Electrophysiological patterning of the heart

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Chapter 4

Developmental basis for electrophysiological heterogeneity in the ventricular and outflow tract myocardium as a substrate for life-threathening ventricular arrhythmias

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Abstract

Reentry is the main mechanism of life-threatening ventricular arrhythmias, including ventricular fibrillation and tachycardia. Its occurrence depends on the simultaneous presence of an arrhythmogenic substrate (a pre-existing condition), and a 'trigger', and is favored by electrophysiological heterogeneities. In the adult heart, electrophysiological heterogeneities of the ventricle exist along the apico-basal, left-right and transmural axes. Also, conduction is preferentially slowed in the right ventricular outflow tract, especially during pharmacologic sodium channel blockade. We propose that the origin of electrophysiological heterogeneities of the adult heart lies in early heart development. The heart is formed from several progenitor regions: the first heart field predominantly forms the left ventricle, while the second heart field forms the right ventricle and outflow tract. Furthermore, the embryonic outflow tract consists of slowly conducting tissue until it is incorporated into the ventricles and develops rapidly conducting properties. The sub-epicardial myocytes and sub-endocardial myocytes run distinctive gene programs from their formation onwards. This review discusses the hypothesis that electrophysiological heterogeneities in the adult heart result from persisting patterns in gene expression and function along the cranial-caudal and epicardial-endocardial axes of the developing heart. Understanding the developmental origins of electrophysiological heterogeneity contributing to ventricular arrhythmias may give rise to new therapies.

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Introduction

Sudden cardiac death is generally preceded by reentry-based ventricular tachycardia or fibrillation.[1,2] These arrhythmias occur by the simultaneous presence of an initiating event (the trigger) and a pre-existing arrhythmogenic substrate.[3] While a short refractory period in combination with slow conduction may provide the substrate for the maintenance of reentry, unidirectional block is required for the initiation of reentry.[4] Unidirectional block can be caused by pre-existing heterogeneity in excitability, conduction or refractoriness.[5] In the healthy heart, electrophysiological heterogeneities allow efficient contraction and relaxation. However, under pathological conditions, electrophysiological heterogeneities may underlie dysfunction and life-threatening cardiac arrhythmias.[5] For example, during acute myocardial ischemia, heterogeneities in extracellular potassium concentration cause heterogeneities in excitability and refractoriness and arrhythmias.[6-8] Also, in hearts with a chronic myocardial infarction, altered tissue architecture and distribution of connexins provide the basis for heterogeneous activation patterns and dispersion in conduction slowing.[9-12] Ventricular arrhythmias induced by heart failure are thought to be facilitated by heterogeneity in repolarization.[13,14] In addition, Brugada syndrome, a hereditary disorder characterized by familial sudden death, absence of (clinically observed) structural abnormalities and characteristic right precordial ST-segment abnormalities, is thought to be caused by transmural or sub-epicardial heterogeneity in action potential duration[15] or heterogeneous conduction delay.[16-18]

In the heart, electrophysiological heterogeneities exist between apex and base, between the right ventricular outflow tract (RVOT) and the right ventricular wall, between the left and right ventricular wall, and between the sub-endocardial and the sub-epicardial myocardium.[19] These electrophysiological heterogeneities result, at least in part, from regional differences in expression of genes encoding connexins and ion channels that are responsible for the shape and duration of the cardiac action potential (Table 1).[20] Various mechanisms underlying heterogeneity in gene expression in the adult are conceivable. Signaling events in the adult may locally affect gene expression and phenotype, as has been indicated for endothelin-signaling or after myocardial infarction.[9,21-23] This review focuses on the possibility that aspects of the extensive phenotypic heterogeneity generated during heart development may persist in the adult heart.

The expression of channel and gap-junction genes is regulated by transcription factors that during embryonic development coordinate the transformation of the cardiac precursor cells of a tubular heart into the adult myocardium.[24-29] Indeed, a causal relation between these locally acting transcription factors and localized expression of genes important for cardiac electrophysiology has been established.[30-35] The mature left ventricle is mainly composed of cells derived from precursor cells of the first heart field, whereas the right ventricular wall and the outflow tract (OFT) are derived from the second heart field.[36] Therefore, electrophysiological differences between the left and right ventricle may be related to the difference in origin of these regions. The OFT is formed in a later stage during development than the right ventricle and maintains its initial slowly

conducting and poorly contracting properties for a longer period during development.[27] Because the embryonic OFT gives rise to the adult RVOT,[37] it has an origin and developmental history different from the ventricles. This may well explain part of its sensitivity in the initiation of reentrant arrhythmias during sodium channel blockade or in patients with structural abnormalities.

In this review we describe the localized expression of genes in the developing and adult ventricles and OFT in relation to the electrophysiological heterogeneity in the adult heart leading to ventricular arrhythmias.

Table 1. Distribution of ion channels

Current	Protein	Gene	Distribution		Species
1_{Na}	α: Nav1.5	a: SCN5A	Epi	◄ Endo [48,84,153]	Rat, Mice, Human
1_{TO}	α: Kv4.2	α: KCND2	Epi	► Endo [32,42,44,45,49,69]	Human, Dog, Rat
	α: Kv3.2*	α: KCND3	LV	◄ RV [42,59]	Guinea-pig, Mice
					Human, Dog
	ß: KChip2	ß: KCNIP2	Epi	► Endo [49,61]	Human, Dog
			RV	◄ RV [61]	Dog
			Base	◄ Base [20]	Human, Dog
1_{Ks}	α: KvLQT1	α: KCNQ1	Epi	◄ Mid [86]	Human, Dog
			LV	◄ RV [61]	Dog
			Base	◄ Apex [20]	Human, Dog
	ß: MinK	ß: KCNE1	Epi	► Mid [86]	Human, Dog
			LV	◄ RV [61]	Dog
			Base	◄ Apex [20]	Human, Dog
1_{Kr}	α: HERG	α: KCNH2	Epi	► Mid [86]	Human
			Epi	= Mid [86]	Dog
	ß: MiRP-1	ß: KCNE2	Epi	= Mid [86]	Human, Dog

