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ABSTRACT

Recent developments in imaging, mapping and ablation techniques have shown that the epicardial region of the heart is a key player in the occurrence of ventricular arrhythmic events in several cardiac diseases such as Brugada syndrome, arrhythmogenic cardiomyopathy or dilated cardiomyopathy. At the atrial level as well, the epicardial region has emerged as an important determinant of the substrate of atrial fibrillation, pointing to common underlying pathophysiological mechanisms. Alteration in the gradient of repolarization between myocardial layers favoring the occurrence of re-entry circuits has largely been described. The fibro-fatty infiltration of the subepicardium is another shared substrate between ventricular and atrial arrhythmias. Recent data have emphasized the role of the epicardial reactivation in the formation of this arrhythmogenic substrate. There are new evidences supporting this structural remodeling process to be regulated by the recruitment of epicardial progenitor cells that can differentiate into adipocytes or fibroblasts under various stimuli. In addition, immune-inflammatory processes can also contribute to fibrosis of the subepicardial layer. A better understanding of such "electrical fragility" of the epicardial area will open perspectives for novel biomarkers and therapeutic strategies. In this review article, a pathophysiological scheme of epicardial-driven arrhythmias will be proposed.

I. INTRODUCTION

Recent developments in imaging, mapping and ablation techniques have shown that the epicardial region of the heart is a key player in the occurrence of ventricular arrhythmic events in several cardiac diseases such as Brugada syndrome[1], arrhythmogenic cardiomyopathy (ACM)[2] or dilated cardiomyopathy.[3] At the atrial level as well, the epicardial region has emerged as an important determinant of the substrate of atrial fibrillation (AF), the most frequent cardiac arrhythmia in clinical practice. Taken together, these observations raise questions whether distinct mechanisms underlie the "tissue fragility" of the epicardial region and whether they could provide new therapeutic targets for cardiac arrhythmias. The present article will describe specific features of the epicardial region that can contribute to activation of arrhythmogenic processes, it will review evidences for an epicardial origin of cardiac arrhythmias, and discuss the role of epicardial reactivation in the formation of the arrhythmogenic substrate.

II. DEFINITIONS AND SPECIAL FEATURES OF THE EPICARDIAL REGION

1. Anatomy and histology

The epicardial area is delineated by the **epicardium,** the outer mesothelial layer of the heart. The epicardium contains multipotent progenitors that can undergo epithelial-to-mesenchymal transition (EMT), migrate into the subepicardium and, during cardiac ontogeny, give rise to multipotent mesenchymal epicardium-derived cells (EPDCs).[4] At that point, the EPDCs can differentiate into smooth muscle cells, coronary vessels[5] or myocardial fibroblasts[6] and, less importantly, into coronary endothelial cells[7] and cardiomyocytes.[8] The epicardium has also important signalling functions and exchanges paracrine factors with the neighbouring

myocardium. Quiescent in the healthy adult heart, the epicardium can be reactivated, becoming a source of myofibroblasts and of growth and angiogenic factors.[9]

The **subepicardium** rapidly increases in volume from embryonic day 6 to day 11 during mesenchymal cell invasion. It is mainly composed of connective tissue, located between the epicardium and the myocardium with a predominance of collagen I and collagen III compared to the rest of the myocardium.[10] It contains also mesenchymal cells such as smooth muscle and Cajal-like cells together with lymphocytes, mast cells, macrophages, fibroblasts, nerves and capillary (Figure 1). Myocardial trabeculations are present within the subepicardium, yet with a distinct spatial organization well visualized by clinical imaging techniques and characterized by a transmural orientation and a rotation of 120° on the axis of myocytes from epicardium to endocardium.[11,12]

The adipose tissue localized between the myocardium and the visceral pericardium, referred to as the **epicardial adipose tissue** (EAT), can be considered as another component of the epicardial area. Firstly, EAT is histologically tightly associated with the epicardium and the neighbouring myocardium that it can infiltrate.[13] Secondly, epicardial progenitors are the source of adipocytes that compose EAT.[14] Finally, EAT differs from paracardiac adipose tissue located at the outer surface of the fibrous pericardium by its embryologic origin, its biological properties and its vascularization from coronary arteries. However, the difficulty in distinguishing these two types of cardiac adipose tissue using clinical imaging techniques explains that they are often confused in studies. There is no barrier between EAT and the subepicardial myocardium such that peptides and adipokines can freely diffuse between the two tissues. For instance, EAT is an important source of free fatty acids used for myocardial energetic metabolism; it releases twice as much fatty acid as pericardial depots and also protects the heart against toxic levels of fatty acids.[13] EAT is a brown adipose tissue, expressing the uncoupling protein-1 located at the mitochondria inner membrane, a protein regulating heat

production, permitting EAT to protect the heart against hypothermia.[13] Moreover, EAT regulates myocardial oxidative stress by releasing adiponectine that inhibits nicotinamide adenine dinucleotide phosphate oxidase activity.[15]

2. Special electrical features of the epicardial region

The presence of distinct electrophysiological properties of the subepicardial myocardium also call for the individualization of this cardiac region. A repolarization gradient exists between the subepicardial and subendocardial myocardium in the human heart as well as in the hearts of other animal species.[16–18] This gradient prevents retrograde depolarization of subendocardial layers by operating as a secure lock against the occurrence of re-entry circuits.

This repolarization gradient is due to the distinct electrical properties of cardiomyoyctes between myocardial layers. Action potential (AP) of subepicardial cardiomyocytes is of shorter duration compared to other cardiomyocytes[19,20], having a more pronounced rate dependency indicated by the persistent suppression of the plateau phase with premature beats, *i.e.* AP duration shortening with decreasing S1S2 interval between S1 steady-state pacing and premature S2.[16,18] Moreover, upon increased extracellular $[K+]_o$, AP shortening is predominant in subepicardial layers resulting in tall positive T waves whereas upon decreased $[K+]_o$, epicardial AP lengthens and T waves flatten.[19]

At the cellular level, the repolarization gradient is generated by a regional difference of the fast component of the voltage-dependent outward current, I_{to}, of much higher density in subepicardial than subendocardial layers.[18] This current governs the early repolarization phase[18] of the AP, the notch, and tunes the duration of the plateau phase to heart rate.[21] It is the functional expression of *shaker* voltage-gated potassium Kv 4.2 and Kv 4.3 channels depending on species.[22,23] In human and large mammal hearts, the molecular basis for transmural difference of I_{to} is the expression gradient of gene encoding for Kv4.x channels and for KCHiP2, a β-subunit that chaperones ionic channels at the plasma membrane.[24–29] The small

density of delayed potassium currents, Iks and Ikr in M cells, too contributes to the electrical gradient.[30]

Other mechanisms underlying epicardial electrical heterogeneity are the left-handed helix to a right-handed[31] arrangement of muscle fibres and the organization of the laminar structure that drives ionic current flow.[32] Whereas in subendocardial and midwall myocardium, myocytes are grouped together by perimysial collagen into branching layers called myolaminae[33], in the subepicardium, perimysial collagen is present only as longitudinal cords.[34]

The fiber direction of the right ventricle appears to change suddenly at the level of the subepicardium[35] which may make this area prone to conduction delay and block. Additionally, the embryologic origin of the subepicardium in the right ventricular outflow tract appears to differ from other sites which makes this area prone to conduction slowing in mice.[36] Finally, fatty infiltration in the right ventricular subepicardium is more outspoken than at other sites even in normal subjects.[37]

III. CLINICAL EVIDENCE FOR EPICARDIAL DRIVEN ARRHYTHMIAS

1. Arrhythmogenic cardiomyopathy

Arrhythmogenic cardiomyopathy (ACM) classically occurs in young patients by 12-lead ECG QRS-T abnormalities and ventricular arrhythmias. Fibro-fatty replacement progresses from the epicardium to the endocardium and leads to areas of slow conduction that constitute the main substrate for reentrant ventricular tachycardia (VT) in ACM.[38] Other arrhythmia mechanisms such as focal epicardial activities have also been described in ACM patients.[39] The epicardial origin of ventricular arrhythmias (VA) associated with ACM has been particularly highlighted by ablation procedures, showing the epicardial predominance of low voltage areas and abnormal electrograms such as fractioned or late potentials, when endocardium may be normal, especially in the earlier stages of the disease.[2] Interestingly, endocardial unipolar low-voltage

zones (< 5.5 mV) were shown to predict epicardial substrate in patients with ACM, with respect to size and location.[40,41] This predictive value of endocardial unipolar mapping was also confirmed in a recent study in which RV epicardial scar region significantly correlated with endocardial unipolar low-voltage zones (< 5.5 mV).[42]

Noninvasive epicardial mapping with Electrocardiographic Imaging (ECGI) is also suitable for mapping electrophysiologic substrate on the epicardial surface. Andrews *et al*.[43] performed ECGI and late gadolinium enhancement cardiac magnetic resonance in 20 ACM patients. Compared with controls, ACM patients had significantly longer ventricular activation duration and prolonged mean epicardial activation-recovery intervals. ECGI also showed varied epicardial activation breakthrough locations and regions of nonuniform conduction and fractionated electrograms that colocalized with late gadolinium enhancement scar.

