Boer PA, Soufan AT *et al*. A caudal proliferating growth center contributes to both poles of the forming heart tube. *Circ Res* 2009;**104**:179–188.
18. Anderson RH, Christoffels VM, Moorman AF. Controversies concerning the anatomical definition of the conduction tissues. *Anat Rec B New Anat* 2004;**280**:8–14.
19. Christoffels VM, Moorman AFM. Development of the cardiac conduction system: why are some regions of the heart more arrhythmogenic than others?. *Circ Arrhythm Electrophysiol* 2009;**2**:195–207.
20. Alcolea S, Theveniau-Ruissy M, Jarry-Guichard T, Marics I, Tzouanacou E, Chauvin JP *et al*. Downregulation of connexin 45 gene products during mouse heart development. *Circ Res* 1999;**84**:1365–1379.
21. Van Mierop LH. Location of pacemaker in chick embryo heart at the time of initiation of heartbeat. *Am J Physiol* 1967;**212**:407–415.
22. Mommersteeg MT, Hoogaars WM, Prall OW, de Gier-de Vries C, Wiese C, Clout DE *et al*. Molecular pathway for the localized formation of the sinoatrial node. *Circ Res* 2007;**100**:354–362.
23. Wiese C, Grieskamp T, Airik R, Mommersteeg MT, Gardiwal A, de Gier-de Vries C *et al*. Formation of the sinus node head and differentiation of sinus node myocardium are independently regulated by Tbx18 and Tbx3. *Circ Res* 2009;**104**:388–397.
24. Horsthuis T, Buermans HP, Brons JF, Verkerk AO, Bakker ML, Wakker V *et al*. Gene expression profiling of the forming atrioventricular node using a novel tbx3-based node-specific transgenic reporter. *Circ Res* 2009;**105**:61–69.
25. Aanhaanen WT, Mommersteeg MT, Norden J, Wakker V, de Gier-de Vries C, Anderson RH *et al*. Developmental origin, growth, and three-dimensional architecture of the atrioventricular conduction axis of the mouse heart. *Circ Res* 2010;**107**:728–736.
26. Habets PE, Moorman AF, Clout DE, van Roon MA, Lingbeek M, van Lohuizen M *et al*. Cooperative action of Tbx2 and Nkx2.5 inhibits ANF expression in the atrioventricular canal: implications for cardiac chamber formation. *Genes Dev* 2002;**16**:1234–1246.
27. Moorman AF, Schumacher CA, de Boer PA, Hagoort J, Bezstarosti K, van den Hoff MJ *et al*. Presence of functional sarcoplasmic reticulum in the developing heart and its confinement to chamber myocardium. *Dev Biol* 2000;**223**:279–290.
28. Soufan AT, van den Berg G, Ruijter JM, de Boer PA, Van Den Hoff MJ, Moorman AF. A regionalized sequence of myocardial cell growth and proliferation characterizes early chamber formation. *Circ Res* 2006;**99**:545–552.
29. Lyons I, Parsons LM, Hartley L, Li R, Andrews JE, Robb L *et al*. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2–5. *Genes Dev* 1995;**9**:1654–1666.
30. Benson DW, Silberbach GM, Kavanaugh-McHugh A, Cottrill C, Zhang Y, Riggs S *et al*. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. *J Clin Invest* 1999;**104**:1567–1573.
31. Kasahara H, Wakimoto H, Liu M, Maguire CT, Converso KL, Shioi T *et al*. Progressive atrioventricular conduction defects and heart failure in mice expressing a mutant Csx/Nkx2.5 homeoprotein. *J Clin Invest* 2001;**108**:189–201.
32. Kasahara H, Benson DW. Biochemical analyses of eight NKX2.5 homeodomain missense mutations causing atrioventricular block and cardiac anomalies. *Cardiovasc Res* 2004;**64**:40–51.
33. Meysen S, Marger L, Hewett KW, Jarry-Guichard T, Agarkova I, Chauvin JP *et al*. Nkx2.5 cell-autonomous gene function is required for the postnatal formation of the peripheral ventricular conduction system. *Dev Biol* 2007;**303**:740–753.
