Modulators of proteostasis: therapeutic targets and diagnostic markers to halt and reverse atrial fibrillation

Marion, D.M.S.

2021

document version
Publisher's PDF, also known as Version of record

Chapter 3

Diagnosis and therapy of atrial fibrillation: the past, the present and the future

Denise M. S. van Marion[1], Eva A. H. Lanters[2], Marit Wiersma[1], Maurits A. Allessie[3], Bianca B. J. J. M. Brundel[1,4#], Natasja M. S. de Groot[2#]

[1]Department of Clinical Pharmacy and Pharmacology, Groningen University Institute for Drug Exploration (GUIDE), University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[2]Department of Cardiology, Erasmus Medical Center, Rotterdam, The Netherlands

[3]Cardiovascular Research Institute Maastricht, Maastricht, The Netherlands

[4]Department of Physiology, Institute for Cardiovascular Research, VU University Medical Center Amsterdam, The Netherlands

Journal of Atrial Fibrillation. 2015 Aug; 8(2):1216

Keywords: atrial fibrillation, heat shock protein, diagnosis, therapy

Abstract

Atrial fibrillation (AF) is the most common age-related cardiac arrhythmia. It is a progressive disease, which hampers successful treatment. The progression of AF is caused by the accumulation of damage in cardiomyocytes which makes the atria more vulnerable for AF. Especially structural remodeling and electrical remodeling, together called electropathology, are sustainable in the atria and impair functional recovery to sinus rhythm after cardioversion.

The exact electropathological mechanisms underlying persistence of AF are at present unknown. High resolution wavemapping studies in patients with different types of AF showed that longitudinal dissociation in conduction and epicardial breakthrough were the key elements of the substrate of longstanding persistent AF. A double layer of electrically dissociated waves propagating transmurally can explain persistence of AF (Double Layer Hypothesis) but the molecular mechanism is unknown. Derailment of proteostasis – defined as the homeostasis in protein synthesis, folding, assembly, trafficking, guided by chaperones, and clearance by protein degradation systems – may play an important role in remodeling of the cardiomyocyte. As current therapies are not effective in attenuating AF progression, step-by-step analysis of this process, in order to identify potential targets for drug therapy, is essential. In addition, novel mapping approaches, enabling assessment of the degree of electropathology in the individual patient, are mandatory to develop patient-tailored therapies. The aims of this review are to 1) summarize current knowledge of the electrical and molecular mechanisms underlying AF, 2) discuss the shortcomings of present diagnostic instruments and therapeutic options and 3) to present potential novel diagnostic tools and therapeutic targets.

Introduction

The first electrocardiogram (ECG) of atrial fibrillation (AF) was recorded by Einthoven in 1906 [1]. Nowadays, AF is one of the most common arrhythmias with a prevalence varying from <0.1% to >12% in the elderly which is expected to be doubled in patients over 55 years by 2060 [2, 3]. AF is originally known as a disease of the ageing population. However, an increasing prevalence is seen in young adults, especially in endurance athletes [4] and patients with congenital heart disease [5]. Hence, a continuous rise in the number of AF associated hospitalizations and healthcare costs is to be expected [6]. Several treatment modalities have been developed, but all are associated with high recurrence rates or negative side effects. The aims of this review are to 1) summarize current knowledge of the electrical and molecular mechanisms underlying AF, 2) discuss the shortcomings of present diagnostic instruments and therapeutic options and 3) to present potential novel diagnostic tools and targets for future therapy.

Deficiencies in diagnostic tools of atrial fibrillation

AF is usually diagnosed by a surface ECG or Holter recording. However, diagnosis of new onset, paroxysmal or asymptomatic AF can be challenging. An ECG only captures several seconds of the heart rhythm and episodes of AF can therefore be easily missed. The use of long-term ambulatory electrocardiography devices or implantable loop recorders increases the chance of detecting AF paroxysms. In addition, these devices also allow determination of the total duration of all AF episodes within a specific time frame, the so-called AF burden. However, electrocardiographic recordings do not provide any information on the mechanism underlying AF. Recent studies [7-10] suggest that body surface mapping arrays, containing 252 electrodes, may be useful to identify driver regions in patients with AF. Yet, none of the currently available recording techniques can determine the degree and extensiveness of atrial electropathology. Hence, when a patient presents with AF, we have no

diagnostic tool available for evaluating the mechanism underlying AF and determining the stage of the disease at any time in the process.

Mechanisms of atrial fibrillation: from past to the present

Experiments performed by Gordon Moe [11] nearly 60 years ago provided the basis for the ongoing debate on the underlying cause for AF. In isolated canine atria, he showed that AF could be due to either *fibrillatory conduction* (AF caused by an ectopic focus with a high frequency discharge resulting in non-uniform excitation of the atria) or *true fibrillation* (AF persists independently from the site where it was initiated). In 1959, Moe [11] introduced the so-called Multiple Wavelet Hypothesis which further described the features of true fibrillation. In this hypothesis, Moe postulated that persistence of AF depended on the average number of wavelets. With the total number of wavelets being increased, the probability of extinguishment and thus termination of AF would become smaller. Twenty-six years later, Allessie et al. [12] performed the first experimental evaluation of Moe's multiple wavelet hypothesis. In a canine right atrium, during 0.5 second of acutely induced AF, he demonstrated in series of consecutive excitation maps that there was a continuous beat-to-beat change in activation pattern. The critical number of wavelets in both right and left atria necessary to perpetuate AF was estimated to be between three and six. Ever since, numerous experimental and clinical mapping studies [11, 13-21], reporting on perpetuation of AF, are supportive on either a focal (repetitive ectopic discharges) or re-entrant mechanism (mother-wave, rotor, multiple wavelets). In the past years, most clinical studies reported on the presence of rotors in patients with various types of AF [20].