* Kv4.3 is not important for I_{TO} in mice

Distribution connexins

	Protein	Gene	Distribution		Species
-	Cx43	Gja1	Epi	◄ Endo [87]	Dog
			Epi	= Endo [90]	Rat
			RV	◄ RVOT [91]	Rabbit
-	Cx40	Gja5	Epi	◄ Endo [88]	Mouse

Regional heterogeneity in repolarization

Repolarization time is determined by local activation times and local action potential duration, which in turn is defined by a critical balance of inward and outward ion currents. When electrical coupling is absent, as in isolated myocytes, the intrinsic action potential duration is only determined by the expression of ion channels. Differences in action potential duration have been observed between myocytes isolated from the apex or the base, the left or the right ventricle, the sub-endocardial or the sub-epicardial myocardium.[38-45] These differences find their origin in intercellular differences in the level of expression of the genes encoding α- and β-subunits of ion channels carrying repolarizing currents: I_{Kr}, I_{Ks}, I_{TO}, I_{K1} and I_{CaL} (Figure 1 and Table 1).[46]

Transmural heterogeneity in action potential duration

In the myocardium of the ventricular wall, three electrophysiologically different cell types are present along the transmural axis: sub-endocardial, mid-myocardial and sub-epicardial myocytes.[38,47] Experiments on isolated cells demonstrated heterogeneity in action potential duration along this axis. Compared to sub-epicardial and sub-endocardial myocytes, mid-myocardial myocytes (M-cells) from dog and human hearts show more action potential prolongation during bradycardia, ischemia, and after application of various drugs.[41,43] Also, in man,[41,42] dog,[43] rat[44,45] and guinea pig,[39] action potential durations of sub-endocardial myocytes are longer than sub-epicardial myocytes. These differences likely result from higher expression of Kv4.2 and Kv4.3 in
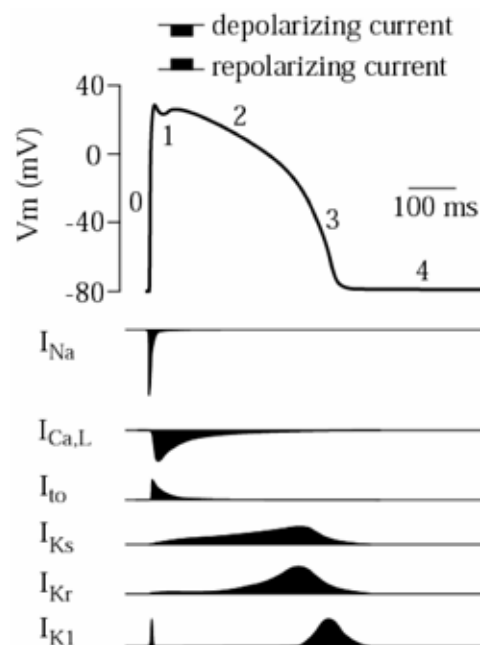

Figure 1. The cardiac action potential and its underlying ion currents. The top panel shows a schematic representation of a ventricular action potential. The numbers indicate the different phases of the action potential. The bottom panel illustrates the time course of underlying ion currents discussed in this review. I_{Na}, fast sodium current; I_{TO}, transient outward current; I_{Ks}, slowly delayed rectifying current; I_{Kr}, rapid delayed rectifying current; I_{K1}, inward rectifying current and I_{CaL}, L-type calcium current.

the sub-epicardium[32,40,42,44,45,48,49] which causes larger I_{TO} and consequently, shorter action potential duration. Furthermore, in human, Cav1.2 is expressed higher in the sub-epicardium than in the sub-endocardium. In mice, however, a transmural gradient of $I_{Ca,L}$ is not present.[44] In rodents, I_{Ks} and I_{Kr} are less important for action potential duration. However, in guinea pig, I_{Ks} and I_{Kr} are smaller in sub-endocardial myocytes than in mid- and sub-epicardial myocytes.[39] Furthermore, I_{K1} is smaller in cat sub-epicardium[50] but not in sub-epicardium of guinea pig[51] and dog.[52] The transmural gradient in action potential duration found in isolated myocytes is present in isolated perfused ventricular wedge preparations of the dog heart as well, especially at long cycle lengths. In this setting, the sub-endocardial cardiac action potential is longer than the sub-epicardial action potential.[43] In contrast, transmural repolarization times in the intact dog heart are not different.[53,54] The presence of an identifiable M-cell layer in intact myocardium is being disputed.[53]

Action potential duration in the apex and base

The action potential duration of isolated dog myocytes from the apex is shorter than that of myocytes from the base.[20] This is caused by larger I_{TO} and I_{Ks} in myocytes isolated from the apex than isolated from the base. In line with this, in dog the expression of α and β subunits of these channels are higher in apical myocytes than in basal myocytes.[20] In contrast, in rabbit,[40] ferret,[55] and rat,[19] action potential duration is longer in myocytes isolated from the apex than in myocytes isolated from the base. In rabbit, this can be explained by larger I_{kr} in myocytes isolated from the apex than in myocytes from the base.[40]

In the intact pig heart, monophasic action potentials recorded from the apex and base are not different, although apical action potential duration becomes shorter than those from the base when afterload increases.[56] Under physiological conditions, little is known about the apico-basal heterogeneity in action potential duration in the intact heart, although in dog, repolarization time is longer at the base than at the apex.[53,57,58] Difference in action potential duration between the left and right ventricle A transmural gradient in action potential duration is present in both the left and right ventricle.[41,42] For all cell-types across the myocardial wall, the left ventricular action potential is longer than the right ventricular action potential.[33,39,41-45] This corresponds with a larger I_{TO} in myocytes isolated from the right ventricle when compared to the left ventricle.[43,59] In addition, I_{Ks} is larger in the mid-myocardium of the right ventricle than mid-myocardium of the left ventricle in dog hearts.[60] This is in line with higher levels of KCNE1 protein in the right ventricle than in the left ventricle.[61] Data on the action potential duration in the RVOT in relation to that in other parts of the heart are lacking. [31] The electrophysiological properties of the ventricular septum can be divided into a right ventricular part and a left ventricular part.[62] The action potential recorded from sub-endocardial myocytes from the right side of the septum is shorter than from the left side of the septum. This correlates with higher expression of KChiP2, KCNQ1 in the right ventricular part of the septum when compared to the left ventricular part.[62]