34. Moskowitz IP, Kim JB, Moore ML, Wolf CM, Peterson MA, Shendure J *et al*. A molecular pathway including Id2, Tbx5, and Nkx2–5 required for cardiac conduction system development. *Cell* 2007;**129**:1365–1376.
35. Gutierrez-Roelens I, De Roy L, Ovaert C, Sluysmans T, Devriendt K, Brunner HG *et al*. A novel CSX/NKX2–5 mutation causes autosomal-dominant AV block: are atrial fibrillation and syncopes part of the phenotype? *Eur J Hum Genet* 2006;**14**:1313–1316.
36. Postma AV, Dekker LR, Soufan AT, Moorman AF. Developmental and genetic aspects of atrial fibrillation. *Trends Cardiovasc Med* 2009;**19**:123–130.
37. Masani F. Node-like cells in the myocardial layer of the pulmonary vein of rats: an ultrastructural study. *J Anat* 1986;**145**:133–142.
38. Perez-Lugones A, McMahon JT, Ratliff NB, Saliba WI, Schweikert RA, Marrouche NF *et al*. Evidence of specialized conduction cells in human pulmonary veins of patients with atrial fibrillation. *J Cardiovasc Electrophysiol* 2003;**14**:803–809.
39. Mommersteeg MT, Soufan AT, de Lange FJ, van den Hoff MJ, Anderson RH, Christoffels VM *et al*. Two distinct pools of mesenchyme contribute to the development of the atrial septum. *Circ Res* 2006;**99**:351–353.
40. Mommersteeg MT, Brown NA, Prall OW, de Gier-de Vries C, Harvey RP, Moorman AF *et al*. Pitx2c and Nkx2–5 are required for the formation and identity of the pulmonary myocardium. *Circ Res* 2007;**101**:902–909.
41. Christoffels VM, Mommersteeg MT, Trowe MO, Prall OW, de Gier-de Vries C, Soufan AT *et al*. Formation of the venous pole of the heart from an Nkx2–5-negative precursor population requires Tbx18. *Circ Res* 2006;**98**:1555–1563.
42. Cheng S, Keyes MJ, Larson MG, McCabe EL, Newton-Cheh C, Levy D *et al*. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. *JAMA* 2009;**301**:2571–2577.
43. Kispert A, Hermann BG. The Brachyury gene encodes a novel DNA binding protein. *EMBO J* 1993;**12**:4898–4899.
44. Bruneau BG, Logan M, Davis N, Levi T, Tabin CJ, Seidman JG *et al*. Chamber-specific cardiac expression of Tbx5 and heart defects in Holt-Oram syndrome. *Dev Biol* 1999;**211**:100–108.
45. Christoffels VM, Habets PE, Franco D, Campione M, de Jong F, Lamers WH *et al*. Chamber formation and morphogenesis in the developing mammalian heart. *Dev Biol* 2000;**223**:266–278.
46. Boogerd CJ, Dooijes D, Ilgun A, Mathijssen IB, Hordijk R, van de Laar IM *et al*. Functional analysis of novel TBX5 T-box mutations associated with Holt-Oram syndrome. *Cardiovasc Res* 2010;**88**:130–139.
47. Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, Caron S *et al*. A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. *Cell* 2001;**106**:709–721.

48. Hiroi Y, Kudoh S, Monzen K, Ikeda Y, Yazaki Y, Nagai R *et al*. Tbx5 associates with Nkx2–5 and synergistically promotes cardiomyocyte differentiation. *Nat Genet* 2001;**28**:276–280.
49. Fijnvandraat AC, Lekanne Deprez RH, Christoffels VM, Ruijter JM, Moorman AF. TBX5 overexpression stimulates differentiation of chamber myocardium in P19C16 embryonic carcinoma cells. *J Muscle Res Cell Motil* 2003;**24**:211–218.
50. Moskowitz IP, Pizard A, Patel VV, Bruneau BG, Kim JB, Kupershmidt S *et al*. The T-Box transcription factor Tbx5 is required for the patterning and maturation of the murine cardiac conduction system. *Development* 2004;**131**:4107–4116.
