The development of the venous pole of the heart

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Publication date
2009

Chapter 2

Atrial fibrillation: a developmental point of view

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Heart Rhythm, Oct. 2009; in press

Abstract

The myocardial sleeves of the systemic venous tributaries and the pulmonary veins are known to be common anatomic substrates for atrial fibrillation. Rapidly evolving evidence has shown that a substantial part of the paroxysmal variant of this abnormal rhythm has a familial heritage, and the number of genes found to be involved is increasing. One of the mechanisms underlying the condition is ectopic pacemaking activity. Knowledge of the normal embryological development of the atrial myocardium, in particular the myocardial sleeves clothing the systemic venous tributaries and the pulmonary veins at their junctions with the atrial chambers, may contribute to the understanding of the origins of such ectopic pacing. In this respect, it is now well established that the myocardial sleeves of the systemic venous tributaries have a distinct origin, and program of gene expression, when compared to the pulmonary venous myocardium. The myocardium clothing the pulmonary veins, however, is particularly susceptible to changes in the levels of gene expression, with the changes then favouring the presence of genes responsible for pacemaking. Only recently has interest developed in the genetic and heritable bases of atrial fibrillation, and much is still to be learned. Better understanding of both the developmental and genetic factors, nonetheless, will surely be helpful in the diagnosis, prevention, and treatment of this troublesome arrhythmia. With this in mind, therefore, we have reviewed the current knowledge concerning the initial development of the pulmonary venous myocardium, emphasising its crucial differences from the systemic venous myocardium.

Introduction

Atrial fibrillation is the most common cardiac arrhythmia encountered in clinical practice. Its prevalence increases with age, so that approximately 1% of the total population, and one-tenth of those surviving to reach the age of 80 years, suffer from this abnormal rhythm.[1] In over nine-tenths of instances of the paroxysmal variant, the anatomic substrate is found in the myocardial sleeves clothing the pulmonary veins,[2] with the rhythm held to be due to either re-entry, triggered activity, or ectopic pacemaker activity.[3] Evidence is now accruing to show that genetic and congenital defects are also involved in its development. Population-based studies have demonstrated a significant heritable component, with studies of genetic association, genetic variants, or polymorphisms revealing a large number of associated genes.[4] In this review, we discuss the development of the pulmonary venous myocardium, emphasising that the sleeves clothing the pulmonary, as opposed to the systemic venous tributaries, have fundamentally different developmental heritages and patterns of gene expression.[5-7] We believe that review of the relevant genetic and morphologic knowledge concerning this early embryological development will help clinicians understand why certain regions in the heart are the favoured sites for arrhythmogenesis. This, in turn, should guide rational treatment of atrial arrhythmias.

The anatomy of the myocardium of the atrial chambers and venous connections

The right and left atrial chambers have the same basic components, namely a body, a venous component, a vestibule, and an appendage.[8] The cavities are separated by the septum, which is formed largely by the primary septum, representing the floor of the oval fossa, and the so-called secondary septum, which is a superior infolding of the atrial walls.[9] Many consider the right atrial appendage to be but the tip of the pectinated extension from the atrial cavity, but this is not the case in developmental terms. The appendage forms the entirety of the anterior atrial wall (Figure 1A). This part is readily distinguished from the remainder of the atrium by the pectinated nature of its walls, with the terminal crest, or crista terminalis, marking the border with the smooth-walled systemic venous component (Figure 1B). The myocardium of this venous component surrounds the orifices of the superior and inferior caval veins and the coronary sinus, with sleeves of limited extent clothing the venous lumens at their junctions with the atrium. The sinus node is located in this myocardium at the junction of the superior caval vein with the right atrium. The vestibule of the right atrium, also smooth-walled, inserts into the leaflets of the tricuspid valve at the atrioventricular