Arrhythmic substrate cannot always be targeted by endocardial ablation, especially as layered and confined epicardial circuits of ventricular tachycardia were found in ACM patients.[44] Given the high rate of VT recurrence after endocardial ablation[45], epicardial ablation was widely tested in ACM patients.[46] Several studies have shown the superiority of a combined endo/epicardial approach to endocardial-only ablation.[47] In a study comparing endocardial-alone ablation to endo-epicardial ablation, freedom from ventricular arrhythmias was observed at 3-year follow-up in 85% of the patients who benefitted from the combined approach and only 53% of the patients with endocardial-alone ablation.[48] Berruezo et al.[49] evaluated a combined endo-epicardial VT ablation approach associated with conducting channel elimination (scar dechanneling) ; freedom from VT recurrence was obtained in 90% of ACM patients.

2. <u>Brugada syndrome</u>

The other cardiac arrhythmia with a well-established epicardial substrate is Brugada syndrome (BrS). In a systematic review, Fernandes *et al*[50] showed that BrS patients undergoing both epicardial and endocardial mapping had exclusive epicardial substrate in 93% of cases.

Simultaneous noninvasive epicardial and endocardial mapping further establishes the epicardial predominance of electrical abnormalities.[51] Noninvasive epicardial mapping with Electrocardiographic Imaging was also conducted in 25 BrS patients and 6 patients with right bundle-branch block (RBBB) for comparison. Unlike patients with RBBB, BrS patients had delayed activation localized to the right ventricular outflow tract, and fractionation, or repolarization abnormalities on RVOT electrograms.[52]

Accordingly, targeting of epicardial rather than endocardial substrate appeared much more effective to prevent VT/VF in BrS. Brugada *et al*.[53] performed right ventricular epicardial mapping of patients with BrS and identifed low-voltage (<1.5 mV) and abnormal electrograms areas with abnormally prolonged fragmented epicardial potentials on the anterior right free wall and RVOT. Radiofrequency ablation of this area resulted in the suppression of the BrS ECG pattern and suppressed VT/VF inducibility. Data on 135 patients with BrS showed that elimination of abnormal epicardial electrograms led to a normalisation of the BrS ECG pattern in all but two patients.[1] In another study in which 28 symptomatic BrS patients underwent RV epicardial mapping, an anterior RVOT abnormal epicardial substrate was found in all patients.[54] Abnormal electrograms covered the entire RVOT epicardium and extended to the RV body in more than half of the patients (53%). The chosen endpoint was to eliminate all abnormal electrograms detected during epicardial mapping after the administration of a sodium channel blocker. Only 3/28 patients had recurrent VF episodes and required reablation.[54]

3. Scar related cardiomyopathy

Following the first description of the technique in 1996[55], transthoracic epicardial catheter ablation was mainly used to treat patients with Chagas disease[55] or with VT related to inferior myocardial infarction.[56] The technique and its indication have expanded over recent decades. In a multicentre study including all patients undergoing VT ablation, epicardial mapping and/or ablation was required in 17% of patients and 35% of those presented with non ischemic

cardiomyopathy (NICM).[3] Lateral subepicardial and anteroseptal intramural arrhythmogenic substrate are very common in NICM, related to presence of interstitial fibrosis that is usually more diffuse and patchy than in ICM.[57] In a series of 22 patients with NICM who failed prior endocardial ablation and/or had ECG suggesting an epicardial origin, Cano *et al*.[58] found larger low voltage and dense scar areas within the epicardium compared to the endocardium. Almost half of the patients, showed an abnormal epicardial voltage map in contrast with a normal endocardial map. Despite the expansion of epicardial ablation, long-term outcomes after VT ablation in NICM remain poor when compared to ICM, possibly due to the diffuse and deep location of arrhythmic substrate. In the HELP-VT registry study, VT free-survival at 1-year follow-up was 40.5% in patients with NICM and 57% in patients with ICM, even though ablation procedures were performed with an epicardial access when necessary.[59] These data reflect the highly complex substrate for VT in NICM and the further progress that remains to be made. Myocarditis related ventricular arrhythmia are also likely to be related to an epicardial substrate, as suggested by Dello Russo *et al*.[60] In this case series of 20 patients with drug refractory VT in the context of proven myocarditis, epicardial ablation was necessary in 30% of patients.[60] These findings are consistent with contrast-enhanced cardiac magnetic resonance (CMR) imaging of subepicardial distribution of late enhancement in patients with active myocarditis.[61]

4. Atrial fibrillation and epicardial region

The thin atrial wall is a heterogeneous structure with electrical dissociation between endocardium and epicardium and transmural muscle fibres connecting those distinct layers[62] that can constitute the substrate for re-entries. Indeed, high-density mapping of long standing persistent atrial fibrillation (AF) performed during cardiac surgery[63,64] has recorded epicardial-endocardial breakthroughs (EEB), a source of "focal" fibrillation waves favouring AF persistence, that originate from the epicardial surface. Furthermore, during the progression from

paroxysmal to persistent AF, secondary to fibro-fatty infiltration, endo-epi dissociation[65] and low voltage areas have been shown to increase. After the restoration of sinus rhythm, EEB can still be recorded with epicardial-endocardial asynchrony and the muscular connections between the endo-epicardial layers clearly indicating the presence of an epicardial substrate.[66] In this line, Pak *et al*.[67] showed that epicardial catheter ablation with a pericardial approach was effective in patients with redo-AF ablation procedure at risk for PV stenosis. Another option is the hybrid AF ablation combining minimally invasive surgery and percutaneous electrophysiology study.[68] In a meta-analysis of 563 patients, long-term success rate (sinus rhythm after a mean of 26 months) after hybrid ablation ranged from 60% to 87%.[69]

5. Value and limitations of the different epicardial mapping techniques

Contact electro-anatomical mapping systems have emerged as essential tools for precise mapping and ablation of cardiac arrhythmias. These magnetic and or impedance-based systems use dedicated mapping catheters that are introduced within the cardiac chamber of interest or the pericardial space for electrophysiological analysis. Once in place, they are able to determine the mechanism and delineate the site of origin of the arrhythmia with a precision of a few millimeters. As discussed above, ventricular endocardial unipolar low-voltage electrograms were shown to predict epicardial substrate location, notably in ACM[40] and nonischemic left ventricular cardiomyopathy.[41] Ventricular epicardial contact mapping using these technologies also clearly demonstrated the epicardial origin of numbers arrhythmias in BrS, ACM and NICM.

The inability for catheters to reach the targeted site in order to efficiently suppress the arrhythmia is one of the limitations of this technology. This is particularly true for epicardial substrate for which an epicardial access is required. Due to past medical (pericarditis) or surgical (cardiac surgery) history, the virtual space between the 2 pericardium sheets could be inaccessible via a transcutaneous puncture. Closely located coronary arteries to the site of

interest or surrounding subepicardial adipose tissue may also limit the ability to successfully suppress the arrhythmogenic substrate.

Finally, noninvasive epicardial mapping with Electrocardiographic Imaging (ECGI) is another effective option for mapping electrophysiologic substrate on the epicardial surface. Despite considerable recent development, validation of ECGI is still challenging[70] as some important discrepancies have been found between ECGi and epicardial contact mapping which should theoretically produce identical maps.

IV. FIBRO FATTY INFILTRATION AND EPICARDIAL ARRHYTHMIAS

Alteration of repolarization gradient and action potential heterogeneity between the subepicardial and subendocardial myocardium are well established arrhythmogenic mechanisms. Brugada syndrome is an archetypal example with two main models, repolarization and depolarization[71,72], with a key role of the epicardium layer in both of them. The fibro-fatty infiltration of myocardial layer appears as another major shared pathophysiological mechanism between several epicardial driven arrhythmias.

a- Adipose tissue infiltration at the ventricular level : the paradigm of ACM.