The effect of intercellular electrical coupling on repolarization heterogeneity In the intact heart, differences in action potential duration are smaller than observed in isolated myocytes,[63] mainly because adjacent myocytes are electrically coupled by gap junctions. [48,53,54,57,58,64] As electrical coupling reduces action potential differences throughout the heart, uncoupling exacerbates these differences. For instance, when electrical coupling is experimentally reduced in healthy intact hearts, electrotonic interaction decreases, and differences in intrinsic action potential durations emerge.[65] This causes heterogeneity in repolarization, which is a substrate for reentry.[66] In a rabbit model of heart failure, heterogeneous down-regulation of connexins causes uncoupling of the myocytes, which leads to heterogeneity in refractoriness and conduction throughout the heart.[67] The latter may explain the increased incidence of arrhythmias during acute myocardial ischemia in this model. Also, in patients with moderate heart failure, arrhythmias are an important cause of death.[5] Although hypertrophy in heart failure per se is pro-arrhythmic,[13,68] the interstitial fibrosis seen in patients with heart failure functionally separates myocytes and adds to the down-regulation of connexin 43 (Cx43), the main gap-junction subunit in the ventricular working myocardium.[11,12,69,70] Thus, changes in intercellular coupling and the intercellular matrix may modulate the preexisting heterogeneities in repolarization.

Heterogeneity in conduction

Normal cardiac conduction is anisotropic and conduction velocity in the direction of the fibers, called longitudinal conduction, is about 2 times faster than transversal conduction velocity.[71,72] Conduction velocity of the activation front is approximately 0.5 m/s in the left ventricular myocardium. In mice, conduction velocity is slightly lower in the right ventricular wall,[73] and there are indications that in the response to Na^+ channel blockade, the right ventricular wall responds with more conduction slowing than the left ventricular wall.[17,69,74] Data on conduction velocities in the apex in comparison to those in the base are lacking.

Myocardial conduction velocity depends on the availability of Na^+ channels and intercellular electrical coupling by gap junctions.[75] The type of connexin present in the gap junctions determines the conductance (reciprocal of resistance) of the channel.[76-78] Also, modification of the channel by (de)phosphorylation can alter the conductance.[79] In *Cx40-*deficient mice*, atrial conduction velocity decreases with 35%[80] and a 95% reduction of Cx43 reduces conduction velocity with 18% in the ventricle.[81] The alpha-subunit Nav1.5 of the voltage dependent cardiac sodium channel, responsible for the generation of the inward Na^+ current, is encoded by *SCN5A*. In mice with 50% reduction in *SCN5A* expression, conduction velocity in the right ventricle decreases with 19%.[82] Furthermore, pharmacological blockade of gap junctions or Na^+ channels leads to slowing of conduction as well.[83] Therefore, heterogeneity in expression of Na^+ channels and/or connexins may lead to regional differences in conduction velocity, which makes some parts of the heart more vulnerable to become the origin of reentrant arrhythmias.[4] Taken together, heterogeneity in expression level of voltage gated ion channels and connexins, and also tissue architecture, may contribute to regional differences in conduction

velocity. The right ventricular wall may therefore be more prone to conduction slowing.

Expression pattern of Nav1.5 / SCN5A

In rat, Nav1.5 is more abundant in sub-endocardial than in sub-epicardial myocardium of the ventricular wall,[84] and in prenatal[85] and adult mice, both *SCN5A* transcripts and Nav1.5 protein are higher in the sub-endocardium than in the sub-epicardium (unpublished observations). In human and dog, Nav1.5 is more abundant in the mid-myocardium than in the sub-epicardium.[86] Consequently, the upstroke velocity is lower in isolated sub-epicardial myocytes.[84,86] In contrast, in an isolated wedge preparation of the left ventricle from dog, the upstroke velocity is higher in the sub-epicardial than in the sub-endocardial part of the wall.[87] However, the sub-epicardium of the wedge preparations are probably less-well coupled, due to the endocardium-high transmural gradient of Cx43, which in turn may explain this phenomenon.[87]

Expression pattern of connexins

Connexins are heterogeneously expressed throughout the heart.[88,89] The main connexin present in ventricular myocardium is Cx43,[85,89] which is however absent from the bundle branches, atrioventricular bundle and atrioventricular node.[88] In mouse and dog, Cx43 is less abundant in myocytes of the sub-epicardium than in myocytes of the mid- and sub-endocardial layers.[87] This transmural gradient in Cx43 expression is species dependent, because the gradient is absent in rat.[90] In rabbit myocardium, Cx43 is less abundant in the RVOT than in the left ventricular free wall.[91] The expression of Cx40 is restricted to the sub-endocardial Purkinje fibers, the bundle branches and the atrioventricular bundle.[88] Expression of Cx30.2 is restricted to the nodes of the conduction system.[92] Furthermore, the expression of Cx45 is low in the ventricular myocardium and no transmural gradient is observed.[93,94] Differences in connexin expression between the adult left and the right ventricle have not been reported, although the Cx40-expressing ventricular conduction system is more extensive in the left compared to the right ventricle.[95]

Conduction and RVOT in disease

Data on conduction velocity in the RVOT are lacking. However, in the Brugada Syndrome and arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), activation of the RVOT is delayed. This may be caused by either conduction slowing or structural block.[96] In these diseases, ventricular arrhythmias are often initiated in the RVOT.[97] The Brugada syndrome is defined by right precordial ST-segment elevation in the absence of detectable structural abnormalities. A mutation in *SCN5A* is present in about 30% of the patients with Brugada Syndrome. Two arrhythmogenic mechanisms in Brugada Syndrome have been proposed.[15,18] The repolarization theory states that shortening of action potential duration in the sub-epicardial myocardium of the RVOT causes an increased transmural gradient in action potential duration, leading to a substrate for reentry.[98] The depolarization theory states that regionally slowed conduction in the RVOT leads to unidirectional block and reentry.[17,18] The

presence of small structural changes in the RVOT may underlie this slowed conduction.[16,99] ARVC/D is characterized by fibrofatty replacement of myocardium predominantly in the right (but also the left) ventricle and is associated with mutations in desmoplakin and plakoglobin.[100,101] These proteins are involved in cell-to-cell junctions and cause conduction slowing in mice, and probably also in humans.[100-102] The structural changes are most prominent in the right ventricular wall where also most of the arrhythmias are initiated.