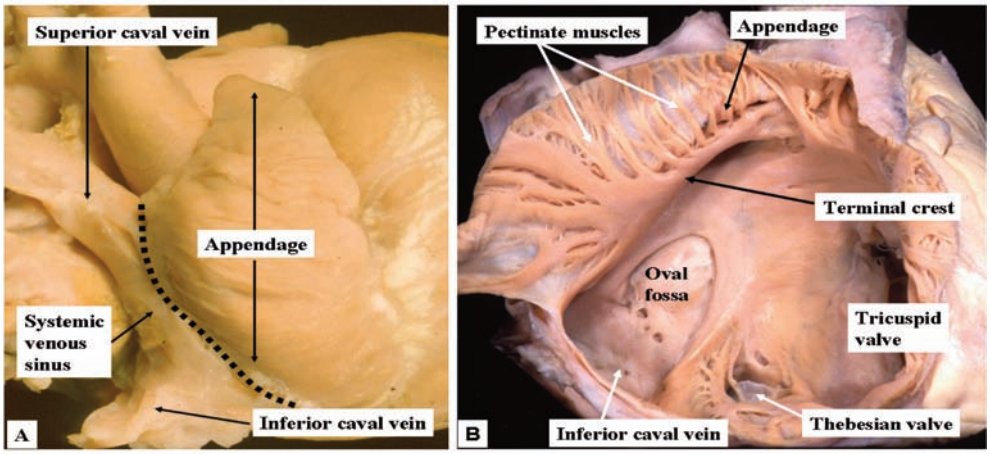

Figure1. The anatomy of the right atrium. Figure 1A shows the external appearance of the right atrium. The terminal groove, or sulcus terminalis, marks the junction between the systemic venous sinus and the extensive right atrial appendage. The entirety of the anterior wall is part of the appendage, and not simply the tip of the triangular extension from the atrial cavity. In Figure 1B, the interior of the atrium has been revealed by making a cut in the appendage parallel to the atrioventricular groove, and reflecting the wall of the appendage upwards. It shows that the appendage is distinguished from the remainder of the atrium on the basis of its ridged walls, the pectinate muscles.

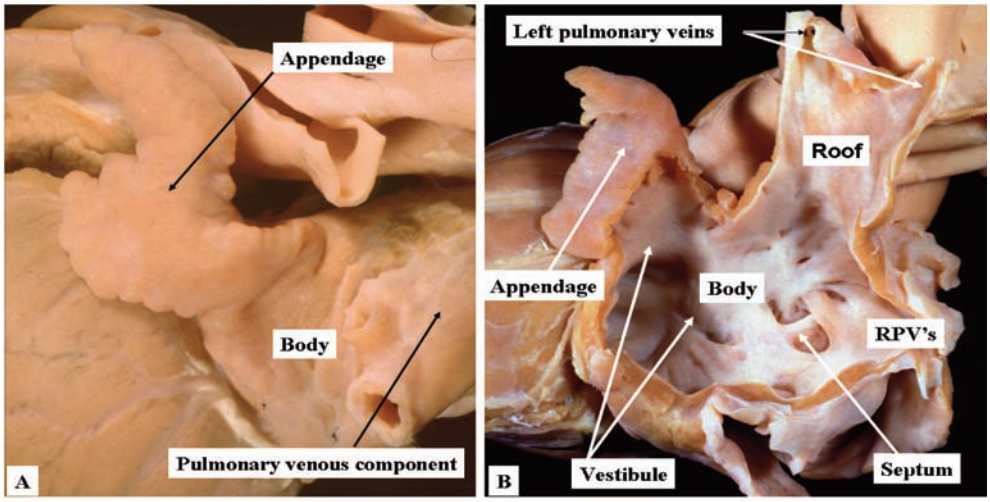

Figure 2. The anatomy of the left atrium. Figure 2A shows the tubular nature of the left appendage. In Figure 2B, the interior of the atrium is shown having reflected the pulmonary venous component, which forms the atrial roof.

junction (Figure 1B). The pectinate muscles of the appendage extend all round the parietal part of the vestibule. The body of the right atrium, the small space between the leftward margin of the systemic venous sinus and the septum, has no discrete anatomic boundaries in the postnatal heart.

The left atrium, in contrast, has a significantly larger body. The appendage is much smaller on the left side (Figure 2A), with pectinate muscles confined within its tubular extent. The larger part of the left atrial cavity, therefore, is smooth-walled (Figure 2B). The pulmonary venous component forms the atrial roof, typically with one vein entering at each of the four corners, albeit that there are many variations in terms of the arrangement of the venous orifices. Myocardial sleeves extend from the atrial roof for short distances along the veins, being longer on the superior than the inferior veins (Figure 3A).[10] These sleeves are composed of working atrial myocardium, albeit with intermingling myocytes arranged in circumferential and longitudinal fashion (Figure 3B, C).