51. Basson CT, Cowley GS, Solomon SD, Weissman B, Poznanski AK, Traill TA *et al*. The clinical and genetic spectrum of the Holt-Oram syndrome (heart-hand syndrome). *N Engl J Med* 1994;**330**:885–891.
52. Postma AV, van de Meerakker JB, Mathijssen IB, Barnett P, Christoffels VM, Ilgun A *et al*. A gain-of-function TBX5 mutation is associated with atypical Holt-Oram syndrome and paroxysmal atrial fibrillation. *Circ Res* 2008;**102**:1433–1442.
53. Mori AD, Zhu Y, Vahora I, Nieman B, Koshiba-Takeuchi K, Davidson L *et al*. Tbx5-dependent rheostatic control of cardiac gene expression and morphogenesis. *Dev Biol* 2006;**297**:566–586.
54. Niederreither K, Vermot J, Messaddeq N, Schuhbaur B, Chambon P, Dolle P. Embryonic retinoic acid synthesis is essential for heart morphogenesis in the mouse. *Development* 2001;**128**:1019–1031.
55. Koshiba-Takeuchi K, Mori AD, Kaynak BL, Cebra-Thomas J, Sukonnik T, Georges RO *et al*. Reptilian heart development and the molecular basis of cardiac chamber evolution. *Nature* 2009;**461**:95–98.
56. Shimizu W. The Brugada syndrome—an update. *Intern Med* 2005;**44**:1224–1231.
57. Kies P, Bootsma M, Bax J, Schalij MJ, van der Wall EE. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: screening, diagnosis, and treatment. *Heart Rhythm* 2006;**3**:225–234.
58. Gage PJ, Suh H, Camper SA. The bicoid-related Pitx gene family in development. *Mamm Genome* 1999;**10**:197–200.
59. Semina EV, Reiter R, Leysens NJ, Alward WL, Small KW, Datson NA *et al*. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. *Nat Genet* 1996;**14**:392–399.
60. Wang J, Klysik E, Sood S, Johnson RL, Wehrens XH, Martin JF. Pitx2 prevents susceptibility to atrial arrhythmias by inhibiting left-sided pacemaker specification. *Proc Natl Acad Sci USA* 2010;**107**:9753–9758.
61. Kirchhof P, Kahr PC, Kaese S, Piccini I, Vokshi I, Scheld HH *et al*. PITX2c is expressed in the adult left atrium, and reducing Pitx2c expression promotes atrial fibrillation inducibility and complex changes in gene expression. *Circ Cardiovasc Genet* 2011;**4**:123–133.
62. Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY *et al*. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* 2003;**299**:251–254.
63. Gomez-Skarmeta JL, Modolell J. Iroquois genes: genomic organization and function in vertebrate neural development. *Curr Opin Genet Dev* 2002;**12**:403–408.
64. Burglin TR. Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res* 1997;**25**:4173–4180.
65. Bruneau BG, Bao ZZ, Tanaka M, Schott JJ, Izumo S, Cepko CL *et al*. Cardiac expression of the ventricle-specific homeobox gene Irx4 is modulated by Nkx2–5 and dHand. *Dev Biol* 2000;**217**:266–277.
66. Christoffels VM, Keijser AG, Houweling AC, Clout DE, Moorman AF. Patterning the embryonic heart: identification of five mouse Iroquois homeobox genes in the developing heart. *Dev Biol* 2000;**224**:263–274.
67. Cheng CW, Chow RL, Lebel M, Sakuma R, Cheung HO, Thanabalasingham V *et al*. The Iroquois homeobox gene, Irx5, is required for retinal cone bipolar cell development. *Dev Biol* 2005;**287**:48–60.
68. Frank-Hansen R, Larsen LA, Andersen P, Jespersgaard C, Christiansen M. Mutations in the genes KCND2 and KCND3 encoding the ion channels Kv4.2 and Kv4.3, conducting the cardiac fast transient outward current (ITO,f), are not a frequent cause of long QT syndrome. *Clin Chim Acta* 2005;**351**:95–100.