Electropathology associated with persistence of atrial fibrillation

High-resolution wavemapping studies [22] of AF in patients with valvular heart disease and longstanding persistent AF, demonstrated that a large proportion of fibrillation waves were so-called focal waves. These waves appeared in the middle of the mapping area and could not be explained by fibrillation waves propagating in the epicardial plane. Focal fibrillation waves appeared scattered throughout the mapping area and were not repetitive (Figure 1).

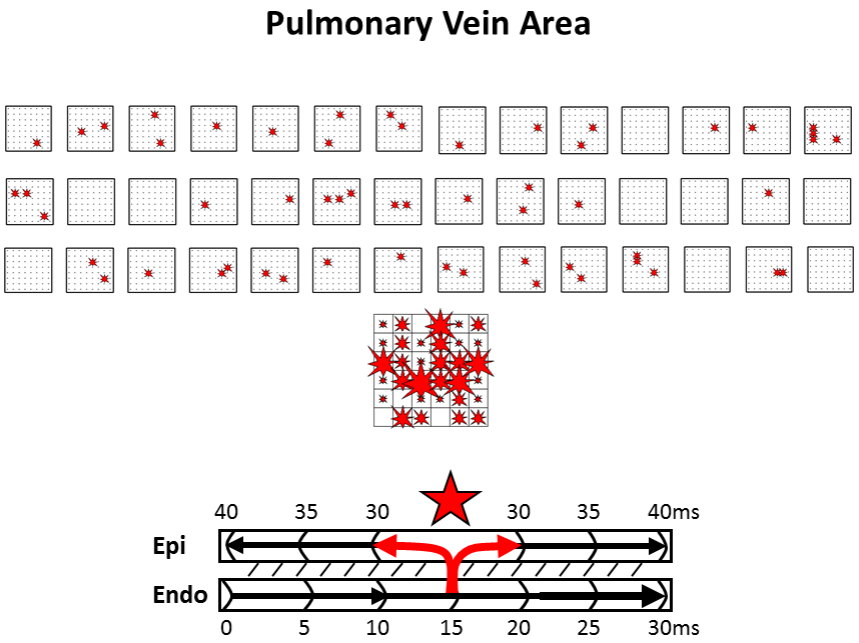

Figure 1. Epicardial breakthrough

Upper panel: beat-to-beat variation in spatiotemporal distribution of epicardial breakthrough waves ('focal waves') during 6 seconds of persistent AF in a small area of 1.25X1.25cm between the pulmonary veins. Each asterisk indicates a breakthrough site. The large map shows all 55 epicardial breakthroughs sites. The size of the asterisk is proportional to the number of epicardial breakthroughs occurring at that site. The breakthrough map demonstrates a wide distribution of these focal waves; none of these breakthrough waves occurred, however, repetitively.

Lower panel: schematic presentation of excitation of the endo- and epicardial layer explaining how transmural conduction from the endocardium to the epicardium gives rise to an epicardial breakthrough wave. Hence, the endocardial layer serves in this case as a source for 'new' fibrillation waves in the epicardial layer [22].

The coupling interval was longer than the dominant AF cycle length, and unipolar electrograms at the epicardial origin of these waves exhibited R-waves [22]. Hence, characteristics of these focal fibrillation waves strongly suggest that they originated from endo-epicardial breakthrough. These findings were supported by a report from Lee et al. [23] who observed that more than one-third of the fibrillation waves in patients with persistent AF were of 'focal' origin without any area sustaining focal activity. Based on our observations, we recently introduced a new mechanism explaining persistence of AF independently of the presence of foci or re-entrant circuits in our Double Layer Hypothesis [22, 24]. The "Double Layer Hypothesis" states that the substrate of longstanding persistent AF in humans is caused by progressive endo-epicardial dissociation, transforming the atria into an electrical double layer of dissociated waves that constantly 'feed' each other (Figure 1). Whereas in patients with short-lasting episodes of AF, the endo- and epicardial layers are still activated synchronously, in patients with longstanding persistent AF, the endo- and epicardial layers of the atrial wall are activated asynchronously. Over time, due to electrical and structural remodeling of the atria, the atrial wall is gradually transformed into a double layer of narrow anatomically delineated pathways. The exact molecular mechanisms underlying electrical dissociation are, however, unknown.

Molecular mechanisms underlying electropathology AF

As mentioned above, AF is a progressive disease, which can be explained by the fact that AF itself induces alterations in both function and structure of the cardiomyocyte. These alterations induce an arrhythmogenic substrate which facilitates perpetuation of AF episodes [25].