In both Brugada Syndrome and ARVC/D the right ventricular free wall and the RVOT are primarily affected and related to arrhythmogenesis originating from these parts of the heart. We surmise that the embryonic origin of the right ventricle explains right ventricular susceptibility to arrhythmias.

Table 2. Patterning along the cardiac axes during development

Axes in adult	Underlying developmental process	Factors involved	ref
Epi-Endo	The endocardium induces trabeculation of the sub-endocardial myocardium. The epicardium signals to the sub-epicardial myocardium to form compact myocardium.	Irx3, Irx5, Hey2, Fgf4, Fgf9, Fgf16, Fgf20, Fgf29, endothelin, Neuregulin, retinoic acid, Notch	32,84,141,146
LV-RV-RVOT (cranial-caudal)	The LV, RV, and OFT are formed along the cranial-caudal axis. The LV is formed from FHF precursor cells whereas the RV and RVOT are formed from SHF precursor cells. These heart fields are molecularly distinctive.	Isl1, Hand1, Hand2, Tbx1 Fgf8, Fgf10, Foxh1, Notch, Nkx2.5, Mef2c	36,132,155
Apex-Base	The base is composed of myocytes which differentiated later in development than myocytes from the apex. Therefore myocytes of the base have been longer exposed to differentiation inhibiting factors in the AVC and OFT than myocytes from the apex.	Bmp2, Tbx2, Nkx2.5, Hey1, Hey2, Sp4/HF-1b	I35,148-150, 156
RV-RVOT	The RVOT is composed of the embryonic OFT. The myocardium of the OFT maintains the slowly conducting phenotype longer than the myocardium of the RV.	ITbx2, Tbx3, Tbx5	35,37,120

Regionalized gene expression and electrophysiological heterogeneity in the developing heart

Many, if not all, genes encoding ion channels and connexins are expressed heterogeneously in the embryonic heart, their expression regulated by factors that are heterogeneously expressed as well. Some transcription factors, have been linked to regionalized expression of genes involved in electrophysiology, including cardiac homeobox factor Nkx2-5, the T-box transcription factors Tbx5, Tbx2 and Tbx3, and the Iroquois homeobox factor Irx5.[30-33,103-105] Another contributing factor to heterogeneity is the developmental origin of the heart. The left ventricle is largely derived from the first heart field, which is molecularly and phenotypically different from the second heart field that gives rise to the right ventricle and OFT (Table 2). Moreover, the prenatal OFT remains undifferentiated and slowly-conducting until it is incorporated into the RVOT. Therefore, we suppose that differences in gene expression, tissue structure, size, composition and function in the adult heart find their origin in heart development.

Role of transcriptional regulation of electrophysiologal heterogeneity deduced from the development of the conduction system

The involvement of transcriptional regulation in heterogeneous expression of target 'electrophysiological' genes in the ventricle and RVOT is partly based on the role of specific transcription factors in the localized regulation of these genes in the early developing heart and conduction system. Pioneering human genetic studies and subsequent functional follow-up studies revealed critical roles of Nkx2-5 and Tbx5 in the formation, function and gene regulation of the atrioventricular conduction system.[31,103,105-109] Nkx2-5 is broadly expressed in the heart, indicating that it acts through regulation of, or interactions with, locally acting factors, such as Id2, Tbx5 and Tbx3.[30,31,34,105] The sinoatrial and atrioventricular node are slowly conducting and spontaneously active structures, corresponding to low expression levels of *Cx40*, *Cx43* and *SCN5A*.[104,110-112] The same holds for the embryonic atrioventricular bundle and proximal bundle branches, which, however, during the fetal period develop fast conductive properties and high expression of *Cx40* and *SCN5A*[30,111,112] (in contrast to published data we observed high levels of expression of Nav1.5 / *SCN5A* in the developing and adult atrioventricular bundle). Recent studies have revealed that Tbx5, together with Nkx2-5, are crucial for the expression of *Cx40*.[31,105] The nodal character and low expression of *Cx40*, *Cx43*, *SCN5A* and many other genes in the sinoatrial node and early developing atrioventricular bundle, depends on repression
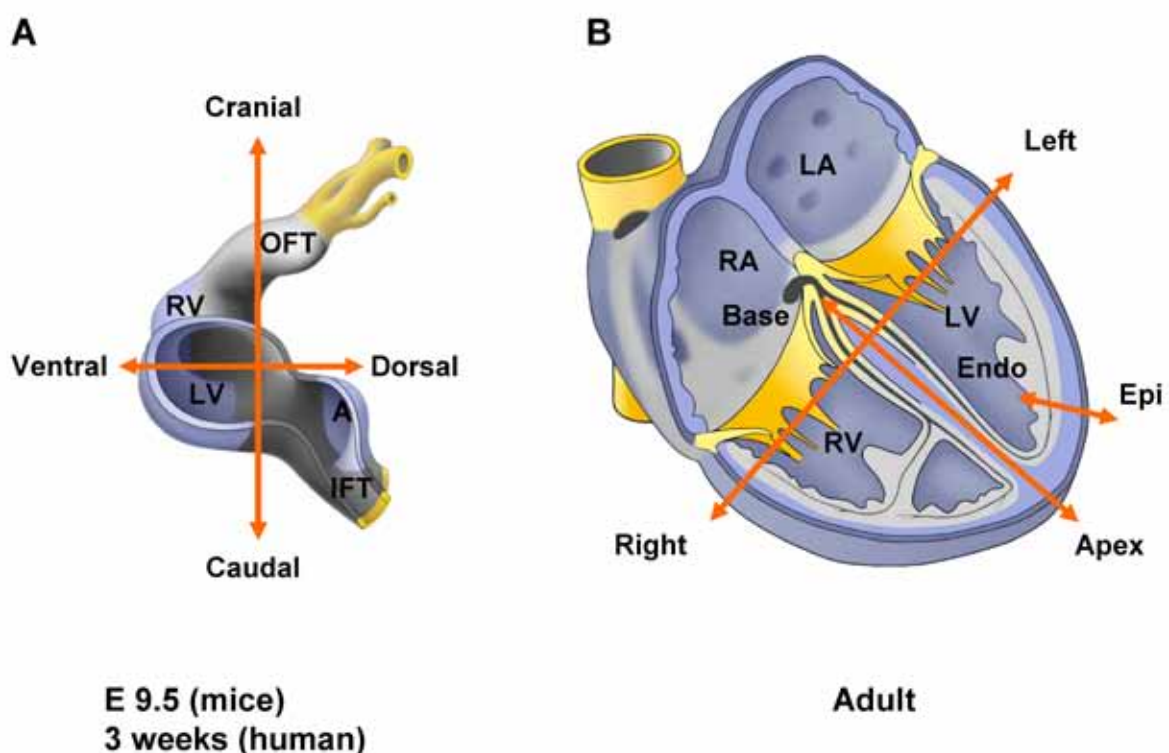