Morphological and genetic knowledge concerning the development of the atrial chambers

The identity of the working atrial myocardium of the morphologically right as opposed to the left atrium is established by the expression of genes responsible for left-right asymmetry. *Pitx2c* is one of best-known of these genes.[11] In absence of the functional product of this gene, the heart develops two morphologically right appendages, along with bilateral sinus nodes.[12] Genes such as *lefty1* and *sonic hedgehog* produce midline barriers that, in the normal embryonic heart, prevent the spread of gene products responsible for morphological leftness across the midline. Knocking out these genes produces hearts with isomeric left appendages.[11] The distinct atrial components also have their own transcriptional profiles. These profiles distinguish the myocardium of the appendages, the atrial septum including the body of the left atrium, the floor of the systemic venous sinus, and the venous inlets.[13] These profiles also confirm that the appendage forms the entirety of the pectinated atrial wall. The myocardium of the pulmonary venous component of the left atrium, including the venous sleeves, in contrast, has a distinct phenotype that is more comparable to that of the atrial septum and the body of the left atrium. The entirety of the walls between the left valve of the systemic venous sinus and the left appendage, including the primary atrial septum and pulmonary myocardium, is derived from the mediastinal myocardium (Figure 4A).[13,14] These walls are characterized by the expression of the fast-conducting connexin *Cx40*, and by the lack of expression of *Nppa*, which encodes atrial natriuretic factor.[15] *Cx40* and *Nppa*, in contrast, are expressed in the entirety of the walls of both

atrial appendages.[7] These atrial components also differ in terms of the properties of their ionic currents.[6] The establishment of this complicated atrial genetic make-up can be understood only by establishing the mechanisms of early development.

The basics of early cardiac development

The heart starts as a simple contractile myocardial tube. Initially, this tube is composed of slowly conducting myocytes, all possessing intrinsic pacemaker activity. In the postnatal heart, this phenotype is retained in the myocytes of the sinus and atrioventricular nodes, which are poorly coupled, have a poorly developed sarcoplasmatic reticulum, and few contractile elements (Figure 4B). It is only subsequent to addition of cells at the poles of the heart tube, and dorsally through the so-called dorsal mesocardium,[16] that it becomes possible to recognize the cardiac chambers.[13] The chamber myocardium, when formed, has a fast-conducting working myocardial phenotype. The myocardium at the venous pole, however, along with the region interposed between the developing chambers, the atrioventricular canal, initially retains the nodal-like phenotype (Figure 4B). Not all of this nodal-like myocardium eventually forms the cardiac nodes, and the phenotype of the embryonic nodal-like myocardium that will form the nodes will differentiate considerably during further development.[17] Hence, it is better to describe this nodal-like myocardium as primary myocardium, thus differentiating it from the chambers myocardium, which proliferates rapidly to form the atrial appendages along with the apical ventricular components.[13] The primary myocardium between these developing areas of chamber myocardium does not increase much in volume during prenatal life, and as discussed, parts persist to become the cardiac nodes.[13]

Most of the systemic venous myocardium, however, although initially having a primary phenotype, becomes working atrial myocardium when assessed histologically. In morphologic terms, this embryological systemic venous sinus, or sinus venosus, is made up of right and left myocardial sinus horns. The right horn eventually becomes the proximal myocardial parts of the superior and inferior caval veins, along with the floor of the systemic venous sinus. The left horn persists as the left-sided superior caval vein in the mouse, but becomes the coronary sinus in the human.