Both post-mortem studies and surgical ablation procedures have revealed the presence of adipose depots in the right ventricle of ACM patients with a progressive replacement of myocardium by fibro-fatty infiltrates and the progressive loss of right ventricle (RV) muscle fibres[73] starting from the subepicardium and then progressively extending to the subendocardium thereby become transmural (Figure 2).[73,74] Clusters of mononuclear cells in the fatty infiltrates corresponding to immune cells have been observed in fibro-fatty infiltrates leading to the notion of lymphocytic myocarditis[75] further supported by the recent observation of clinical myocarditis in patients with ACM.[76]

Some degree of fibro-fatty remodelling has also been observed in the subepicardial layer of the right ventricle outflow track in BrS patients. In a post-mortem study, RVOT histological sections of BrS patients showed an increased epicardial surface collagen that was thicker than that in control hearts. This epicardial fibrosis infiltrated into the underlying epicardial myocardium, admixed with fat. BrS cases also had reduced Cx43 expression in the RVOT when compared with controls.[77]Ohkubo et al.[78] also performed endomyocardial biopsy in 25 patients with BrS. Moderate to severe fatty infiltration was observed in 5 patients and significant fibrosis infiltration in 4. Noteworthy, right ventricular cardiomyopathy with fibro-fatty replacement was observed in patients with Brugada like-ST segment elevation.[79] This histological feature together with the delayed activation of the RVOT points to a phenotypic overlap between ACM and BrS.[80,81]

b- Fibro-fatty infiltration of the atrial myocardium and the substrate of AF

Strikingly similar fibro-fatty remodelling of the subepicardium resembling the histology of the ACM heart has been described in the atria of patients suffering from AF (Figure 2).[82,83] In healthy adult atria, the epicardium is mainly a cell monolayer with thin, extracellular matrix in contact with the myocardium or adipose tissue. However, with ageing or in patients with hypertension[84], mitral valve diseases[85] or AF, the epicardium can become thick and adipose tissue fibrotic, resulting in fibro-fatty infiltrates.[83] In a model of persistent AF in sheep, the degree of fibrosis of the subepicardial adipose tissue follows the progression of AF from paroxysmal to permanent.[83] As in ventricle, clusters of inflammatory cells can be observed at the epicardial site in the transition zone between adipocytes and fibrosis in both human and sheep atria. Immuno-histochemistry analysis of these cell clusters revealed a predominance of CD3+ T lymphocytes, with the vast majority of them CD8+ cytotoxic T cells, displaying functional cytotoxic activity through granzyme B against adipocytes.[83] Indeed, such an immune component mediated by CD8+ cytotoxic T cells has been described for other visceral adipose

tissues notably in obese patients and is considered as a major mechanism underlying the fibrosis of adipose tissue.[86]

c- Evidences for the arrhythmogenicity of fibro-fatty infiltration of myocardial layers

By comparing histology with electrical mapping of the right ventricular outflow tract, it has been possible to establish a relationship between arrhythmogenicity and the different distributions of subepicardial fibro-fatty infiltrations. For instance, 80 consecutive ACM patients in sinus rhythm were classified into three groups according to the type of fibro-fatty infiltration, referred to in this study as scar gradient (<10%: transmural, 10-20%: intermediate, >20%: horizontal).[42] Patients with horizontal scars experienced significantly more syncope, sustained VT and fatal VA when compared to patients with transmural and intermediate scar gradient, independently of the right ventricular volume. Horizontal extension of epicardial scars was an independent predictor of life-threatening VA whereas patients with transmural scars had a greater number of clinical and EP induced PVCs that was not correlated with fatal VA.[42] A heterogeneous distribution of scars between subepicardial and subendocardial layers was well described in ACM patients, with a predominance of scar area and late abnormal ventricular potentials within the subepicardium layer compared to the endocardium.[2,44] Moreover, the dense fibrotic infiltrates, characteristic of ACM, may electrically isolate the epicardium from the endocardium. When studying the transmural right ventricular activation pattern, it was observed that the epicardium is activated with a major delay in ACM patients without direct transmural spread from the endocardium but with a laminar activation pattern from the border to the central scar favouring re-entry circuits.[44]

Pieroni et al.[87] described the relationship between electroanatomic abnormalities and pathological substrate in BrS patients. 3-dimensional electroanatomic mapping-guided RVOT biopsies were performed in 20 patients and histopathological abnormalities including fibrosis

were found in 15 of them and could provide an explanation for the areas of low voltage recorded in those patients.

In a goat model of AF, EEB incidence and degree of endo-epicardial dissociation increased with increasing AF substrate complexity.[65] EED and breakthroughs also correlated with the degree of epicardial fibrosis[88] as evidenced using a 3D computational model of human atria integrating MR images and histo-anatomical data. Slow electrical conduction was recorded in the region of the human right atrial myocardium infiltrated by fibro-fatty tissue and has been attributed to the deleterious effect of adipose tissue on normal myocyte-myocyte coupling.[89]

Several mechanisms can underlie the arrhythmogenicity of the fibro-fatty infiltration of subepicardial myocardium.[90] First, by infiltrating subepicardial myocardial layers, adipocytes and fibrosis disrupt normal myocyte-myocyte coupling leading to local conduction slowing / block and to the formation of myocardial area of low voltage.[89] These altered conduction properties pave the way for reentry circuits within this remodelled area (Graphical Abstract).

The adipose tissue can also secrete a myriad of cytokines and adipokines such as leptine, but also small extracellular vesicles[91] that can regulate directly the cardiac electrical properties[92] or indirectly, for instance, by modulating the oxidative stress of the myocardium.[93] Finally, the altered electrical properties caused by fibro-fatty infiltrates subepicardial myocardial layers could disturb the normal endo-epi repolarization gradient.

However, the difficulty to reproduce experimentally the precise composition of cardiac tissue with controlled texture of fibrosis and adipose tissues explains the lack of direct evidences for the arrhythmogenicity of fibro-fatty infiltration. To overcome this limitation, computational model of myocardial tissue have been used providing arguments for reduction in conduction velocity, enhanced spiral wave periodicity and increased break-up with the degree of fibro fatty infiltration.[90]

V. EPICARDIAL REACTIVATION AND FIBRO-FATTY INFILTRATION OF THE SUBEPICARDIUM

Several explanations have been proposed for the apparent replacement of myocardium by adipose tissue notably in the context of ACM. This includes transdifferentiation of cardiomyocytes[94], proliferation and differentiation of cardiac progenitors[95], or a mesenchymal origin.[96] For instance, in explanted ACM hearts, mesenchymal stromal cells were found to contribute to fibro-fatty infiltrates suggesting an epicardial EMT origin.[96] The link between genetic defects underlying ACM and fibro-fatty remodelling of right ventricle myocardium is still actively investigated and the description of "the connexome" had provided some clues. Connexome is a network of proteins localized at the intercalated disks (ID) that includes desmosome proteins, gap junction and ionic channels. It plays a crucial role in the normal electrical and mechanical coupling between myocytes and also in the maintenance of differentiated myocardium through various signalling pathways that depend on the integrity of myocyte-myocyte contacts including Wnt/β-catenin or Hippo-Yap pathways. It is to be noted that the Hippo-Yap pathway was reported to be activated during ACM as the result of changes of the expression of several ID proteins.[97]

a-Epicardial reactivation and fibro fatty infiltration of the atrial myocardium

The study of the origin of EAT and of fibro-fatty infiltrates in the atria has provided strong evidence for the role played by the epicardium in this remodelling process (Graphical Abstract). Firstly, cells expressing markers of an epicardial origin such as Wilm's tumor (Wt-1) and Tbx18 have been detected in the fibro- fatty infiltrates of subepicardium of human atria. Secondly, using a genetic lineage tracing as Wt-1-$^{CreERT2+/-}$Rosa$^{tdT+/-}$ mouse model, it has been possible to track EPDCs in situ and to show their migration into the subepicardium and their differentiation into adipocytes or fibroblasts during various atrial remodelling.[14,98] Single cell RNA-sequencing analysis revealed the heterogeneity of atrial epicardial cells with at least 8 clusters.[98]

The analysis of inference trajectory of atrial EPDCs identifies, at least, two differentiation pathways, one towards adipocyte and another towards myofibroblast with an apparent progression from fat accumulation to fibrosis. Atrial natriuretic peptide (ANP) and angiotensin-II (Ang-II) have been shown to be important regulators of the epicardial reactivation and to control the signalling pathways that regulate differentiation lineages of atrial EPDCs (aEPDCs).[14,98] They operate as a switch to induce differentiation of aEPDCs into fibroblasts or adipocytes, respectively. For instance, the atrial natriuretic peptide, at low concentration, activates cGMP-dependent protein kinase (PKG) that regulates the expression of transcription factors C/EBPα and PPARγ.[14]

These peptides are locally secreted explaining why areas with a thin epicardial layer co-exist with thick and fibrotic epicardial areas.[98] This could contribute to focal structural reactivation of the epicardium and accumulation of fat or fibrosis. Finally, both Ang-II and ANP up-regulated their own receptors indicating a positive feedback of the agonists on their signalling pathways to secure a long-lasting response. Numerous factors can trigger epicardial reactivation and EMT such as transforming growth factor-β, fibrobast growth factors, platelet derived growth factors, Notch, retinoic acid or thymosin-beta-4 (Graphical Abstract).[100,101]

b- Are their evidences for reactivation of the epicardium at the ventricle level?