Figure 2. Axis of patterning in the embryonic and adult heart. Transition of axes of patterning in the heart during development. A, On embryonic day 9.5 the heart tube consists of a caudally positioned inflow tract and a cranial positioned outflow tract. The ventricles expand at the ventral side and the atria at the dorsal side. B, The position of the left and right ventricle and outflow tract in the adult heart are related to the cranial-caudal axis of the embryonic heart tube. The apical-basal axis corresponds to the dorsal-ventral axis of the embryonic heart tube. R/LA, right/left atrium; R/LV, right /left ventricle; Epi↔Endo, epicardial to endocardial axis

by Tbx3. Tbx3 is selectively expressed in the conduction system and directly interacts with the regulatory DNA of *Cx43*.[30,104] Moreover, Tbx3 and Tbx2 both suppress the above mentioned genes in the atrioventricular canal, out of which the atrioventricular node will develop.[30,33,35,113] Overall, the role of Nkx2-5 and T-box transcription factors in the heart, and their role in the direct regulation of genes important for electrophysiology has been well established.
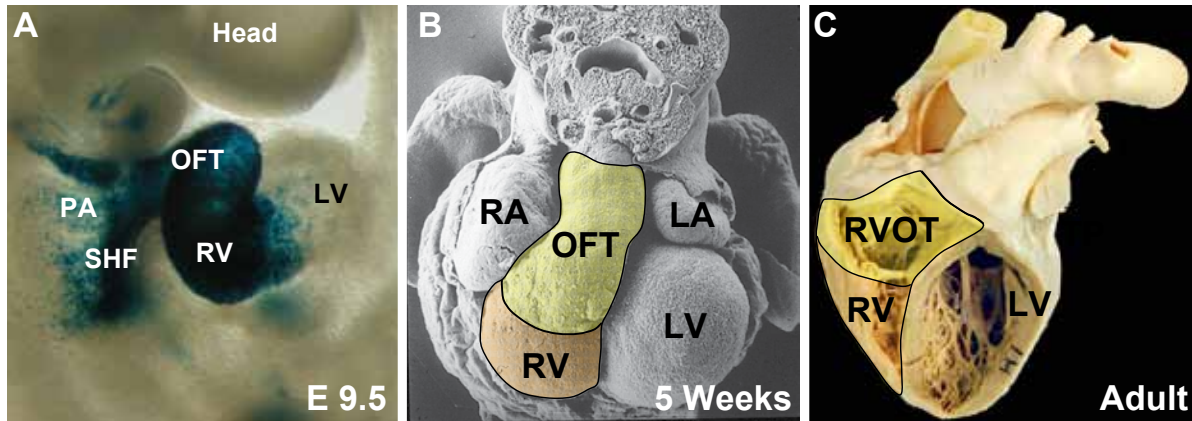

Figure 3. The embryonic OFT forms the RVOT. A, Whole mount X-Gal staining of an embryonic day 9.5 *Mlc1v-nlacZ-24* embryo, expressing a *lacZ* enhancer trap of the *Fgf10* gene. It reveals that the *Fgf10* positive second heart field does not contribute to the left ventricle (courtesy of Dr R. Kelly, Marseille, France). B, Scanning electron microscope image of a human heart at five weeks of development. The right ventricle (orange) is composed of working myocardium demonstrating fast conduction and rapid contraction. The embryonic OFT (yellow) is composed of slow conducting primary myocardium. C, The embryonic OFT is incorporated in the right ventricle and forms the RVOT of the adult heart. R/LA, right/left atrium; R/LV, right/left ventricle; OFT, outflow tract; SHF, second heart field; PA, pharyngeal arches; RVOT, right ventricular outflow tract; Ao, aorta; PT, pulmonary trunk.

Axes of polarity in the embryonic and adult heart

In the embryo, three axes of polarity can be defined: the cranio-caudal axis (also called rostral-caudal, anterior-posterior), the dorso-ventral axis and the left-right axis. These axes can also be applied to the early heart tube. The relation between the axes in the looped embryonic heart and the adult heart are shown in Figure 2 and Table 2, together with the nomenclature of the axes at these stages. Signaling and other regulatory processes are polarized along these axes.[27] For example, the anterior side of the developing heart receives, or has received, different regulatory signals compared to the caudal side.