During the last decennia, various researchers aimed to identify the molecular mechanisms that underlie cardiomyocyte remodeling and AF progression. Although several pathways, especially related to ion channel remodeling, have been described, the exact molecular mechanisms driving AF remodeling and progression are still unidentified.

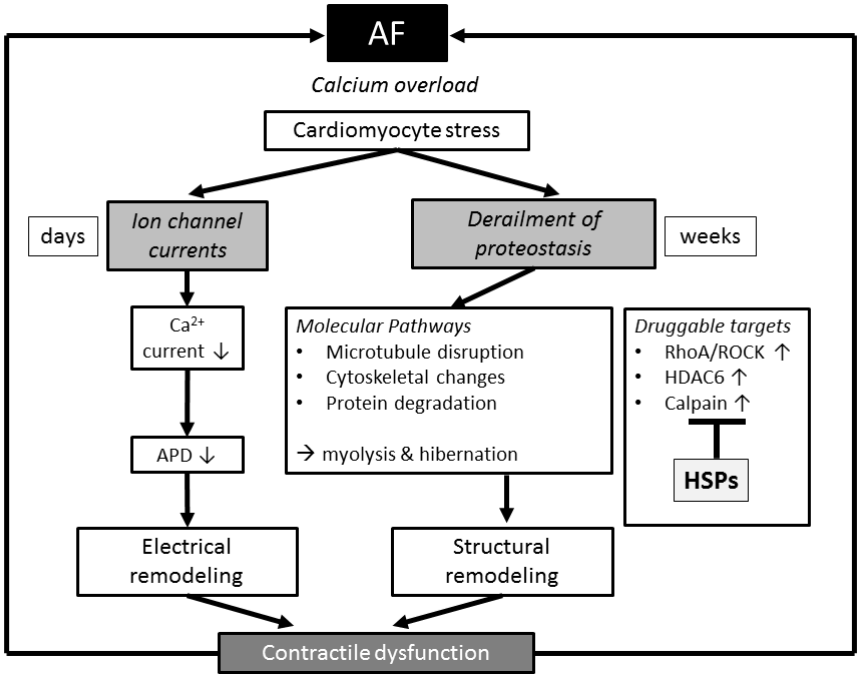

Figure 2. Overview of AF-induced cardiomyocyte remodeling

AF induces time-related progressive remodeling. First, AF causes a stressful cellular Ca^{2+} overload, which results in a direct inhibition of the L-type Ca^{2+} channel, shortening of action potential duration and contractile dysfunction. These changes have an early onset and are reversible. The early processes protect the cardiomyocyte against Ca^{2+} overload but at the expense of creating a substrate for persistent AF. When AF persists, derailment of proteostasis occurs, which result in microtubule disruption, cytoskeletal changes and degradation of proteins. The targets involved in proteostasis are RhoA/ROCK, HDAC6 and calpain. In addition, HSP induction has been found to counteract these targets. Derailment of proteostasis results in structural remodeling, myolysis/hibernation, and consequently impaired contractile function and AF persistence. Thus drugs that normalize proteostasis via inhibition of RhoA/ROCK, calpain, and HDAC6, but also via induction of cardio-protective HSPs are of therapeutic interest for future treatment of clinical AF.

The general concept is that during AF, cardiomyocytes are subjected to rapid and irregular excitation causing calcium overload in the cells which leads to fast and reversible electrical remodeling and slower, irreversible structural remodeling (Figure 2). The cardiomyocyte responds to a calcium overload by the functional downregulation of L-type Ca^{2+} current channels, which causes the shortening of action potential duration (APD) and electrical remodeling, thereby providing a further substrate for AF [26-30]. Also, several other ion channel currents are affected either on the expression level or phosphorylation and redox status [31-33]. In addition, various kinases and phosphatases

become activated and regulate the function of ion channels and other downstream target proteins, for example transcriptions factors, various calcium handling proteins (such as RyR2, Sarcoplasmic Reticulum Ca^{2+} ATPases (SERCA), or Na^{+}/Ca^{2+} exchanger) and the actin cytoskeleton [34-38]. When AF persists beyond a few days, irreversible structural remodeling occurs, especially hibernation [39] (Figure 2). Various research groups [39-41] showed that hibernation is a form of tissue adaptation. It is defined as the ability of the cardiomyocytes to turn into a non-functional phenotype featuring irreversible degradation of the myofibril structure (myolysis), which leads to loss of atrial contraction.

While the early electrical remodeling is reversible [30] a 'second factor' underlies the persistence of AF, having a time course comparable to AF-induced structural changes (hibernation/myolysis) in the atrial cardiomyocytes [42]. Thus, the prevention of structural remodeling represents a key target to attenuate cardiomyocyte remodeling and dysfunction and may improve the outcome of (electrical) cardioversion to normal sinus rhythm. We have strong indications that derailment of proteostasis represents this 'second factor' that underlies AF progression [38, 39, 43-46].