An epicardial reactivation has been also reported at the ventricle level in adult heart. For instance, following acute myocardial infarction an intense reactivation of the epicardium with recruitment of progenitor cells contributes to the scar formation and to the fibrotic remodeling of the ventricle myocardium. In this line, the inhibition of embryonic Wt1-Cre lineage mobilization is associated with the reduction in the number of epicardium-derived fibroblasts colonizing the post-myocardial injury scar.[101]

Epicardial progenitor cells are also a source of adipocytes that compose the fat depot of atrio-ventricular groove. Furthermore, depending on the activation PPARγ signalling pathway,

epicardial progenitors can differentiate into adipocytes and colonize too the post-myocardial infarction scar.[102] An epicardial reactivation due to local mechanical stretch is likely to explain fibro-fatty scars development in NICM patients and should be investigated.

c-Role of desmosome proteins in the differentiation of cardiac progenitors into adipocytes

Several evidences indicate a role of desmosomal proteins in fatty infiltration of the venticule myocardium. First, desmosome proteins are present in fibro-adipogenic progenitors whereas some mutations of desmosomal proteins favour their differentiation into adipocytes.[103] Second, in a population of non-excitable cardiac-resident cells, plakophiline has been shown to regulate intracellular lipid accumuation. Finally, epicardial progenitor cells derived from iPS cells, generated from skin fibroblasts obtained in patients suffering from ACM and harboring Plakophilin-2 mutations, spontaneously differentiated into adipocytes. The activating enhancer binding protein 2 alpha (AP2α) could be the transcription factor involved in the adipogenic differentiation of EPDCs.[104] Further studies using epicardial progenitor lineage models harboring desmosomal protein gene mutations are necessary to establish firmly the role played by epicardial reactivation in the ventricle subepicardial remodeling during ACM.

Despite solid arguments for an epicardial origin of fibro-fatty infiltration of myocardium and several evidences for the arrhythmogenicity of such fibro-fatty infiltrates, a direct causal role of epicardial reactivation in the occurrence of cardiac arrhythmias remains to be established. Furthermore, there is no evidence for a reactivation of the epicardium during BrS.

VI. CONCLUSION

Progress in the mapping of cardiac arrhythmias, notably ventricular tachycardia, has revealed the crucial role played by the epicardial region in the activation of arrhythmogenic mechanisms. This has led to important practical implications with the development of noninvasive and percutaneous catheter based epicardial mapping and ablation procedures. It has also given rise to intense research activity to better understand why the epicardial region can be a source of arrhythmias. It turns out that this region is characterised by distinct electrical and histological characteritics that under some circumstances can become a risk factor for arrhythmias. For instance, the epicardial origin of adipose tissue explains likely the propensity of the infiltration of subepicardial myocardial layers by fibro-fatty tissue as observed during several epicardial-driven arrhythmias and that has been shown to be potentially arrhythmogenic. In this line, the reactivation of the epicardium and the capacity of progenitor cells to differentiate into adipocytes or fibroblasts could be an early event in the pathophysiology of certain cardiac arrhythmias. Promising anti-arrhythmic strategies emerge from these novel pathological insights , one of them could target the accumulation of epicardial adipose tissue and its replacement by fibrosis. Progress will come also from the development of biological and imaging biomarkers that could detect the electrical fragility of the epicardial region.

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LIST OF ABBREVIATIONS

AF : atrial fibrillation
APD : action potential duration
ACM : arrhythmogenic cardiomyopathy
BRs : Brugada Syndrome
EAT : epicardial adipose tissue
EEB : epicardial-endocardial breakthroughs
EMT : epithelial-to-mesenchymal transition
EPDCs : epicardial derived cells
EP : electrophysiological
ID : intercalated disks
MMPs : Matrix metalloproteinases
NICM : non ischemic cardiomyopathy
RV : right ventricule
RVOT : right ventricular outflow tract
VT : ventricular tachycardias

REFERENCES

1. Pappone C, Brugada J, Vicedomini G, Ciconte G, Manguso F, Saviano M, Vitale R, Cuko A, Giannelli L, Calovic Z, Conti M, Pozzi P, Natalizia A, Crisà S, Borrelli V, Brugada R, Sarquella-Brugada G, Guazzi M, Frigiola A, Menicanti L, Santinelli V. Electrical Substrate Elimination in 135 Consecutive Patients With Brugada Syndrome. *Circ Arrhythm Electrophysiol* 2017;**10**:e005053.

2. Garcia Fermin C., Bazan Victor, Zado Erica S., Ren Jian-Fang, Marchlinski Francis E. Epicardial Substrate and Outcome With Epicardial Ablation of Ventricular Tachycardia in Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia. *Circulation*; 2009;**120**:366–375.

3. Sacher F, Roberts-Thomson K, Maury P, Tedrow U, Nault I, Steven D, Hocini M, Koplan B, Leroux L, Derval N, Seiler J, Wright MJ, Epstein L, Haissaguerre M, Jais P, Stevenson WG. Epicardial Ventricular Tachycardia Ablation: A Multicenter Safety Study. *J Am Coll Cardiol* 2010;**55**:2366–2372.

4. Gise A von, Pu WT. Endocardial and epicardial epithelial to mesenchymal transitions in heart development and disease. *Circ Res* 2012;**110**:1628–1645.

5. Dettman RW, Denetclaw W, Ordahl CP, Bristow J. Common epicardial origin of coronary vascular smooth muscle, perivascular fibroblasts, and intermyocardial fibroblasts in the avian heart. *Dev Biol* 1998;**193**:169–181.

6. Männer J. Does the subepicardial mesenchyme contribute myocardioblasts to the myocardium of the chick embryo heart? A quail-chick chimera study tracing the fate of the epicardial primordium. *Anat Rec* 1999;**255**:212–226.

7. Katz TC, Singh MK, Degenhardt K, Rivera-Feliciano J, Johnson RL, Epstein JA, Tabin CJ. Distinct Compartments of the Proepicardial Organ Give Rise to Coronary Vascular Endothelial Cells. *Dev Cell* 2012;**22**:639–650.

8. Cai C-L, Martin JC, Sun Y, Cui L, Wang L, Ouyang K, Yang L, Bu L, Liang X, Zhang X, Stallcup WB, Denton CP, McCulloch A, Chen J, Evans SM. A myocardial lineage derives from Tbx18 epicardial cells. *Nature* 2008;**454**:104–108.

9. Zhou B, Honor LB, He H, Ma Q, Oh J-H, Butterfield C, Lin R-Z, Melero-Martin JM, Dolmatova E, Duffy HS, Gise A von, Zhou P, Hu YW, Wang G, Zhang B, Wang L, Hall JL, Moses MA, McGowan FX, Pu WT. Adult mouse epicardium modulates myocardial injury by secreting paracrine factors. *J Clin Invest*; 2011;**121**:1894–1904.

10. Tidball JG. Distribution of collagens and fibronectin in the subepicardium during avian cardiac development. *Anat Embryol (Berl)* 1992;**185**:155–162.

11. Tseng W-YI, Wedeen VJ, Reese TG, Smith RN, Halpern EF. Diffusion tensor MRI of myocardial fibers and sheets: Correspondence with visible cut- face texture. *J Magn Reson Imaging*; 2003;**17**:31–42.

12. Scollan DF, Holmes A, Winslow R, Forder J. Histological validation of myocardial microstructure obtained from diffusion tensor magnetic resonance imaging. *Am J Physiol* 1998;**275**:H2308-2318.

13. Iacobellis G, Corradi D, Sharma AM. Epicardial adipose tissue: anatomic, biomolecular and clinical relationships with the heart. *Nat Clin Pract Cardiovasc Med* 2005;**2**:536–543.

14. Suffee N, Moore-Morris T, Farahmand P, Rücker-Martin C, Dilanian G, Fradet M, Sawaki D, Derumeaux G, LePrince P, Clément K, Dugail I, Puceat M, Hatem SN. Atrial natriuretic peptide regulates adipose tissue accumulation in adult atria. *Proc Natl Acad Sci* 2017;**114**:E771–E780.

15. Antonopoulos Alexios S., Margaritis Marios, Verheule Sander, Recalde Alice, Sanna Fabio, Herdman Laura, Psarros Costas, Nasrallah Hussein, Coutinho Patricia, Akoumianakis Ioannis, Brewer Alison C., Sayeed Rana, Krasopoulos George, Petrou Mario, Tarun Akansha, Tousoulis Dimitris, Shah Ajay M., Casadei Barbara, Channon Keith M., Antoniades Charalambos. Mutual Regulation of Epicardial Adipose Tissue and Myocardial Redox State by PPAR-γ/Adiponectin Signalling. *Circ Res*; 2016;**118**:842–855.

16. Litovsky SH, Antzelevitch C. Rate dependence of action potential duration and refractoriness in canine ventricular endocardium differs from that of epicardium: role of the transient outward current. *J Am Coll Cardiol* 1989;**14**:1053–1066.

17. Ueda N, Zipes DP, Wu J. Functional and transmural modulation of M cell behavior in canine ventricular wall. *Am J Physiol-Heart Circ Physiol*; 2004;**287**:H2569–H2575.

18. Litovsky SH, Antzelevitch C. Transient outward current prominent in canine ventricular epicardium but not endocardium. *Circ Res* 1988;**62**:116–126.

19. Antzelevitch C, Sicouri S, Litovsky SH, Lukas A, Krishnan SC, Di Diego JM, Gintant GA, Liu DW. Heterogeneity within the ventricular wall. Electrophysiology and pharmacology of epicardial, endocardial, and M cells. *Circ Res* 1991;**69**:1427–1449.