In the adult heart, the RVOT and the left and the right ventricular walls are anatomically different. These three components are formed along the caudo-cranial axis of the developing heart, and, hence, the differences between these components are rooted in development. Further, adult apex-to-base differences will arise from cranio-caudal and dorso-ventral patterning in the embryonic heart. The adult left ventricular base, for example, is derived from the transition between the developing atrioventricular canal and the left ventricular border, whereas the apex derives from the embryonic left ventricle positioned ventral from the atrioventricular canal. Along the left-right axis, another layer of patterning acts on top of the other axes and therefore affects all cardiac phenotypes. The left and right ventricle, for example, each have a left-derived and a right-derived

component, and are regionally exposed to transcription factor Pitx2c-mediated signaling.[114,115]

Progenitors of the left and right ventricle and of the outflow tract

The early embryonic heart is a simple tube with an outer layer of cardiomyocytes, an inner layer of endocardium, with interposed cardiac jelly. The cells of the primitive heart tube originate from a progenitor population, called the first heart field. These cells differentiate to myocardial cells and fuse at the midline generating a linear heart tube with a caudal inflow and a cranial OFT. At this stage, the heart tube rapidly grows primarily by addition of cardiac progenitors of the so-called second heart field to the inflow tract, OFT and dorsal mesocardium. These second heart field-derived cells will largely form the right ventricle, OFT and atria.[36] The ventricular septum forms at the border between the two heart fields.[116-118] During the elongation phase, the heart tube loops and ventricular working myocardium differentiates at the ventral side (outer curvature). First, the left, and then, cranial to this, the right ventricular myocardium forms. The atrioventricular canal, OFT and inner curvature initially do not differentiate into working myocardium.[119] With further development, most of the muscular OFT will be absorbed in the right ventricle and form the RVOT, whereas a small part will form the connection between the left ventricle and the aorta.[37,120] (Figure 3)

The left and right ventricle have a different morphology, tissue architecture, geometry and function, and myocytes of the right and left ventricle differentiate to similar but not identical working phenotypes. It is conceivable that differences in the regulatory- and gene programs of progenitors of the left and right ventricle contribute to the differences in their phenotypes. Although not much is known about these contributions, transcription factors regionally regulate genes involved in cardiac electrophysiology. In the developing heart, the transcription factor *Hand1* is expressed in the first heart field and, subsequently in the left ventricular outer curvature.[29] Hand1 is required for expression of *Cx40*, *Nppa*, *Cited1* and other genes in the embryonic left ventricle. Loss of *Hand1* in the myocardium causes left ventricular defects and dysregulation of gene expression. In contrast, *Hand2* is expressed in the second heart field and subsequently in the right ventricle and OFT.[29] Loss of *Hand2* results in hypoplasia of the right ventricle and OFT.[121] Reduction in Hand-dose, by deleting one or both *Hand* alleles, results in complete absence of expression of *Cx40* and *Nppa*.[122] Accordingly, these genes are up-regulated in hearts over-expressing *Hand* genes.[123,124] Taken together, while Hand1 and Hand2 play partially overlapping roles in ventricular differentiation, expansion and gene expression,[122] their individual expression patterns demonstrate that morphogenesis and gene regulation in the left ventricle and in the right ventricle and OFT, are governed by locally distinct regulatory mechanisms.

Tbx5 is expressed in a caudo-cranial gradient in the tubular heart and is absent from the second heart field.[31,125] During and after chamber expansion, *Tbx5* expression is confined to the left ventricle, in a transmural gradient (high expression at the endocardial side), whereas some remaining expression is observed in the right ventricular trabeculea.[125]

The OFT is devoid of detectable *Tbx5* expression. Tbx5 is required for heart formation and chamber expansion, and is necessary for the activity of a number of genes, including *Cx40*.[31] The expression pattern of *Cx40* mimics that of *Tbx5*.[31,113] Because of the important regulatory functions of Tbx5 and its patterned expression, it contributes to the difference in gene program and identity of the left ventricle and right ventricle. The expression of *Tbx5* is regulated by retinoic acid.[126] Retinoic acid signals to the caudal progenitors of the heart tube, thereby providing them with caudal identity and fate information.[127] This signal most likely underlies the caudal-high expression of *Tbx5* in the fusing and looping heart tube.

The outflow tract: a slowly conducting primitive structure

The embryonic heart tube elongates by recruiting second heart field progenitors. Like the myocytes of the initial heart tube, these added cardiomyocytes have a poorly developed contractile and sarcoplasmic reticular apparatus, and are poorly coupled (mainly by Cx45). [27,128,129] Specific regions of the heart tube differentiate into ventricular working myocardium and initiate expression of genes permitting fast conduction, including Cx40 and Cx43. In contrast, the embryonic OFT does not initiate this gene program, and retains the original slowly conducting and poorly contracting properties much longer.[27,130] The embryonic OFT therefore displays long-lasting contractions, thought to prevent regurgitation of the blood from the circulation to the ventricle prior to the formation of the semilunar valves.[131] *In vivo* labeling studies have shown that the proximal and distal embryonic OFT eventually differentiates further and gives rise to the trabeculated free wall and the smooth-walled conus of the RVOT.[37,120] Although the regulatory mechanisms that control the phenotype of the OFT are only partially understood, a number of molecular pathways controlling its development has been identified.[132,133] The OFT is almost devoid of Tbx5, strictly required for *Cx40* expression and for working myocardial differentiation.[31] At the same time, Tbx2, a repressor that competes with Tbx5 for many of the same target genes, is expressed in the OFT, but not in the ventricular wall. Tbx2 suppresses *Cx40* and *Cx43* expression and chamber differentiation (Figure 4).[33,35,113] Moreover, Tbx2 is functionally equivalent to Tbx3, which suppresses working myocardial differentiation and imposes the nodal (= embryonic myocardial) gene program on myocardium.[104] This gene program includes suppression of ion channels, connexins and contractile proteins that are normally highly expressed in the working myocardium.[104] Hence, Tbx2 possibly suppresses working myocardium differentiation in the OFT, contributing to its persisting embryonic phenotype (Figure 4). *Cx43* expression is absent from the fetal OFT (our unpublished observations) but is expressed in the RVOT of adults, albeit to a lesser extent than in the free wall of the RV.[91] This suggests that insufficient up-regulation of *Cx43* and possibly other genes in the RVOT before birth can result in heterogeneous expression in the right ventricular wall. *Tbx2* is gradually down-regulated towards the end of gestation, which may contribute to the gradual up-regulation of *Cx43* in the RVOT myocardium.[35]