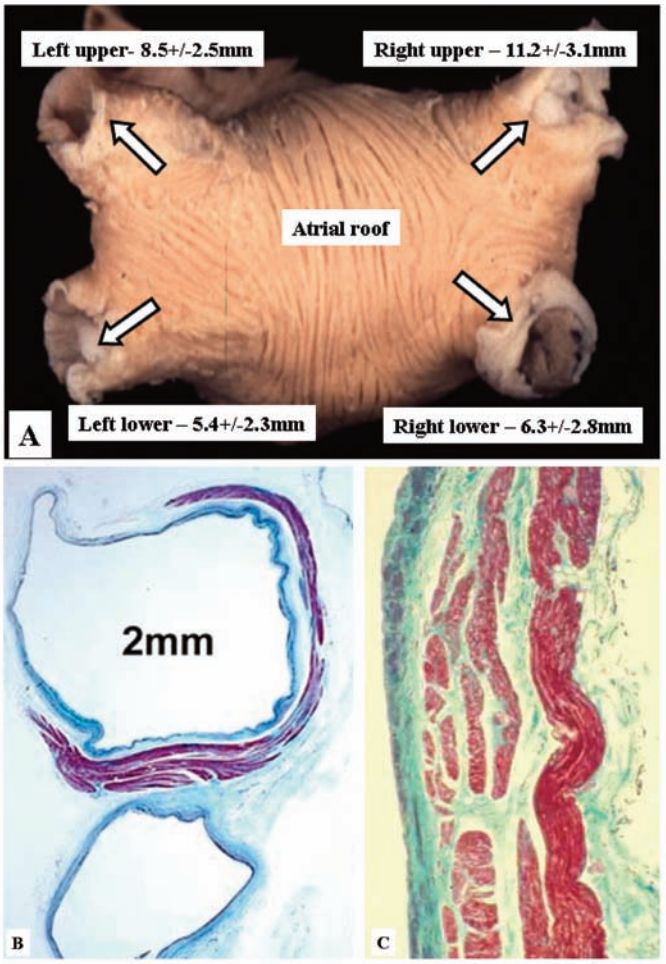

Figure 3. The structure of the pulmonary venous sinus. Figure 3A shows the roof of the left atrium, with each of the four pulmonary veins entering a corner of the atrial roof. The epicardium has been removed to show the alignment of the atrial myocytes, which extend to varying distances as sleeves along the pulmonary veins. The numbers show the extent of these sleeves as shown by Ho and colleagues.[9] Figure 3B shows the cross-sectional extent of one of the sleeves, and Figure 3C shows that the walls are made up of working atrial myocytes, with no evidence of histological specialisation.