Derailed proteostasis: novel concept of cardiomyocyte remodeling

Proteostasis is defined as the homeostasis in protein synthesis, folding, assembly, trafficking, guided by chaperones, and clearance by protein degradation systems [47-50]. Healthy proteostasis is controlled by an exquisitely regulated network of molecular components and cellular pathways, the protein quality control (PQC) system [47, 51]. Cells, including cardiomyocytes, are very sensitive to changes in the intra- and extracellular environment, induced by stressors, including AF. Stressors can cause derailment in the proteostasis by altering the stability of proteins, leading to protein damage, unfolding and breakdown, as observed for cardiac troponins and structural proteins [38, 43]. In the heart, various chaperones, especially heat shock proteins (HSPs), are expressed to ensure a healthy cardiomyocyte proteostasis and optimal function of the heart. For example HSPB1, HSPB6, HSPB7 and

HSPB8 are important members of the PQC system and attenuate derailment of proteostasis in AF by assisting in the refolding of unfolded proteins [38, 51], prevention of AF-induced damage to contractile proteins [44, 52] and attenuation of protein breakdown [43]. In this way, HSPs normalize the proteostasis and protect the cardiomyocyte against remodeling and AF progression.

Molecular pathways underlying derailed proteostasis

Recently, several molecular pathways were found to induce derailment of proteostasis. These pathways include the persistent activation of calpain, activation of RhoA/ROCK pathway and the activation of HDAC6.

Investigators found proof for a role of persistent activation of the calcium overload-induced protease, calpain, to underlie impairment of proteostasis and AF progression in experimental cardiomyocyte, and *Drosophila* model systems for AF [43, 52, 53], but also in human permanent AF [39]. In experimental studies it was observed that calpain activation causes the degradation of contractile and structural proteins, and subsequently contributes to structural cardiomyocyte remodeling (myolysis) and dysfunction and AF progression [43, 53]. The role of calpain was confirmed in human AF. Here, a significant induction in calpain activation was observed in patients with permanent AF, compared to patients with paroxysmal AF and controls in sinus rhythm [39]. Furthermore, patients with permanent AF revealed induced amounts of myolysis which correlated significantly with calpain activity levels, suggesting a role for calpain in derailment of cardiomyocyte proteostasis, structural remodeling and AF progression.

Also, during AF, RhoA-GTPases are activated. RhoA-GTPases represent a family of small GTP-binding proteins that are involved in cell cytoskeleton organization, migration, transcription and proliferation. They have an important role as regulators of the actin cytoskeleton in cardiomyocytes [54] and trigger the initiation of AF [55, 56]. RhoA-GTPases activation results in conduction disturbances and cardiac

dysfunction [57, 58]. A recent study [38] revealed that in AF RhoA-GTPases become activated, resulting in the activation of its downstream effector ROCK and thereby stimulate the polymerization of G-actin to filamentous F-actin stress bundles. These stress bundles impair calcium homeostasis and contribute to contractile dysfunction, cardiomyocyte remodeling and AF progression [38].

Furthermore, recently it was found that histone deacetylases (HDACs), such as HDAC6, are implicated in AF-induced cardiomyocyte remodeling [43]. HDACs affect cardiomyocyte proteostasis by epigenetically regulating protein expression and modulating various cytoplasmic proteins, including α-tubulin, a structural protein from the microtubule network [59-61]. By using mutant constructs, AF-induced contractile dysfunction and structural remodeling was proven to be driven by HDAC6 via de-acetylation of α-tubulin and finally breakdown of microtubules by calpain. This effect of HDAC6 was observed in tachypaced HL-1 atrial cardiomyocytes, *Drosophila*, dogs and confirmed in patients with permanent AF [43]. HDAC6 inhibition by tubacin conserved the microtubule homeostasis and prevented depolymerized α-tubulin from calpain-mediated degradation. These results indicate a key role for HDAC6 in the derailment of cardiomyocyte proteostasis in experimental and clinical AF.

So, three key pathways in AF-induced structural and functional remodeling have been identified, and all these pathways impair a healthy proteostasis of the cardiomyocyte.

Induction of HSPs normalize proteostasis

To maintain a good functioning PQC system, numerous chaperones are expressed to ensure a healthy cardiomyocyte proteostasis [38]. HSPs are under the control of heat shock transcription factor 1 (HSF1), and represent important chaperones in proteostatic control [47, 62]. During excessive stress situations such as AF, HSP levels were found to become exhausted [44]. This finding suggests that upregulation of HSP levels might normalize proteostasis and improve cardiomyocyte function in AF. In clinical studies, induced HSP levels showed protection against AF initiation and progression. HSPA1A

atrial expression levels were found to correlate with reduced incidence of post-operative AF in patients in sinus rhythm undergoing cardiac surgery [63, 64]. In another clinical study [65], a potent heat shock response (HSR) and high HSPB1 levels have been associated with restoration of normal sinus rhythm in patients with permanent AF after mitral valve surgery. Higher atrial HSPB1 levels were found to be related to shorter AF duration and less myolysis when comparing paroxysmal versus persistent AF and sinus rhythm [44, 66]. These findings suggest that HSPs become activated after AF episodes, and exhaust in time in a stress-related manner [44]. Consequently, PQC is lost and incorrect/damaged proteins accumulate in cardiomyocytes, inducing or accelerating remodeling, in turn resulting in AF progression and recurrence. Next to AF, also a loss of PQC is recognized to contribute to the deterioration of heart function, reduction of stress tolerance, and the possibility of reducing the threshold for manifestation of cardiac disease [67].