Taken together, the wall of the adult right ventricle is formed by the incorporation
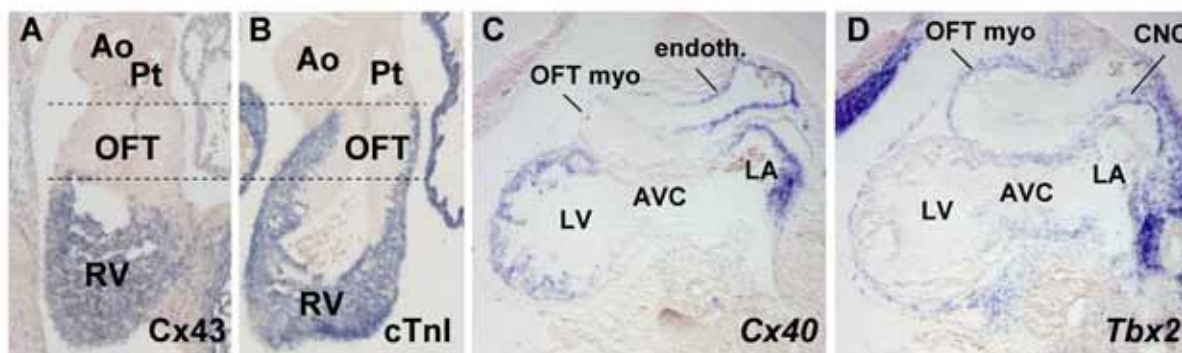
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Figure 4. Expression pattern of Cx43, Cx40 and Tbx2 in the embryonic heart. A,B, In situ hybridizations of a mouse heart at embryonic day 14.5. A, *Cx43* expression is absent from the myocardium of the embryonic outflow tract. B, *cardiac troponin I* (*cTnI*) expression labels all cardiomyocytes. C,D, In situ hybridisation of a mouse heart at embryonic day 10. C, *Cx40* is expressed in the myocardium of the ventricle and atrium but no in the myocardium of the outflow tract and atrioventricular canal. D, *Tbx2* is only expressed in the myocardium of the OFT and atrioventricular canal and not in that of the atrium and the ventricle. LA, left atrium; LV, left ventricle; CNC, cardiac neural crest; AVC, atrioventricular canal; myo, myocardium; endoth, endothelium.

of an initially slowly conducting embryonic OFT into the rapidly conducting working myocardium of the right ventricle. We suggest that remnants of the embryonic OFT phenotype and expression profile in the adult RVOT determine the electrophysiological and structural characteristics that makes the right ventricle more vulnerable for arrhythmias.

Cardiac neural crest cells

The development of the conduction system and OFT, and the function of the embryonic heart, are influenced by cardiac neural crest (CNC) cells.[134,135] CNC cells migrate into the OFT around embryonic day 9.5 (mouse) and form the aorticopulmonary septum that divides the arterial pole into systemic and pulmonary outlets.[136] Furthermore, these cells form the smooth muscle tunics of the great arteries and the parasympathic innervation of the heart, and have been implicated in the development of the conduction system.[134,135] CNC cells do not materially contribute to myocardium, but play an important signaling role in the development of the conduction system and OFT. Ablation of CNC in chicken, results in defects of the atrioventricular bundle, Purkinje fiber ands OFT.[134,135,137,138] OFT misalignment and persistent truncus arteriosus, abnormal patterning of the great arteries, and abnormal myocardial function are part of these defects. Thus, congenital defects in the OFT or the great vessels mediated by CNC may affect cardiac electrophysiology indirectly. The local action of CNC cells implicates them also in regionalized gene regulation, most notably in the OFT and RVOT in the adult. However, the mechanistic link between CNC and heterogeneity in electrophysiology of the RVOT is not clear.

Transmural patterns in signaling and gene expression

Transmural heterogeneities in gene expression and phenotype arise as soon as the ventricular wall is formed. (Figure 5) The primitive epithelial-like myocytes of the initial embryonic ventricle generate a series of progeny which grows vertically to form ridge-like protrusions (trabeculae). The peripheral layer proliferates much faster, which results in cone- or wedge-shaped sectors

spanning the entire ventricular wall.[139] The myocardium and the endocardium exchange signals, including Notch, neuregulin and endothelin signaling, which regulate trabeculae formation, proliferation and differentiation at the endocardial side.[140-144] These trabeculae form papillary muscles, ventricular septum, Purkinje fibers, and are incorporated in the compact wall. The Purkinje fibers maintain automaticity, which later in live may trigger ventricular fibrillation.[145] An epicardial cell layer is formed around the myocardial wall and signals (e,g, Fgf- and retinoic acid signaling) to the myocardium to form the compact wall. The epicardium thereby controls gene regulation and maturation of the underlying sub-epicardial myocardium.[139,141] Most genes expressed in the ventricular wall show mild to profound differences in the expression level across the ventricular wall, including *Cx40*, *Cx43*, *Serca2a*, *SCN5A* and *Kcnd2* (encodeing Kv4.2).[48,84] For example, *Cx40* is expressed across the entire wall of the left ventricle until the compact wall starts to form, and expression gradually becomes confined to the sub-endocardial Purkinje fibers.[95,128]

The combination of different signals and responses across the ventricular wall results in transmurally patterned expression of transcription factors and of their target genes. For example, the transmural pattern of *Tbx5* is most likely responsible for the transmural pattern of *Cx40*.[31] In chicken, endothelin signaling from the endocardium and coronary endothelium positively regulates *Cx40* expression in the sub-endocardial and sub-endothelial myocytes and functional maturation of the Purkinje fibers.[142] *Hey2*, encoding a helix-loop-helix transcription factor, is expressed in a transmural gradient high at the sub-epicardial side. In the absence of Hey2, *Nppa* is ectopically expressed in the compact ventricular wall, whereas normally it is only expressed in the trabeculae.[146] Proof of principle that transmural patterns of transcription factors are functionally important has been provided by Constantini et al.[32] They demonstrated that the Iroquois (Irx) homeobox transcription factor family member *Irx5*, which is initially expressed in the ventricular endocardium,[147] subsequently becomes expressed in a sub-endocardium-high transmural gradient across the ventricular wall.[32] Irx5 was found to suppress the expression of Kv4.2, the alpha-subunit of the ion channel carrying I_{TO}, which is a modulator of the action potential shape. *Irx5*-deficient mice express Kv4.2 homogeneously across the wall, do not have a detectable T-wave and are prone to arrhythmias.[32] Several family members of *Irx5* are expressed in specific patterns in the ventricles.[147] *Irx3* is expressed in a sub-endocardium-high transmural pattern like *Irx5*, and *Irx1* and *Irx2* are specifically expressed in the ventricular septum. Their possible contribution to electrophysiological heterogeneity awaits testing. These examples suggest that transmural patterning in gene expression and function in the adult ventricular wall finds its origin in development, and that errors in the regulation of these patterns (e.g. due to mutations in genes involved in their regulation), may render individuals more prone to arrhythmias.