20. Sicouri S, Antzelevitch C. A subpopulation of cells with unique electrophysiological properties in the deep subepicardium of the canine ventricle. The M cell. *Circ Res* 1991;**68**:1729–1741.

21. Tseng GN, Hoffman BF. Two components of transient outward current in canine ventricular myocytes. *Circ Res* 1989;**64**:633–647.

22. Niwa N, Nerbonne JM. Molecular determinants of cardiac transient outward potassium current (I(to)) expression and regulation. *J Mol Cell Cardiol* 2010;**48**:12–25.

23. Dixon Jane E., Shi Wenmei, Wang Hong-Sheng, McDonald Christine, Yu Hangang, Wymore Randy S., Cohen Ira S., McKinnon David. Role of the Kv4.3 K+ Channel in Ventricular Muscle. *Circ Res*; 1996;**79**:659–668.

24. An WF, Bowlby MR, Betty M, Cao J, Ling H-P, Mendoza G, Hinson JW, Mattsson KI, Strassle BW, Trimmer JS, Rhodes KJ. Modulation of A-type potassium channels by a family of calcium sensors. *Nature*; 2000;**403**:553–556.

25. Rosati B, Pan Z, Lypen S, Wang H-S, Cohen I, Dixon JE, McKinnon D. Regulation of KChIP2 potassium channel β subunit gene expression underlies the gradient of transient outward current in canine and human ventricle. *J Physiol* 2001;**533**:119–125.

26. Guo W, Li H, Aimond F, Johns DC, Rhodes KJ, Trimmer JS, Nerbonne JM. Role of heteromultimers in the generation of myocardial transient outward K+ currents. *Circ Res* 2002;**90**:586–593.

27. Deschênes I, DiSilvestre D, Juang GJ, Wu RC, An WF, Tomaselli GF. Regulation of Kv4.3 current by KChIP2 splice variants: a component of native cardiac I(to)? *Circulation* 2002;**106**:423–429.

28. Zicha S, Xiao L, Stafford S, Cha TJ, Han W, Varro A, Nattel S. Transmural expression of transient outward potassium current subunits in normal and failing canine and human hearts. *J Physiol*; 2004;**561**:735.

29. Balse E, Steele DF, Abriel H, Coulombe A, Fedida D, Hatem SN. Dynamic of Ion Channel Expression at the Plasma Membrane of Cardiomyocytes. *Physiol Rev*; 2012;**92**:1317–1358.

30. Liu DW, Antzelevitch C. Characteristics of the delayed rectifier current (IKr and IKs) in canine ventricular epicardial, midmyocardial, and endocardial myocytes. A weaker IKs contributes to the longer action potential of the M cell. *Circ Res* 1995;**76**:351–365.

31. Sengupta PP, Korinek J, Belohlavek M, Narula J, Vannan MA, Jahangir A, Khandheria BK. Left Ventricular Structure and Function: Basic Science for Cardiac Imaging. *J Am Coll Cardiol* 2006;**48**:1988–2001.

32. Hooks Darren A., Trew Mark L., Caldwell Bryan J., Sands Gregory B., LeGrice Ian J., Smaill Bruce H. Laminar Arrangement of Ventricular Myocytes Influences Electrical Behavior of the Heart. *Circ Res*; 2007;**101**:e103–e112.

33. LeGrice IJ, Smaill BH, Chai LZ, Edgar SG, Gavin JB, Hunter PJ. Laminar structure of the heart: ventricular myocyte arrangement and connective tissue architecture in the dog. *Am J Physiol* 1995;**269**:H571-582.

34. Pope AJ, Sands GB, Smaill BH, LeGrice IJ. Three-dimensional transmural organization of perimysial collagen in the heart. *Am J Physiol - Heart Circ Physiol* 2008;**295**:H1243–H1252.

35. Vetter FJ, Simons SB, Mironov S, Hyatt CJ, Pertsov AM. Epicardial fiber organization in swine right ventricle and its impact on propagation. *Circ Res* 2005;**96**:244–251.

36. Boukens Bas J., Sylva Marc, de Gier-de Vries Corrie, Remme Carol Ann, Bezzina Connie R., Christoffels Vincent M., Coronel Ruben. Reduced Sodium Channel Function Unmasks Residual Embryonic Slow Conduction in the Adult Right Ventricular Outflow Tract. *Circ Res*; 2013;**113**:137–141.

37. Tansey DK, Aly Z, Sheppard MN. Fat in the right ventricle of the normal heart. *Histopathology* 2005;**46**:98–104.

38. Berruezo A, Acosta J, Fernández-Armenta J, Pedrote A, Barrera A, Arana-Rueda E, Bodegas AI, Anguera I, Tercedor L, Penela D, Andreu D, Perea RJ, Prat-González S, Mont L. Safety, long-term outcomes and predictors of recurrence after first-line combined endoepicardial ventricular tachycardia substrate ablation in arrhythmogenic cardiomyopathy. Impact of arrhythmic substrate distribution pattern. A prospective multicentre study. *Europace*; 2017;**19**:607–616.

39. Miljoen H, State S, Chillou C de, Magnin-Poull I, Dotto P, Andronache M, Abdelaal A, Aliot E. Electroanatomic mapping characteristics of ventricular tachycardia in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Europace*2005;**7**:516–524.

40. Polin GM, Haqqani H, Tzou W, Hutchinson MD, Garcia FC, Callans DJ, Zado ES, Marchlinski FE. Endocardial unipolar voltage mapping to identify epicardial substrate in arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Heart Rhythm* 2011;**8**:76–83.

41. Hutchinson MD, Gerstenfeld EP, Desjardins B, Bala R, Riley MP, Garcia FC, Dixit S, Lin D, Tzou WS, Cooper JM, Verdino RJ, Callans DJ, Marchlinski FE. Endocardial unipolar voltage mapping to detect epicardial ventricular tachycardia substrate in patients with nonischemic left ventricular cardiomyopathy. *Circ Arrhythm Electrophysiol* 2011;**4**:49–55.

42. Lin C-Y, Lin Y-J, Li C-H, Chung F-P, Lo M-T, Lin C, Chang H-C, Chang S-L, Lo L-W, Hu Y-F, Chang Y-T, Lin C-H, Chen Y-Y, Walia R, Te ALD, Yamada S, Wu T-J, Chen S-A. Heterogeneous distribution of substrates between the endocardium and epicardium promotes ventricular fibrillation in arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Europace* 2018;**20**:501–511.

43. Andrews CM, Srinivasan NT, Rosmini S, Bulluck H, Orini M, Jenkins S, Pantazis A, McKenna WJ, Moon JC, Lambiase PD, Rudy Y. Electrical and Structural Substrate of Arrhythmogenic Right Ventricular Cardiomyopathy Determined Using Noninvasive Electrocardiographic Imaging and Late Gadolinium Magnetic Resonance Imaging. *Circ Arrhythm Electrophysiol* 2017;**10**:e005105.

44. Haqqani Haris M., Tschabrunn Cory M., Betensky Brian P., Lavi Nimrod, Tzou Wendy S., Zado Erica S., Marchlinski Francis E. Layered Activation of Epicardial Scar in Arrhythmogenic Right Ventricular Dysplasia. *Circ Arrhythm Electrophysiol* 2012;**5**:796–803.

45. Wijnmaalen AP, Schalij MJ, Bootsma M, Kies P, Roos AD, Putter H, Bax JJ, Zeppenfeld K. Patients with Scar-Related Right Ventricular Tachycardia: Determinants of Long-Term Outcome. *J Cardiovasc Electrophysiol* 2009;**20**:1119–1127.

46. Santangeli P, Zado ES, Supple GE, Haqqani HM, Garcia FC, Tschabrunn CM, Callans DJ, Lin D, Dixit S, Hutchinson MD, Riley MP, Marchlinski FE. Long-Term Outcome With Catheter Ablation of Ventricular Tachycardia in Patients With Arrhythmogenic Right Ventricular Cardiomyopathy. *Circ Arrhythm Electrophysiol* 2015;**8**:1413–1421.

47. Romero J, Cerrud-Rodriguez RC, Di Biase L, Diaz JC, Alviz I, Grupposo V, Cerna L, Avendano R, Tedrow U, Natale A, Tung R, Kumar S. Combined Endocardial-Epicardial Versus Endocardial Catheter Ablation Alone for Ventricular Tachycardia in

Structural Heart Disease: A Systematic Review and Meta-Analysis. *JACC Clin Electrophysiol* 2019;**5**:13–24.

48. Bai R, Di Biase L, Shivkumar K, Mohanty P, Tung R, Santangeli P, Saenz LC, Vacca M, Verma A, Khaykin Y, Mohanty S, Burkhardt JD, Hongo R, Beheiry S, Dello Russo A, Casella M, Pelargonio G, Santarelli P, Sanchez J, Tondo C, Natale A. Ablation of ventricular arrhythmias in arrhythmogenic right ventricular dysplasia/cardiomyopathy: arrhythmia-free survival after endo-epicardial substrate based mapping and ablation. *Circ Arrhythm Electrophysiol* 2011;**4**:478–485.