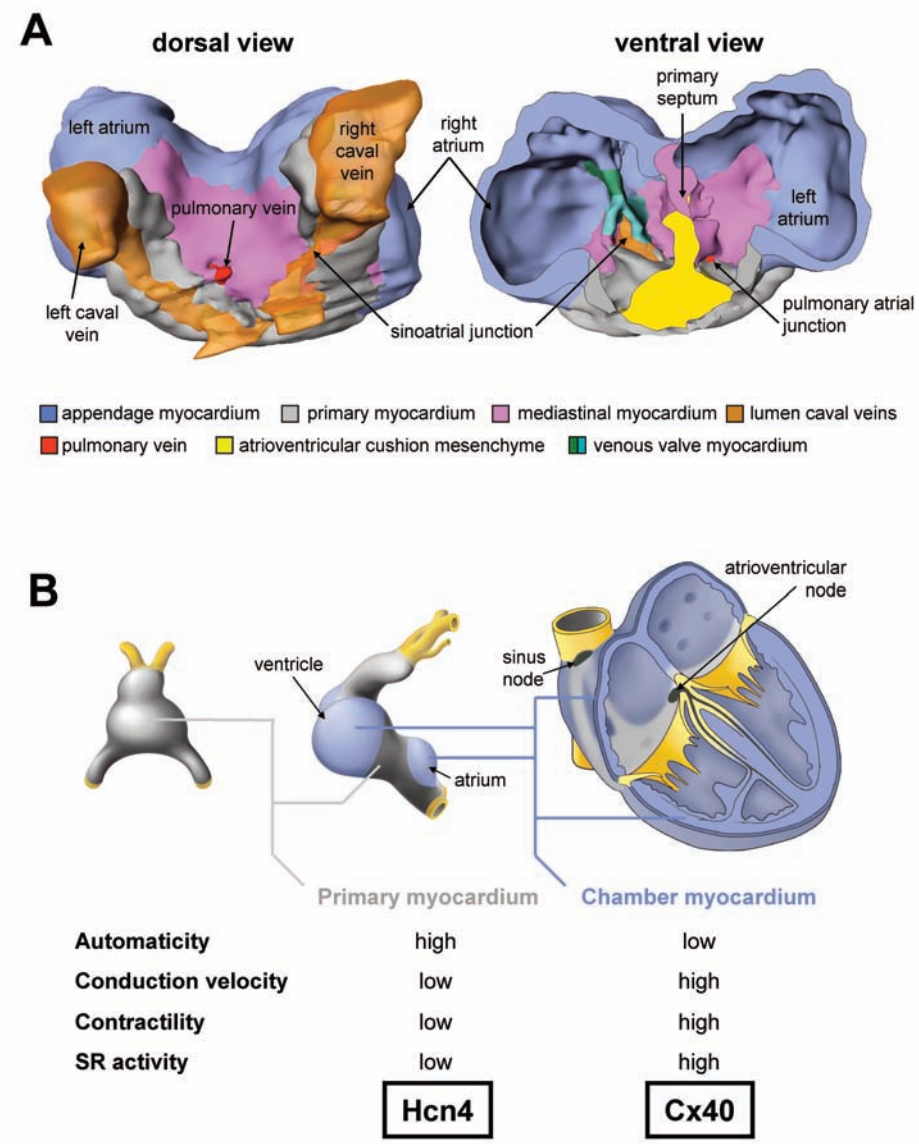

Figure 4. The initial myocardium of the heart tube has pacemaker activity. Panel A is a reconstruction of the atrial chambers of an E11.5 mouse heart with a dorsal and ventral view on the dorsal halves of the atria. The different colors represent myocardial areas with distinct gene expression profiles. Panel B shows that the embryonic myocytes in the early heart tube possess a phenotype typical for the conduction system, with a high automaticity and a low conduction velocity, contractility and sarcoplasmic reticulum activity. The chambers balloon out from the initial heart tube made up of slowly conducting myocytes and immediately initiate a fast conducting working myocardial phenotype. The myocardium at the venous pole, and the region interposed between the developing chambers, the atrioventricular canal, initially retains the conduction system phenotype and will form the cardiac conduction system.

The development of the sinus node

Even in the earliest stages, when the heart is no more than a simple tube, dominant pacemaker activity is always found at the most proximal part of the venous pole.[18] Expression of hyperpolarization-activated pacemaker channel *Hcn4*, which is required for the pacemaker activity in murine embryos,[19] overlaps in the venous pole with that of the cardiac transcription factor *Nkx2-5*. *Nkx2-5*, essential for formation of the heart, is expressed in all atrial and ventricular myocytes, as well as in the atrioventricular conduction system.[20] Although its expression initially overlaps with *Hcn4* at the venous pole, new myocardium is added to the venous pole during development that does not express *Nkx2-5*. This myocardium differentiates from *Tbx18*-expressing precursor cells, which will give rise to the sinus horns[21] and the sinus node.[22] Concomitant with formation of this *Nkx2-5*-negative myocardium, expression of *Hcn4* is extinguished in the *Nkx2-5*-positive myocardium, becoming restricted to the newly formed myocardium of the systemic venous sinus (Figure 5).[12] The *Nkx2-5*-negative walls of the sinus horns and sinus node have, from the outset, a genetic program that is distinct from that of the atrial myocardium, with absence of expression of fast-conduction atrial genes such as *Cx40*.[12,15] In contrast, the systemic venous myocardium specifically expresses *Hcn4*, the T-box transcription factor *Tbx18*,[21] and the short stature homeobox gene *Shox2*.[23] During early development, the entire systemic venous myocardium, including the sinus node, can act as the cardiac pacemaker. During the fetal period, pacemaking activity, and expression of *Hcn4*, become restricted to the developing sinus node, recognised by its expression of the T-box transcription factor *Tbx3*.[12,24] The remainder of the initially primary myocardium of the systemic venous sinus then attains a working phenotype, with up-regulation of fast conducting connexins.[12] The early primary phenotype of this myocardium, including the left horn, explains well the occurrence of ectopic atrial rhythms originating at the mouth of the coronary sinus.