Various *in vitro* and *in vivo* models for tachypacing-induced AF identified HSPs to protect against AF initiation and against the derailment of proteostasis and cardiomyocyte remodeling. HSPs increase SERCA activity and stimulate both the reuptake of Ca^{2+} into the sarcoplasmic reticulum and the removal of Ca^{2+} out of the cardiomyocyte via Na^{+}/Ca^{2+} exchanger [68], suggesting that HSPs attenuate AF progression by protecting against (tachypacing-induced) changes in calcium handling proteins. Several HSPs (including HSPB1) were shown to reduce oxidative stress, thereby potentially preventing or restoring the redox status of the ion channels [69] and preventing damage to the actin cytoskeleton. This protective effect of HSPB1 was found via direct binding to actin filaments and indirectly by preserving the redox status [43, 44, 70-73]. Reducing oxidative stress preserves proteostasis and electrophysiological and contractile function of the cardiomyocyte in AF. Moreover, HSPs prevent calpain activation [39, 53] and thereby attenuate contractile protein degradation and contractile dysfunction.

Deficiencies of present therapy of atrial fibrillation

Therapy of AF is aimed at either rhythm or rate control. Since AF induces electrical, structural, and contractile remodeling, therapy aimed at prevention or restoration of remodeling and consequently restoration of sinus rhythm should be the strategy of first choice [74]. The different AF treatment modalities include pharmacological therapy, electrical cardioversion (ECV), pacemaker implantation combined with His bundle ablation or surgical isolation of the pulmonary veins with or without additional linear lesions/substrate modification (endovascular or surgical). According to the Multiple Wavelet Theory, the stability of the fibrillatory process is determined by the number of simultaneously circulating wavelets. Anti-fibrillatory effects of class IA, IC and III drugs are based on widening of the excitable period (difference between AF cycle length and refractory period). When the excitable period widens, it is less likely that a fibrillation wave encounters atrial tissue, which is still refractory. This in turn decreases the degree of fractionation of fibrillation waves and subsequently also the number of fibrillation waves. It is most likely that when patients with AF have a variable degree of remodeling due to e.g. dissimilar underlying heart diseases or AF episodes of different durations anti-arrhythmic drugs will also widen the excitable gap to a variable degree. This in turn may explain differences in inter-individual responses to anti-arrhythmic drugs. The acute success rate of intravenous chemical cardioversion (CCV) using various drugs, including amiodarone and flecainide, is 58-75% [75, 76] for patients with paroxysmal or persistent AF and is highest when performed in AF <48 hours [76]. Immediate (prior to discharge) AF recurrences were observed in 3% [76] and AF relapsed in 30-40% of patients within one year with continuation of anti-arrhythmic drugs [76]. When CCV is unsuccessful, ECV is the next treatment in line. Immediate restoration of sinus rhythm is achieved in 88-97% [76-78]. Comparable to CCV, AF recurrences are common; sinus rhythm is maintained for one year in only 40-60% of the patients.

Circumferential Pulmonary Vein Isolation (PVI), endovascular or surgical, is aimed at isolating ectopic foci within the myocardial sleeves of the pulmonary veins. Endovascular PVI can be achieved with

radiofrequency current, laser or cryothermal energy. Navigation of the ablation catheters can be performed either manually guided by fluoroscopy or electroanatomical mapping systems, or robotically using remote (non-) magnetic navigation systems [79-81]. Despite the promising acute success rates, one year AF free survival is approximately 40-50% and redo ablations are frequently performed [79-82]. This data is confirmed in a large meta-analysis by Ganesan et al. [83]. In this study, the long-term success rate increased to 79,8%, however only after multiple ablation procedures. The overall complication rate associated with endovascular AF ablation is 5% including phrenic nerve palsy, pulmonary vein stenosis, pericardial effusion and cardiac tamponade [82, 84]. From a theoretical point of view, PVI should be an effective treatment modality for patients with paroxysms of AF triggered by ectopic foci within the pulmonary veins. Recurrences of AF after pulmonary vein isolation can be due to incompleteness of circular lesions, conduction or an arrhythmogenic substrate located outside the pulmonary veins [85]. In addition, an arrhythmogenic substrate may also develop over time as a result of a progressive cardiomyopathy. Different ablation approaches targeting the assumed substrate of AF have therefore been developed in the past years [85] including ablation of ganglionated autonomic plexuses in epicardial fat pads or disruption of dominant rotors in the left or right atrium as recognized by high-frequency Complex Fractionated Atrial Electrograms (CFAE) [86]. Wu et al. [87] concluded in a meta-analysis that CFAE ablation could reduce the recurrence of atrial tachycardia in patients with non-paroxysmal AF after a single procedure. This effect was not observed in patients with paroxysmal AF. The reported one year AF free survival after the first CFAE ablation is only 29% when performed as a standalone procedure [86] and 74% in CFAE ablation additional to PVI [86, 88]. Endovascular ablation of the ganglionic plexi as a standalone procedure in patients with paroxysmal AF is associated with a significantly lower arrhythmia free survival when compared to the PVI [89, 90]. When performed additionally to (repeat) PVI in patients with persistent AF, 16 months success rate rises to 59% [90]. The recurrence rates of these (concomitant) substrate modifications are thus high, indicating that the arrhythmogenic substrate underlying persistence of AF was still not fully understood. Our Double Layer

Hypothesis [22, 24] provides the explanation why, in case the endo- and epicardial layers are electrically dissociated, ablative therapy is not successful anymore.