49. Berruezo A, Fernández-Armenta J, Mont L, Zeljko H, Andreu D, Herczku C, Boussy T, Tolosana JM, Arbelo E, Brugada J. Combined endocardial and epicardial catheter ablation in arrhythmogenic right ventricular dysplasia incorporating scar dechanneling technique. *Circ Arrhythm Electrophysiol* 2012;**5**:111–121.

50. Fernandes GC, Fernandes A, Cardoso R, Nasi G, Rivera M, Mitrani RD, Goldberger JJ. Ablation strategies for the management of symptomatic Brugada syndrome: A systematic review. *Heart Rhythm* 2018;**15**:1140–1147.

51. Rudic B, Chaykovskaya M, Tsyganov A, Kalinin V, Tülümen E, Papavassiliu T, Dösch C, Liebe V, Kuschyk J, Röger S, El- Battrawy I, Akin I, Yakovleva M, Zaklyazminskaya E, Shestak A, Kim S, Chmelevsky M, Borggrefe M. Simultaneous Non- Invasive Epicardial and Endocardial Mapping in Patients With Brugada Syndrome: New Insights Into Arrhythmia Mechanisms. *J Am Heart Assoc Cardiovasc Cerebrovasc Dis* 2016;**5**: :e004095.

52. Zhang Junjie, Sacher Frédéric, Hoffmayer Kurt, O'Hara Thomas, Strom Maria, Cuculich Phillip, Silva Jennifer, Cooper Daniel, Faddis Mitchell, Hocini Mélèze, Haïssaguerre Michel, Scheinman Melvin, Rudy Yoram. Cardiac Electrophysiological Substrate Underlying the ECG Phenotype and Electrogram Abnormalities in Brugada Syndrome Patients. *Circulation* American Heart Association; 2015;**131**:1950–1959.

53. Brugada J, Pappone C, Berruezo A, Vicedomini G, Manguso F, Ciconte G, Giannelli L, Santinelli V. Brugada Syndrome Phenotype Elimination by Epicardial Substrate Ablation. *Circ Arrhythm Electrophysiol* 2015;**8**:1373–1381.

54. Nademanee K. Epicardial substrate ablation for Brugada syndrome. *Heart Rhythm* 2017;**14**:5.

55. Sosa E, Scanavacca M, D'avila A, Pilleggi E. A New Technique to Perform Epicardial Mapping in the Electrophysiology Laboratory. *J Cardiovasc Electrophysiol* 1996;**7**:531–536.

56. Sosa E, Scanavacca M, Avila A d', Oliveira F, Ramires JAF. Nonsurgical transthoracic epicardial catheter ablation to treat recurrent ventricular tachycardia occurring late after myocardial infarction. *J Am Coll Cardiol* 2000;**35**:1442–1449.

57. Roberts WC, Siegel RJ, McManus BM. Idiopathic dilated cardiomyopathy: analysis of 152 necropsy patients. *Am J Cardiol* 1987;**60**:1340–1355.

58. Cano O, Hutchinson M, Lin D, Garcia F, Zado E, Bala R, Riley M, Cooper J, Dixit S, Gerstenfeld E, Callans D, Marchlinski FE. Electroanatomic substrate and ablation outcome for suspected epicardial ventricular tachycardia in left ventricular nonischemic cardiomyopathy. *J Am Coll Cardiol* 2009;**54**:799–808.

59. Dinov Borislav, Fiedler Lukas, Schönbauer Robert, Bollmann Andreas, Rolf Sascha, Piorkowski Christopher, Hindricks Gerhard, Arya Arash. Outcomes in Catheter Ablation of Ventricular Tachycardia in Dilated Nonischemic Cardiomyopathy Compared With Ischemic Cardiomyopathy. *Circulation*; 2014;**129**:728–736.

60. Dello Russo A, Casella M, Pieroni M, Pelargonio G, Bartoletti S, Santangeli P, Zucchetti M, Innocenti E, Di Biase L, Carbucicchio C, Bellocci F, Fiorentini C, Natale A, Tondo C. Drug-refractory ventricular tachycardias after myocarditis: endocardial and epicardial radiofrequency catheter ablation. *Circ Arrhythm Electrophysiol* 2012;**5**:492–498.

61. De Cobelli F, Pieroni M, Esposito A, Chimenti C, Belloni E, Mellone R, Canu T, Perseghin G, Gaudio C, Maseri A, Frustaci A, Del Maschio A. Delayed Gadolinium-Enhanced Cardiac Magnetic Resonance in Patients With Chronic Myocarditis Presenting With Heart Failure or Recurrent Arrhythmias. *J Am Coll Cardiol* 2006;**47**:1649–1654.

62. Schuessler R B, Kawamoto T, Hand D E, Mitsuno M, Bromberg B I, Cox J L, Boineau J P. Simultaneous epicardial and endocardial activation sequence mapping in the isolated canine right atrium. *Circulation* 1993;**88**:250–263.

63. Groot NMS de, Houben RPM, Smeets JL, Boersma E, Schotten U, Schalij MJ, Crijns H, Allessie MA. Electropathological substrate of longstanding persistent atrial fibrillation in patients with structural heart disease: epicardial breakthrough. *Circulation* 2010;**122**:1674–1682.

64. Groot N de, Does L van der, Yaksh A, Lanters E, Teuwen C, Knops P, Woestijne P van de, Bekkers J, Kik C, Bogers A, Allessie M. Direct Proof of Endo-Epicardial Asynchrony of the Atrial Wall During Atrial Fibrillation in Humans. *Circ Arrhythm Electrophysiol* 2016;**9**:e003648.

65. Eckstein J, Maesen B, Linz D, Zeemering S, Hunnik A van, Verheule S, Allessie M, Schotten U. Time course and mechanisms of endo-epicardial electrical dissociation during atrial fibrillation in the goat. *Cardiovasc Res* 2011;**89**:816–824.

66. Mouws Elisabeth M.J.P., Lanters Eva A.H., Teuwen Christophe P., van der Does Lisette J.M.E., Kik Charles, Knops Paul, Bekkers Jos A., Bogers Ad J.J.C., de Groot Natasja M.S. Epicardial Breakthrough Waves During Sinus Rhythm. *Circ Arrhythm Electrophysiol* 2017;**10**:e005145.

67. Pak H-N, Hwang C, Lim HE, Kim JS, Kim Y-H. Hybrid epicardial and endocardial ablation of persistent or permanent atrial fibrillation: a new approach for difficult cases. *J Cardiovasc Electrophysiol* 2007;**18**:917–923.

68. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, Castella M, Diener H-C, Heidbuchel H, Hendriks J, Hindricks G, Manolis AS, Oldgren J, Popescu BA, Schotten U, Van Putte B, Vardas P, Agewall S, Camm J, Esquivias GB, Budts W, Carerj S, Casselman F, Coca A, De Caterina R, Deftereos S, Dobrev D, Ferro JM, Filippatos G,

Fitzsimons D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur Heart J* 2016;**37**:2893–2962.

69. Vroomen M, Pison L. Hybrid ablation for atrial fibrillation: a systematic review. *J Interv Card Electrophysiol* 2016;**47**:265–274.

70. Cluitmans M, Brooks DH, MacLeod R, Dössel O, Guillem MS, Dam PM van, Svehlikova J, He B, Sapp J, Wang L, Bear L. Validation and Opportunities of Electrocardiographic Imaging: From Technical Achievements to Clinical Applications. *Front Physiol* 2018;**9**:1305.

71. Wilde AAM, Postema PG, Di Diego JM, Viskin S, Morita H, Fish JM, Antzelevitch C. The pathophysiological mechanism underlying Brugada syndrome: depolarization versus repolarization. *J Mol Cell Cardiol* 2010;**49**:543–553.

72. Meregalli PG, Wilde AAM, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? *Cardiovasc Res* 2005;**67**:367–378.

73. Gandjbakhch E, Redheuil A, Pousset F, Charron P, Frank R. Clinical Diagnosis, Imaging, and Genetics of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2018;**72**:784–804.

74. Corrado D. CARDIOMYOPATHY: Arrhythmogenic right ventricular cardiomyopathy: diagnosis, prognosis, and treatment. *Heart* 2000;**83**:588–595.

75. Burke Allen P., Farb Andrew, Tashko Gerti, Virmani Renu. Arrhythmogenic Right Ventricular Cardiomyopathy and Fatty Replacement of the Right Ventricular Myocardium. *Circulation*; 1998;**97**:1571–1580.

76. Ader Flavie, Surget Elodie, Charron Philippe, Redheuil Alban, Zouaghi Amir, Maltret Alice, Marijon Eloi, Denjoy Isabelle, Hermida Alexis, Fressart Véronique, Gandjbakhch Estelle. Inherited Cardiomyopathies Revealed by Clinically Suspected Myocarditis. *Circ Genomic Precis Med*; 2020;**13**:e002744.