69. Furushima H, Chinushi M, Okamura K, Iijima K, Komura S, Tanabe Y *et al*. Comparison of conduction delay in the right ventricular outflow tract between Brugada syndrome and right ventricular cardiomyopathy: investigation of signal average ECG in the precordial leads. *Europace* 2007;**9**:951–956.
70. Morita H, Fukushima-Kusano K, Nagase S, Takenaka-Morita S, Nishii N, Kakishita M *et al*. Site-specific arrhythmogenesis in patients with Brugada syndrome. *J Cardiovasc Electrophysiol* 2003;**14**:373–379.
71. de Jong F, Opthof T, Wilde AA, Janse MJ, Charles R, Lamers WH *et al*. Persisting zones of slow impulse conduction in developing chicken hearts. *Circ Res* 1992;**71**:240–250.
72. Black BL. Transcriptional pathways in second heart field development. *Semin Cell Dev Biol* 2007;**18**:67–76.
73. Christoffels VM, Hoogaars WM, Tessari A, Clout DE, Moorman AF, Campione M. T-box transcription factor Tbx2 represses differentiation and formation of the cardiac chambers. *Dev Dyn* 2004;**229**:763–770.
74. Harrelson Z, Kelly RG, Goldin SN, Gibson-Brown JJ, Bollag RJ, Silver LM *et al*. Tbx2 is essential for patterning the atrioventricular canal and for morphogenesis of the outflow tract during heart development. *Development* 2004;**131**:5041–5052.
75. Aanhaanen WT, Brons JF, Dominguez JN, Rana MS, Norden J, Airik R *et al*. The Tbx2+ primary myocardium of the atrioventricular canal forms the atrioventricular node and the base of the left ventricle. *Circ Res* 2009;**104**:1267–1274.
76. Ou B, Nakagawa M, Kajimoto M, Nobe S, Ooie T, Ichinose M *et al*. Heterogeneous expression of connexin 43 in the myocardium of rabbit right ventricular outflow tract. *Life Sci* 2005;**77**:52–59.
77. Deal BJ, Keane JF, Gillette PC, Garson A Jr. Wolff-Parkinson-White syndrome and supraventricular tachycardia during infancy: management and follow-up. *J Am Coll Cardiol* 1985;**5**:130–135.
78. Delhaas T, Sarvaas GJ, Rijlaarsdam ME, Strengers JL, Eveleigh RM, Poulino SE *et al*. A multicenter, long-term study on arrhythmias in children with Ebstein anomaly. *Pediatr Cardiol* 2010;**31**:229–233.
79. Fananapazir L, Tracy CM, Leon MB, Winkler JB, Cannon RO 3rd, Bonow RO *et al*. Electrophysiologic abnormalities in patients with hypertrophic cardiomyopathy. A consecutive analysis in 155 patients. *Circulation* 1989;**80**:1259–1268.
80. Pignatelli RH, McMahon CJ, Dreyer WJ, Denfield SW, Price J, Belmont JW *et al*. Clinical characterization of left ventricular noncompaction in children: a relatively common form of cardiomyopathy. *Circulation* 2003;**108**:2672–2678.
81. Gollob MH, Green MS, Tang AS, Gollob T, Karibe A, Ali Hassan AS *et al*. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. *N Engl J Med* 2001;**344**:1823–1831.
82. Arad M, Maron BJ, Gorham JM, Johnson WH Jr, Saul JP, Perez-Atayde AR *et al*. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *N Engl J Med* 2005;**352**:362–372.
83. Arad M, Moskowitz IP, Patel VV, Ahmad F, Perez-Atayde AR, Sawyer DB *et al*. Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff-Parkinson-White syndrome in glycogen storage cardiomyopathy. *Circulation* 2003;**107**:2850–2856.
84. Sidhu JS, Rajawat YS, Rami TG, Gollob MH, Wang Z, Yuan R *et al*. Transgenic mouse model of ventricular preexcitation and atrioventricular reentrant tachycardia induced by an AMP-activated protein kinase loss-of-function mutation responsible for Wolff-Parkinson-White syndrome. *Circulation* 2005;**111**:21–29.