The genetic program of the systemic venous sinus is responsible for normal pacemaker function

Nkx2-5 is required to establish the boundary between the working atrial myocardium and the sinus node, preventing in dose-dependent manner the sinus nodal phenotype invading the atrial walls.[12] In *Nkx2-5*-deficient embryos, the entire heart tube retains its primary phenotype, with ectopic expression of *Hcn4* and *Tbx3*,[12] and no activation of fast-conduction connexins. Beating is then initiated from the embryonic ventricular

region, rather than from the venous inflow.[25] Like the systemic venous sinus, the sinus node is formed from *Nkx2-5*-negative precursors, and remains largely free of *Nkx2-5* expression.[12] When *Nkx2-5* levels are low, at approximately one-quarter of normal levels, *Hcn4*- and *Tbx3*-expressing cells expand from the sinus node into the atrium, suggesting that atrial cells bordering the sinus node have acquired (or maintained) an nodal phenotype.[12] Over-expression of *Tbx3* in the atrium results in expression of pacemaking genes, including *Hnc4*, in the atrial myocardium, with down-regulation of the atrial genes responsible for fast conduction, causing arrhythmias and ectopic atrial pacemaking. Absence of *Tbx3*, in contrast, causes expansion of the atrial genetic program into the sinus node.[24] *Hcn4* strongly contributes to the "funny" current I_f, which is important for the spontaneous activity of the cardiac pacemaker cells.[19] A strong funny current in atrial cells will normally be overruled by the inward rectifier potassium current I_{K1}, along with the dominant pacemaker current of the sinus node. In a diseased heart, however, it is possible that atrial arrhythmias will occur more readily. Ectopic expression of *Nkx2-5* in the sinus node seems to result in bradycardia,[23,26] suggesting deregulation of the genetic program controlling sinus nodal function. Although sinus nodal dysfunction is typically due to acquired diseases, dysfunction shows familial inheritance in a significant proportion of patients, shown to have mutations in *HCN4*, *SCN5A* and *ANK2*.[27]

The development of the myocardial sleeves surrounding the pulmonary veins

Although ectopic foci in the atria and systemic venous sinus can produce atrial fibrillation, the more frequent origin is now well established as being within the pulmonary venous myocardial sleeves.[2] Initially during early development, the pulmonary veins open to the left atrium through a solitary orifice, which is adjacent to the atrioventricular junction.[28] After the completion of atrial septation, the pulmonary vein tree (a stem and usually four main branches) achieve their muscular sleeves (Figure 3B, C, 6). Thereafter, in human, the stem of the pulmonary myocardial tree is absorbed in the atrial roof. Thereby, the orifices of the pulmonary venous branches become part of the atrial roof, with one vein opening at each of the four corners.[10]

Some have suggested that the pulmonary venous myocardium is derived by outgrowth from the atrial myocardium, but lineage analysis shows this to be very unlikely.[5] The pulmonary myocardium differentiates from the mesenchyme surrounding the dorsal atrial wall, which proliferates to form the myocardial pulmonary venous sleeves.[5] This phase of rapid proliferation is not initiated in absence of *Pitx2c*, and