77. Nademanee K, Raju H, Noronha SV de, Papadakis M, Robinson L, Rothery S, Makita N, Kowase S, Boonmee N, Vitayakritsirikul V, Ratanarapee S, Sharma S, Wal AC van der, Christiansen M, Tan HL, Wilde AA, Nogami A, Sheppard MN, Veerakul G, Behr ER. Fibrosis, Connexin-43, and Conduction Abnormalities in the Brugada Syndrome. *J Am Coll Cardiol* 2015;**66**:1976–1986.

78. Ohkubo K, Watanabe I, Okumura Y, Takagi Y, Ashino S, Kofune M, Sugimura H, Nakai T, Kasamaki Y, Hirayama A, Morimoto S-I. Right ventricular histological substrate and conduction delay in patients with Brugada syndrome. *Int Heart J* 2010;**51**:17–23.

79. Corrado D, Nava A, Buja G, Martini B, Fasoli G, Oselladore L, Turrini P, Thiene G. Familial cardiomyopathy underlies syndrome of right bundle branch block, ST segment elevation and sudden death. *J Am Coll Cardiol* 1996;**27**:443–448.

80. Corrado Domenico, Zorzi Alessandro, Cerrone Marina, Rigato Ilaria, Mongillo Marco, Bauce Barbara, Delmar Mario. Relationship Between Arrhythmogenic Right Ventricular Cardiomyopathy and Brugada Syndrome. *Circ Arrhythm Electrophysiol* 2016;**9**:e003631.

81. Furushima H, Chinushi M, Okamura K, Iijima K, Komura S, Tanabe Y, Okada S, Izumi D, Aizawa Y. 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.

82. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, Chugh SS, Corradi D, D′Avila A, Dobrev D, Fenelon G, Gonzalez M, Hatem SN, Helm R, Hindricks G, Ho SY, Hoit B, Jalife J, Kim Y-H, Lip GYH, Ma C-S, Marcus GM, Murray K, Nogami A, Sanders P, Uribe W, Wagoner DRV, Nattel S, Centurion (Paraguay) DROA, Kuck (Germany) K-H, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on Atrial cardiomyopathies: Definition, characterisation, and clinical implication. *J Arrhythmia*; 2016;**32**:247.

83. Haemers P, Hamdi H, Guedj K, Suffee N, Farahmand P, Popovic N, Claus P, LePrince P, Nicoletti A, Jalife J, Wolke C, Lendeckel U, Jaïs P, Willems R, Hatem SN. Atrial fibrillation is associated with the fibrotic remodelling of adipose tissue in the subepicardium of human and sheep atria. *Eur Heart J*; 2017;**38**:53–61.

84. Medi C, Kalman JM, Spence SJ, Teh AW, Lee G, Bader I, Kaye DM, Kistler PM. Atrial electrical and structural changes associated with longstanding hypertension in humans: implications for the substrate for atrial fibrillation. *J Cardiovasc Electrophysiol* 2011;**22**:1317–1324.

85. John B, Stiles MK, Kuklik P, Chandy ST, Young GD, Mackenzie L, Szumowski L, Joseph G, Jose J, Worthley SG, Kalman JM, Sanders P. Electrical remodelling of the left and right atria due to rheumatic mitral stenosis. *Eur Heart J* 2008;**29**:2234–2243.

86. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* 2009;**15**:914–920.

87. Pieroni M, Notarstefano P, Oliva A, Campuzano O, Santangeli P, Coll M, Nesti M, Carnevali A, Fraticelli A, Iglesias A, Grassi S, Brugada R, Bolognese L. Electroanatomic and Pathologic Right Ventricular Outflow Tract Abnormalities in Patients With Brugada Syndrome. *J Am Coll Cardiol*; 2018;**72**:2747–2757.

88. Gharaviri A, Bidar E, Potse M, Zeemering S, Verheule S, Pezzuto S, Krause R, Maessen JG, Auricchio A, Schotten U. Epicardial Fibrosis Explains Increased Endo–Epicardial Dissociation and Epicardial Breakthroughs in Human Atrial Fibrillation. *Front Physiol* Frontiers; 2020;**11**.

89. Nalliah CJ, Bell JR, Raaijmakers AJA, Waddell HM, Wells SP, Bernasochi GB, Montgomery MK, Binny S, Watts T, Joshi SB, Lui E, Sim CB, Larobina M, O'Keefe M, Goldblatt J, Royse A, Lee G, Porrello ER, Watt MJ, Kistler PM, Sanders P, Delbridge LMD, Kalman JM. Epicardial Adipose Tissue Accumulation Confers Atrial Conduction Abnormality. *J Am Coll Cardiol*;2020;**76**:1197–1211.

90. De Coster T, Claus P, Kazbanov IV, Haemers P, Willems R, Sipido KR, Panfilov AV. Arrhythmogenicity of fibro-fatty infiltrations. *Sci Rep* 2018;**8**:2050.

91. Gan L, Xie D, Liu J, Bond Lau W, Christopher TA, Lopez B, Zhang L, Gao E, Koch W, Ma X-L, Wang Y. Small Extracellular Microvesicles Mediated Pathological Communications Between Dysfunctional Adipocytes and Cardiomyocytes as a Novel Mechanism Exacerbating Ischemia/Reperfusion Injury in Diabetic Mice. *Circulation* 2020;**141**:968–983.

92. Zhao Y, Sun Q, Zeng Z, Li Q, Zhou S, Zhou M, Xue Y, Cheng X, Xia Y, Wang Q, Tu X. Regulation of SCN3B/scn3b by Interleukin 2 (IL-2): IL-2 modulates SCN3B/scn3b transcript expression and increases sodium current in myocardial cells. *BMC Cardiovasc Disord* 2016;**16**:1.

93. Gómez-Hurtado N, Domínguez-Rodríguez A, Mateo P, Fernández-Velasco M, Val-Blasco A, Aizpún R, Sabourin J, Gómez AM, Benitah J-P, Delgado C. Beneficial effects of leptin treatment in a setting of cardiac dysfunction induced by transverse aortic constriction in mouse. *J Physiol* 2017;**595**:4227–4243.

94. Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen H-SV. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 2013;**494**:105–110.

95. Lombardi R, Dong J, Rodriguez G, Bell A, Leung TK, Schwartz RJ, Willerson JT, Brugada R, Marian AJ. Genetic fate mapping identifies second heart field progenitor cells as a source of adipocytes in arrhythmogenic right ventricular cardiomyopathy. *Circ Res* 2009;**104**:1076–1084.

96. Sommariva E, Brambilla S, Carbucicchio C, Gambini E, Meraviglia V, Dello Russo A, Farina FM, Casella M, Catto V, Pontone G, Chiesa M, Stadiotti I, Cogliati E, Paolin A, Ouali Alami N, Preziuso C, Amati G d', Colombo GI, Rossini A, Capogrossi MC, Tondo C, Pompilio G. Cardiac mesenchymal stromal cells are a source of adipocytes in arrhythmogenic cardiomyopathy. *Eur Heart J* 2016;**37**:1835–1846.

97. Chen SN, Gurha P, Lombardi R, Ruggiero A, Willerson JT, Marian AJ. The hippo pathway is activated and is a causal mechanism for adipogenesis in arrhythmogenic cardiomyopathy. *Circ Res* 2014;**114**:454–468.

98. Suffee N, Moore-Morris T, Jagla B, Mougenot N, Dilanian G, Berthet M, Proukhnitzky J, Le Prince P, Tregouet DA, Pucéat M, Hatem SN. Reactivation of the Epicardium at the Origin of Myocardial Fibro-Fatty Infiltration During the Atrial Cardiomyopathy. *Circ Res* 2020;**126**:1330–1342.

99. Hao J, Wang B, Jones SC, Jassal DS, Dixon IM. Interaction between angiotensin II and Smad proteins in fibroblasts in failing heart and in vitro. *Am J Physiol Heart Circ Physiol* 2000;**279**:H3020-3030.

100. Blom JN, Feng Q. Cardiac repair by epicardial EMT: Current targets and a potential role for the primary cilium. *Pharmacol Ther* 2018;**186**:114–129.

101. Quijada P, Trembley MA, Small EM. The Role of the Epicardium During Heart Development and Repair. *Circ Res* 2020;**126**:377–394.

102. Yamaguchi Y, Cavallero S, Patterson M, Shen H, Xu J, Kumar SR, Sucov HM. Adipogenesis and epicardial adipose tissue: A novel fate of the epicardium induced by mesenchymal transformation and PPARγ activation. *Proc Natl Acad Sci* 2015;**112**:2070–2075.

103. Lombardi R, Chen SN, Ruggiero A, Gurha P, Czernuszewicz GZ, Willerson JT, Marian AJ. Cardiac Fibro-Adipocyte Progenitors Express Desmosome Proteins and Preferentially Differentiate to Adipocytes Upon Deletion of the Desmoplakin Gene. *Circ Res* 2016;**119**:41–54.