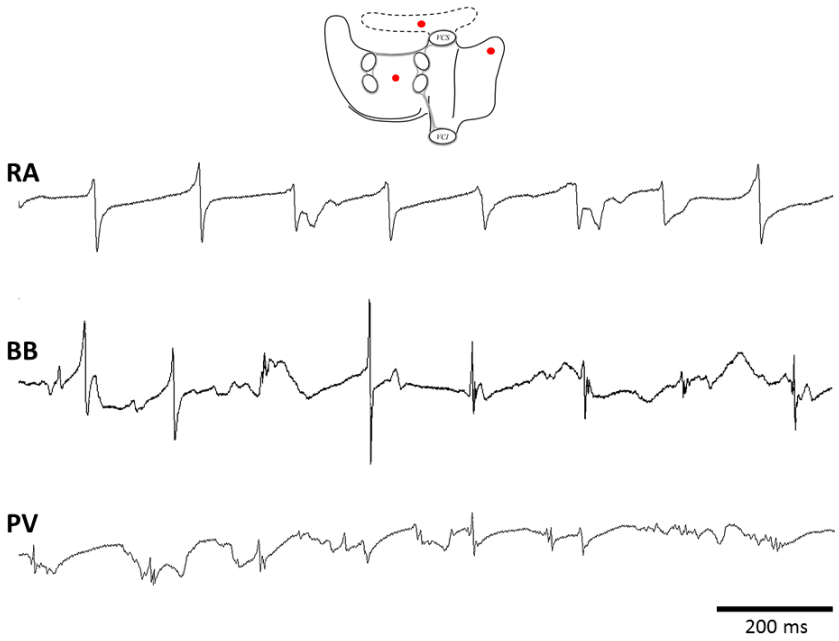

Figure 3. Intra-individual variation in electrogram morphology

Typical examples of unipolar fibrillation electrograms recorded from the middle of respectively the right atrial appendage (RA), Bachmann's Bundle (BB) and the pulmonary vein area (PV) obtained from a patient with mitral valve disease and persistent AF. In the right atrium, the fibrillation potentials contain a single deflection whereas fibrillation potentials recorded from Bachmann's Bundle and the pulmonary vein area contain multiple deflections.

Future diagnostic tools

As large numbers of disorders are associated with AF and patients with AF reveal AF episodes of variable duration, it is most likely that there is a large degree of variation in the degree of atrial remodeling. In addition to this, within a patient, it is also likely that there is intra-atrial variation in the degree of remodeling. Examples of regional differences in morphology of unipolar fibrillation potentials are shown in Figure 3. Hence, knowledge of the degree and extensiveness of the arrhythmogenic substrate in the individual patient is essential in order to evaluate a patient-tailored therapy for AF. For this purpose, we developed custom made mapping software ('wave mapping')

which enabled visualization of the individual fibrillation waves and quantification of the fibrillatory process. By using this software, we compared electrophysiological properties of fibrillation waves recorded during induced AF in patients with normal atria (physiological AF) with persistent AF in patients with valvular heart disease (pathological AF) and demonstrated that electrical dissociation of atrial muscle bundles and epicardial breakthrough of fibrillation waves play a key role in development of the substrate of persistent AF (Figure 4) [24]. In order to diagnose the arrhythmogenic substrate of AF in individual patients, we are currently evaluating a real-time, high resolution, multi-site epicardial mapping approach of the entire atria (Figure 5) as a novel diagnostic tool which can be applied as a routine procedure during cardiac surgery. An approach like this allows quantification of electrophysiological properties of the entire atria. In such manner, we study electropathology throughout the entire atria in patients with and without AF and with a diversity of underlying structural heart diseases. This novel mapping approach will not only be used to gain further insights into the arrhythmogenic substrate of AF, but will also be used to develop novel therapies or to improve existing treatment modalities. For example, it may guide ablative therapy when the arrhythmogenic substrate is confined to a circumscribed region. In addition, data acquired with this mapping approach will also provide the basis for development of less- or non-invasive mapping techniques.