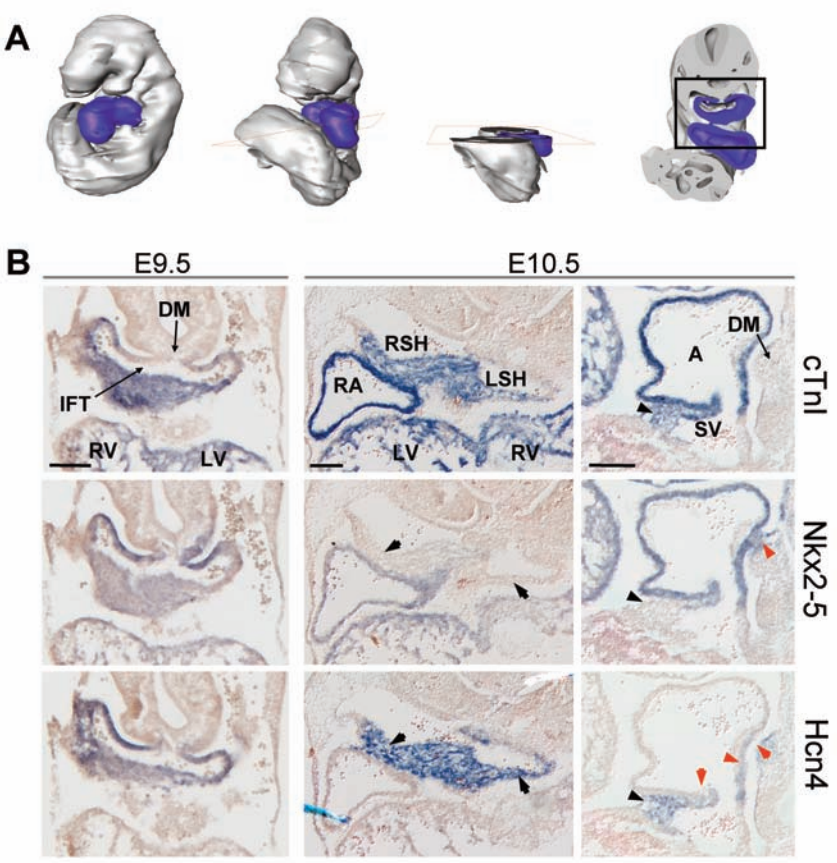

Figure 5. The localization of pacemaker activity is controlled by *Nkx2-5.* Panel A shows a three dimensional reconstruction of an E9.5 mouse embryo. The black box indicates the location of sectioning shown in Panel B. Purple, myocardium. Panel B shows in situ hybridization stained sister sections of an E9.5 mouse heart, with co-expression of *cTnI* (myocardium), *Nkx2-5* and *Hcn4* in the inflow tract. Sections of an E10.5 heart show expression of *Hcn4* selectively in the embryonic sinus venosus and of *Nkx2-5* excluded from the sinus venosus (black arrow heads indicate the sinus venosus myocardium). *Hcn4* staining is used as indication of the location of pacemaker activity. Figure with modifications from ref. 12. DM, dorsal mesocardium; IFT, inflow tract; (L/R)A, (left/right) atrium; L/RV, left/right ventricle; (L/R)SH, (left/right) sinus horn; SV, sinus venosus. Bars represent 100 µm.