104. Kohela Arwa, van Kampen Sebastiaan, Moens Tara, Monshouwer-Kloots Jantine, Molenaar Bas, Wehrens Martijn, Vink Arjan, Chen Huei-Sheng Vincent, van Rooij Eva. Abstract 783: Epicardial Contribution to Arrhythmogenic Cardiomyopathy. *Circ Res* American Heart Association; 2019;**125**:A783–A783.

105. Mallat Z, Tedgui A, Fontaliran F, Frank R, Durigon M, Fontaine G. Evidence of Apoptosis in Arrhythmogenic Right Ventricular Dysplasia. *N Engl J Med*; 1996;**335**:1190–1197.

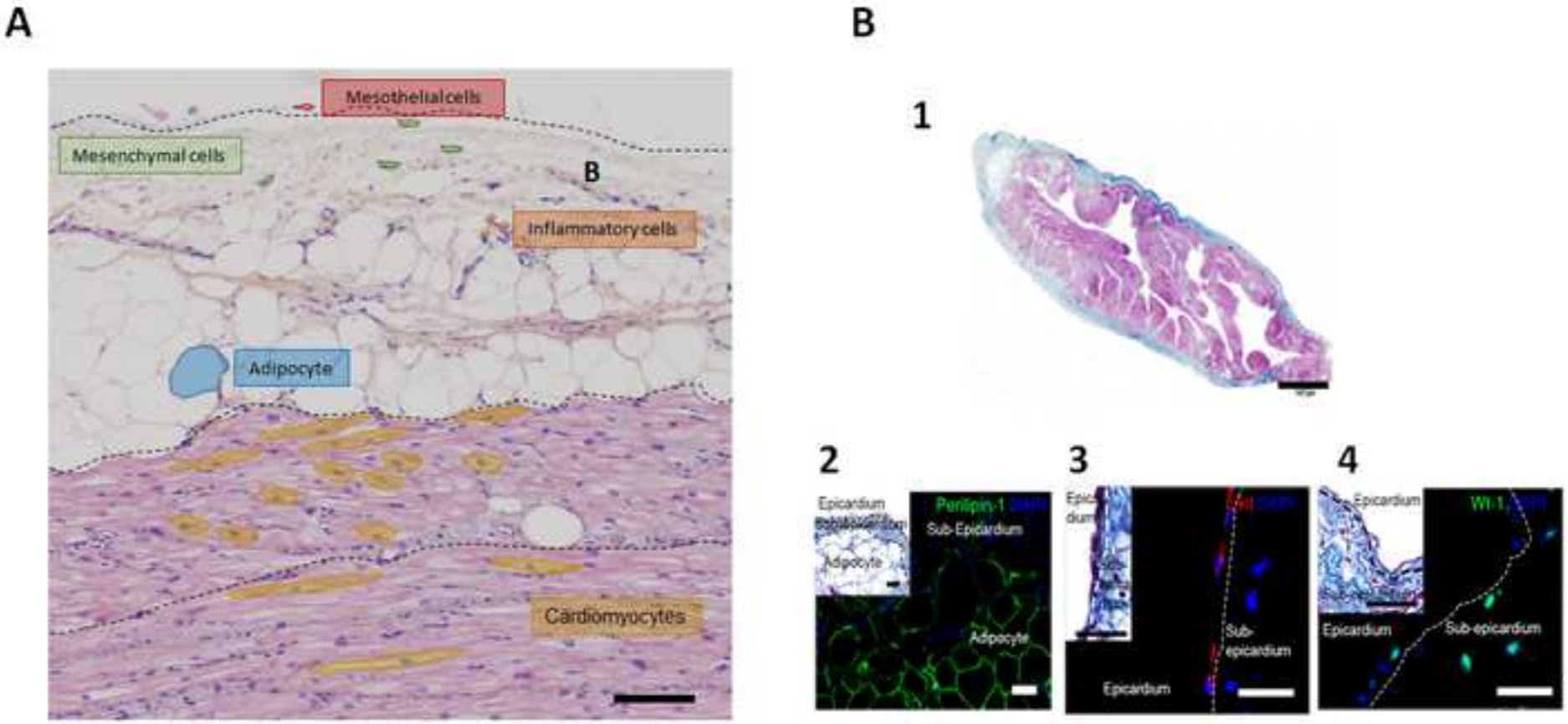
FIGURE LEGENDS

Figure 1. Histology and structural organization of the epicardial area. (A) Schematic representation of a cross section of left atrium showing the interaction between epicardium, adipose tissue and myocardium with distinct orientations of fibers between layers (cross section vs longitudinal myocytes). **(B)** Masson's trichrome staining of human atrial tissue (1) showing the epicardium in blue, myocardium in red and subepicardial adipose tissue in white (Scale bar, 100 µm). At high magnification, immunofluorescence staining of human atrial section reveals the presence of adipocytes expressing perilipin-1 (2) and progenitor cells expressing c-Kit (3) and WT-1 (4) in both epicardium and subepicardium (Scale bars, 10 µm), from Suffee *et al*.[14]

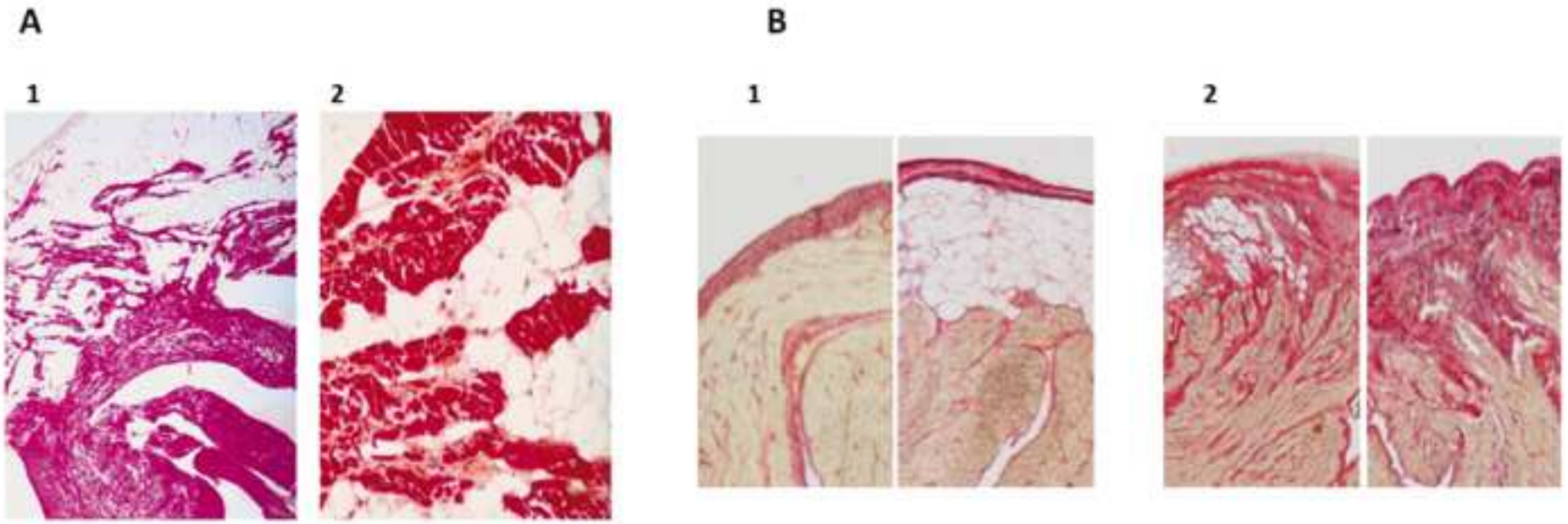
Figure 2. Fibro-fatty infiltration of subepicardial layers of ventricle and atrial myocardium. (A) Hematoxylin–phloxin–safran staining of the right ventricle free wall of a patient with arrhythmogenic cardiomyopathy showing a large amount of adipose tissue occupying subepicardial layers (1, ×10) and isolated strands of myocardium bordered by or embedded in fibrous tissue (2, ×100), from Mallat Z *et al***.** [105] **(B)** Red sirius staining of a human right atrial section showing an example of non-fibrotic remodelled epicardium (1) either without subepicardial adipose tissue or with subepicardial adipose tissue and (2) fibrotic remodelled epicardium with various degree of fibro-fatty infiltration, from Haemers *et al*. [83]

Graphical abstract. From the reactivation of the epicardium to the formation of an arrhythmogenic substrate. Transition from a quiescent epicardium (panel A) to epicardial reactivation followed by fibro-fatty infiltrations of subepicardial myocardial layers (panel B). Potential arrhythmogenic mechanisms (panel C) include i- at the tissue level, conduction

slowing or block and low voltage area favoring formation of electrical reentry circuit within the myocardial wall and ii- at the cellular level, altered myocyte coupling and adipokine- and cytokine- induced abnormal excitation contraction coupling and oxidative stress.



Schematic representation of left atria



Graphical Abstract