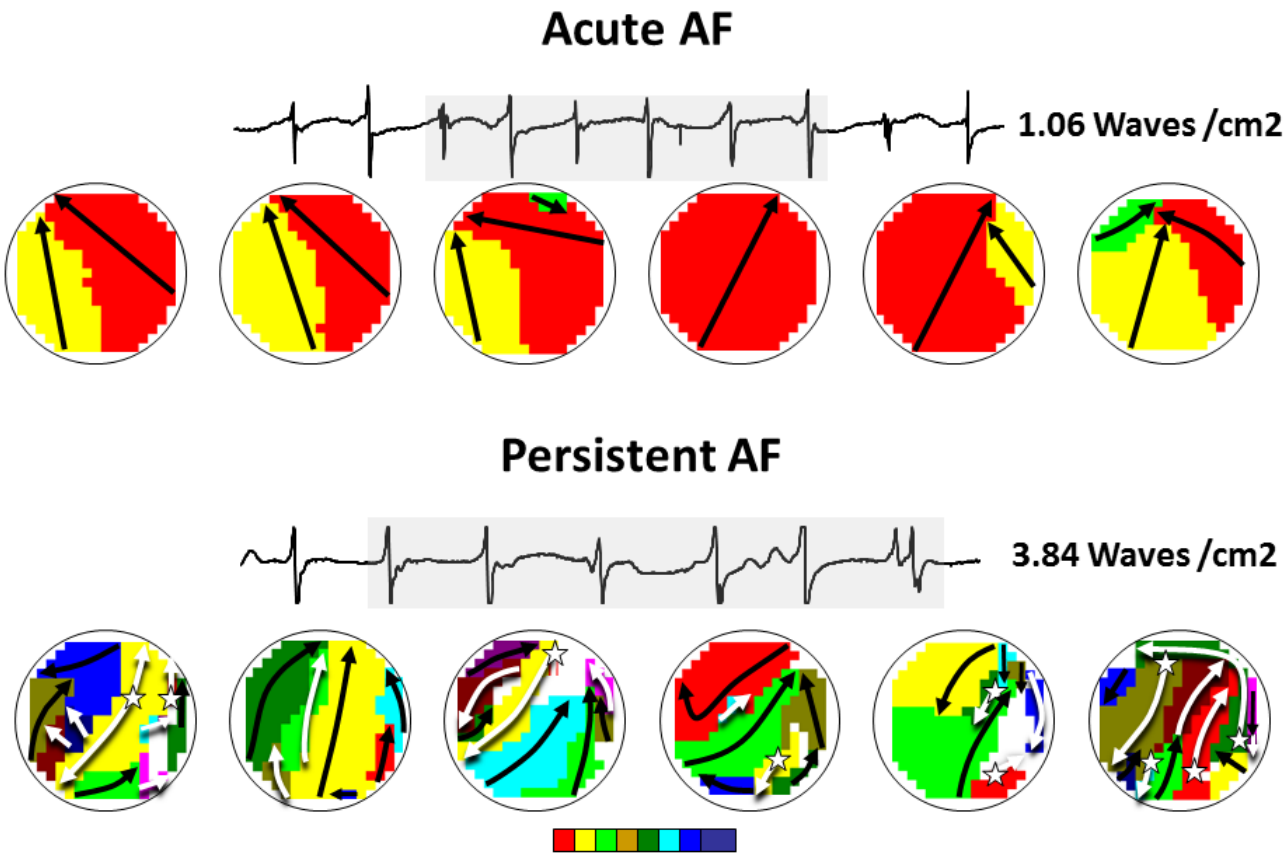

Figure 4. Inter-individual variation in characteristics of fibrillation waves

Examples of six consecutive wavemaps obtained from the right atrial free wall constructed during acute AF (upper panel) and persistent AF (lower panel); unipolar fibrillation electrograms recorded in the middle of the mapping area are shown on top. The mapping area activated by each individual fibrillation is represented by a color; every color indicates the moment of entrance in the mapping area (from red to purple); the arrows indicate the main trajectory of the fibrillation wave (black: peripheral fibrillation wave, white: epicardial breakthrough wave). During acute AF, there are a fewer number of fibrillation waves and the patterns of activation are less complex, compared to persistent AF. In addition, 'focal fibrillation waves' occur more frequently during persistent AF.