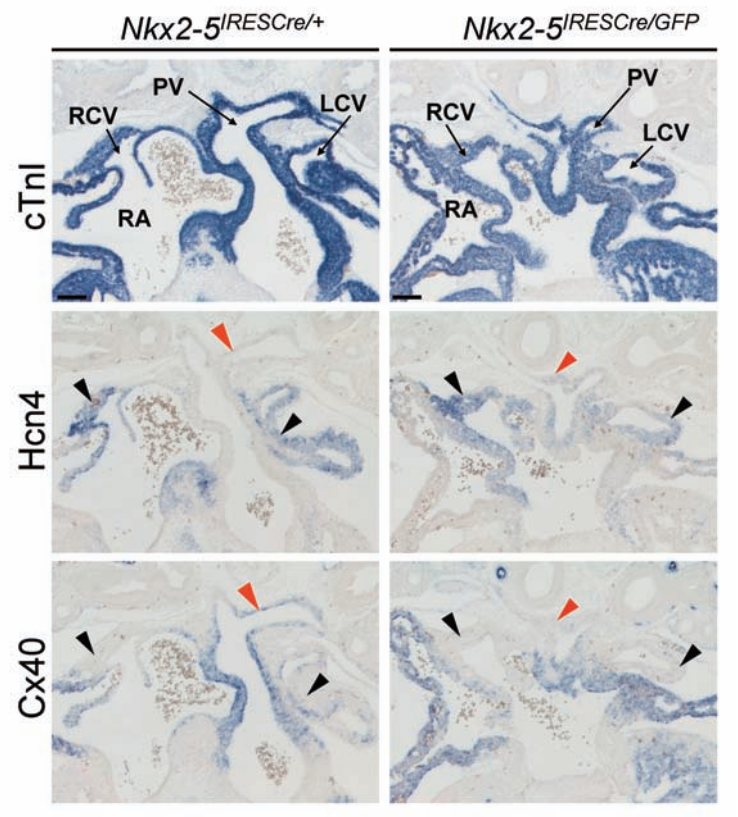

Figure 6. Reduced levels of *Nkx2-5* cause a shift in the pulmonary myocardium to a pacemaker-like gene program. In situ hybridization stained serial sections of an E14.5 *Nkx2-5*$^{IRESCre/+}$ control mouse embryo and a hypomorphic *Nkx2-5*$^{IRESCre/GFP}$ littermate stained for *cTnI* (myocardium), *Hcn4* and *Cx40*. Black arrow heads show the expression of *Hcn4* and absence of *Cx40* in the sinus venosus and the absence of *Hcn4* and presence of *Cx40* in the pulmonary vein myocardium. Red arrow heads show the switch of the pulmonary myocardium to *Hcn4* expression and absence of *Cx40* when *Nkx2-5* levels are reduced. Figure with modifications from ref. 5. R/LCV, right/left caval vein; PV, pulmonary vein. Bars represent 100 µm.

the myocardial sleeves are shown to be lacking.[5] Recently, sequence variants on chromosome 4q25 have been found to be strongly associated with an increased risk for atrial fibrillation. The gene closest to these variants, although separated by more than 50 thousand base pares, is *PITX2*.[29] Atrial fibrillation, even gene mutation-associated atrial fibrillation, of course, occurs long after the end of embryonic development. The developmental factors by themselves, therefore, do not directly or solely cause atrial fibrillation. The abnormal rhythm occurs most likely due to combination of changes in the structural, contractile, and electrophysiological properties of the myocardium, the likelihood of these changes to occur being influenced by the genetic factors.

Ectopic foci: are there nodal cells in the pulmonary venous myocardium?

As we have discussed, it is suggested by some that the pulmonary venous myocardium has a common origin with the myocardium of the systemic venous sinus,[30] but lineage data shows unequivocally that this is not the case. We have reviewed the evidence showing that the progenitors of the systemic venous tributaries and the pulmonary veins are located in different mesenchymal populations.[5,21] From the outset of their development, the systemic and pulmonary venous myocardial sleeves are fundamentally different.[15] The pulmonary myocardium has never been primary myocardium, which forms the cardiac nodes. It has a working myocardial phenotype from its initial formation. The notion of a common origin for the systemic and pulmonary venous tributaries is based in part on the expression of markers associated with the conduction system, such as *HNK-1*, *CCS-lacZ*, and *podoplanin*.[30] Although *CCS-lacZ* marks parts of the conduction system, it is also expressed outside the conduction system, notably in atrial working myocardium, whereas it is absent from the sinus node.[31] Its presence in the pulmonary venous myocardium, therefore, cannot be taken as evidence that the myocardial sleeves are part of the conduction system. It is most unlikely, therefore, that, under normal conditions, pacemaker cells are present in the pulmonary myocardium. Histological examination of the normal pulmonary venous sleeves in the human heart shows the presence only of working atrial myocytes (Figure 3B,C).[10] Ectopic beats arising in the pulmonary venous myocardium, nonetheless, are known to be frequent. Some have suggested the existence of pacemaker-like cells within the sleeves,[32] but others have not confirmed these findings,[7,10] and the purported pacemaker-like cells are markedly different morphologically from the cells of sinus node. More recent findings[33] suggest that acquired changes in the

working myocytes could underscore the ectopic pacemaking. Recently, moreover, embryological data has shown that the pulmonary venous myocardium is relatively sensitive for changes in gene expression. Under normal conditions, the myocardium has an atrial-like gene program, and expresses *Cx40*, with expression of *Hcn4* expression being low or absent. In contrast, when *Nkx2-5* expression is strongly reduced, the pulmonary venous myocardial phenotype shifts to become more pacemaker-like, then expressing Hcn4, but with a reduced expression of *Cx40* (Figure 6).[5] Mutations in human *NKX2-5* have also been suggested to be linked to atrial fibrillation.[34] Although *PITX2* and *NKX2-5* are good candidate genes, the correlation of the developmental roles of these genes with the initiation of atrial fibrillation still has to be proven. Taken together, nonetheless, these findings suggest that small genetic variations in humans could be the trigger for the origin of atrial fibrillation. This fits well with the finding that strong individual variability is present in humans in the extent of the pulmonary venous myocardial sleeves, and in the known clinical susceptibility to atrial fibrillation.

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