4

Apex-to-base heterogeneity

The origin and regulation of heterogeneity over the apex-to-base axis are poorly understood. Transcription factor Sp4/HF-1b is expressed in the heart and neural crest. *Sp4*-deficiency leads to decreased Cx43 expression and slowed conduction in the apical ventricular domain, whereas the basal region is less affected.[148] The underlying mechanism remains to be defined. Fate-map studies in chicken,[120,149] have indicated that cells located initially in the atrioventricular canal or OFT of the early developing heart, will become part of the base of the ventricles. These initially primitive cardiomyocytes differentiate to working myocardium and contribute to the base of the left and right ventricle. Recent genetic lineage analysis using the expression pattern of *Tbx2* to label all primary (non-working) myocardium in the embryonic heart, indicates that the embryonic atrioventricular canal forms the definitive left ventricular free wall, whereas the embryonic left ventricle forms the definitive apex and ventricular septum (our unpublished data). Therefore, the primitive atrioventricular canal and OFT myocardial cells differentiate later during development to ventricular working myocardium than the apex cells. Hence, they will be exposed longer to regulatory pathways such as the Bmp2-Tbx2-Hey2- and Tbx3 pathways,[33,113,150-152] which initially suppress their differentiation and the expression of *Cx40, Cx4 and SCN5A*. Consequently, *Tbx2*-deficiency leads to morphological defects and ectopic expression of connexins and *SCN5A* in the left atrioventricular canal and left ventricular base (our unpublished observations).
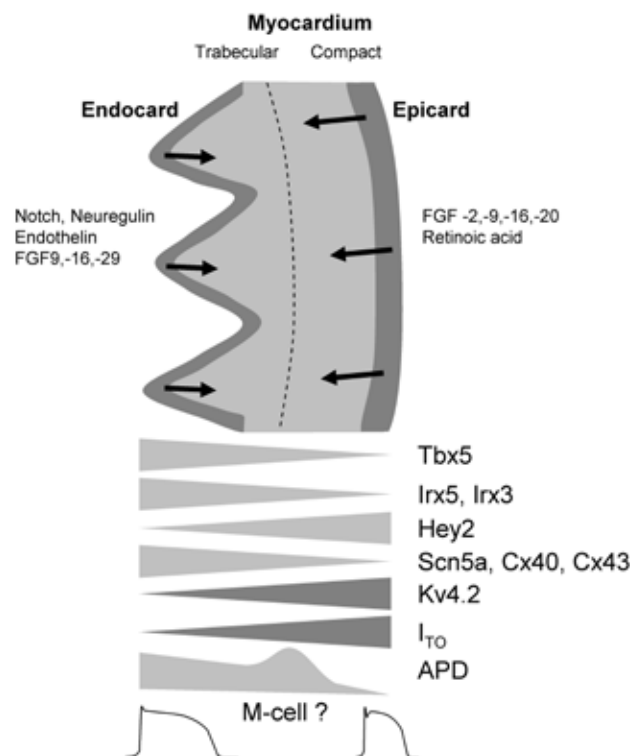

Figure 5. Transmural heterogeneity in expression of transcription factors and ion channels. The endocardium and epicardium regulate growth and differentiation of the myocardium. At the endocardial side trabeculae are formed and at the epicardial side compact myocardium is formed. The signals from endo- and epicardium possibly cause gradients in expression of transcription factors and ion channels. In the adult heart, action potential duration is longer at the sub-endocardial side than at the sub-epicardial side. However, mid-myocardial myocytes with long action potential duration are found in wedge preparations from the canine ventricle

Conclusion and future research

In the adult heart electrophysiological heterogeneity is present along the apico-basal, left-right, and transmural axes of the ventricles. (Table 1) Moreover, the RVOT is electrophysiological different from the left ventricle. Moderate electrophysiological heterogeneity is a prerequisite for cardiac function. However, large heterogeneities may lead to life-threatening arrhythmias. In this review we propose that intrinsic electrophysiological heterogeneity in the developing heart may persist in the adult heart. Electrophysiological heterogeneity along each of the axes is related to distinctive patterning and signaling processes during development (Table 2). The established roles of T-box and other transcription factors in the regulation of the electrophysiological phenotype and regulation of channel genes in the developing conduction system provide support for the idea that these factors have a role in the heterogeneity of the ventricles and RVOT. Moreover, the role of Irx5 in the regulation of the transmural gradient in expression of channel subunits of I_{TO} supports the general idea that transmural electrophysiological gradients across the adult ventricular wall are rooted in development.

This overview provide a framework that could help in designing models to test the hypothesis that development impacts on electrophysiological heterogeneity, as well as to test the idea that inter-individual variations in this heterogeneity affect the likelihood to develop arrhythmias. For example, expression profiles and electrophysiological phenotypes of myocytes from the left and right ventricle and RVOT could be quantified to identify physiologically relevant differences between these compartments. Furthermore, genes encoding proteins for fast conduction could be selectively over-expressed in the OFT to investigate whether arrhythmias with RVOT origin can be prevented.

In conclusion, electrophysiological heterogeneity in disease states or in hereditary syndromes are likely influenced by cardiac developmental processes. Understanding these processes may enable early detection and therapy of life threatening arrhythmias.

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