85. Ko JK, Deal BJ, Strasburger JF, Benson DW Jr. Supraventricular tachycardia mechanisms and their age distribution in pediatric patients. *Am J Cardiol* 1992;**69**:1028–1032.
86. Naheed ZJ, Strasburger JF, Deal BJ, Benson DW Jr, Gidding SS. Fetal tachycardia: mechanisms and predictors of hydrops fetalis. *J Am Coll Cardiol* 1996;**27**:1736–1740.
87. Kolditz DP, Wijffels MC, Blom NA, van der Laarse A, Markwald RR, Schalij MJ *et al*. Persistence of functional atrioventricular accessory pathways in postseptated embryonic avian hearts: implications for morphogenesis and functional maturation of the cardiac conduction system. *Circulation* 2007;**115**:17–26.
88. Hahurij ND, Gittenberger-De Groot AC, Kolditz DP, Bokenkamp R, Schalij MJ, Poelmann RE *et al*. Accessory atrioventricular myocardial connections in the developing human heart: relevance for perinatal supraventricular tachycardias. *Circulation* 2008;**117**:2850–2858.
89. Tallini YN, Ohkura M, Choi BR, Ji G, Imoto K, Doran R *et al*. Imaging cellular signals in the heart in vivo: cardiac expression of the high-signal Ca2+ indicator GCaMP2. *Proc Natl Acad Sci USA* 2006;**103**:4753–4758.
90. McGuire MA, de Bakker JM, Vermeulen JT, Moorman AF, Loh P, Thibault B *et al*. Atrioventricular junctional tissue. Discrepancy between histological and electrophysiological characteristics. *Circulation* 1996;**94**:571–577.
91. Ma L, Lu MF, Schwartz RJ, Martin JF. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. *Development* 2005;**132**:5601–5611.
92. Le Gloan L, Pichon O, Isidor B, Boceno M, Rival JM, David A *et al*. A 8.26Mb deletion in 6q16 and a 4.95Mb deletion in 20p12 including JAG1 and BMP2 in a patient with Alagille syndrome and Wolff-Parkinson-White syndrome. *Eur J Med Genet* 2008;**51**:651–657.
93. Lalani SR, Thakuria JV, Cox GF, Wang X, Bi W, Bray MS *et al*. 20p12.3 microdeletion predisposes to Wolff-Parkinson-White syndrome with variable neurocognitive deficits. *J Med Genet* 2009;**46**:168–175.
94. Gaussin V, Morley GE, Cox L, Zwijsen A, Vance KM, Emile L *et al*. Alk3/Bmpr1a receptor is required for development of the atrioventricular canal into valves and annulus fibrosus. *Circ Res* 2005;**97**:219–226.
95. Rentschler S, Harris BS, Kuznekoff L, Jain R, Manderfield L, Lu MM *et al*. Notch signaling regulates murine atrioventricular conduction and the formation of accessory pathways. *J Clin Invest* 2011;**121**:525–533.
96. Rottbauer W, Baker K, Wo ZG, Mohideen MA, Cantiello HF, Fishman MC. Growth and function of the embryonic heart depend upon the cardiac-specific L-type calcium channel alpha1 subunit. *Dev Cell* 2001;**1**:265–275.
97. Papadatos GA, Wallerstein PM, Head CE, Ratcliff R, Brady PA, Benndorf K *et al*. Slowed conduction and ventricular tachycardia after targeted disruption of the cardiac sodium channel gene Scn5a. *Proc Natl Acad Sci USA* 2002;**99**:6210–6215.
98. Chopra SS, Stroud DM, Watanabe H, Bennett JS, Burns CG, Wells KS *et al*. Voltage-gated sodium channels are required for heart development in zebrafish. *Circ Res* 2010;**106**:1342–1350.
99. Stieber J, Herrmann S, Feil S, Loster J, Feil R, Biel M *et al*. The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. *Proc Natl Acad Sci USA* 2003;**100**:15235–15240.
100. Nerbonne J. Molecular analysis of voltage-gated K+ channel diversity and functioning in the mammalian heart. In: Terjung R, ed. *Comprehensive Physiology*. Wiley; 2010.