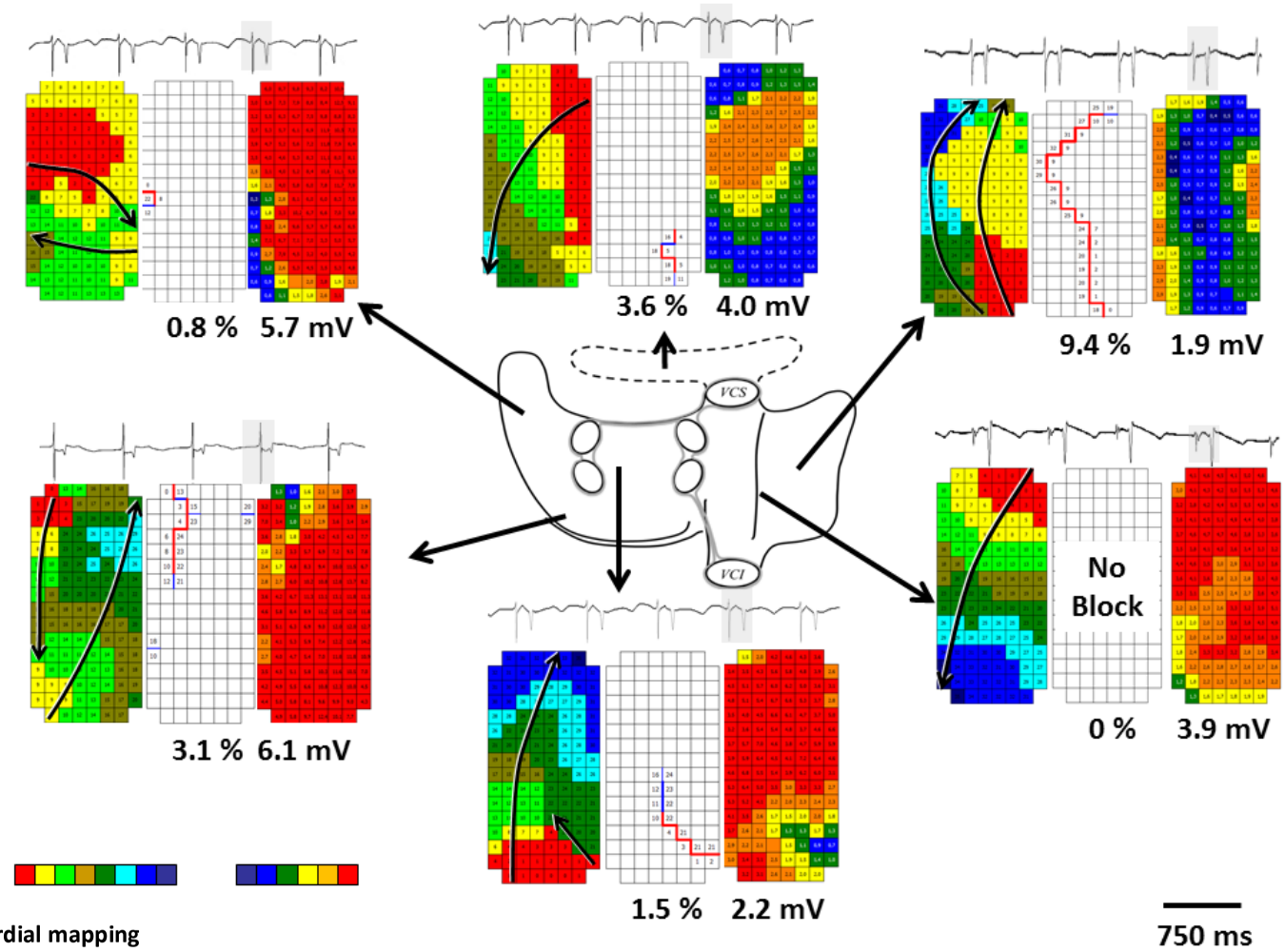

Figure 5. Atrial epicardial mapping

Activation-, conduction block- and voltage maps constructed from Bachmann's Bundle, right atrium, crista terminalis, pulmonary vein area, left atrioventricular groove, left atrial appendage during sinus rhythm, obtained from a patient with coronary artery disease. Electrograms recorded from the middle of the mapping area are shown on top. Arrows in the color-coded activation maps show the main trajectory of the excitation wave. Areas of slow conduction (<18cm/s) and conduction block (<30cm/s) are represented by respectively blue and red lines. Voltage maps show the peak-to-peak amplitude of the atrial potentials.

The future: novel therapeutic targets

Current therapies are directed at suppression of AF symptoms, but are not effective in attenuating AF remodeling, therefore there is a high need to identify novel therapeutic targets which will improve the clinical outcome. Novel targets include RhoA, calpain and HDAC6 inhibition, but also HSP induction. Recent studies revealed the important role of the RhoA/ROCK pathway activation in structural remodeling of cardiomyocytes during AF [38]. To maintain proper cardiac function, RhoA/ROCK inhibitors might be of therapeutic interest. Several RhoA and ROCK inhibitors have been developed. RhoA inhibitors CCG-1423 and Rhosin are studied in the preclinical phase [91, 92]. Fasudil, Ezetimibe and AR-12286 are ROCK inhibitors currently studied in Phase II-IV trials for Raynaud's phenomenon, vascular function study, atherosclerosis and glaucoma (Table 1).

Calpain activation during AF causes the degradation of contractile and structural proteins, resulting in myolysis [38, 39, 43]. *In vitro* studies showed that inhibitors of calpain conserve the cardiomyocyte structure and function and therefore might have beneficial effects in the treatment of AF [43, 53]. Various calpain inhibitors have been developed and preclinically studied. Disadvantages of the current developed inhibitors are that they show poor selectivity for subtypes of calpain and often have a high LogP value and therefore are hard to dissolve in aqueous solutions [93].

HDAC6 inhibition, by tubacin, conserves α-tubulin proteostasis, and prevents its degradation by calpain 1 and thereby protects against loss of calcium transient and cardiac remodeling in experimental model systems for AF. As tubacin is not suitable for *in vivo* studies due to low drug-likeness [94], other promising HDAC6 inhibitors, such as tubastatin A and ACY-1215 have been recently developed [94-96] (Table 1). Interestingly, tubastatin A showed to protect against tachypacing-induced cardiac remodeling in a canine model for AF [52], supporting the use of HDAC6 inhibitors as a novel therapeutic approach in AF.

Table 1. Novel therapeutic targets

Drug	Target	Phase	Indication	Ref (clinical trials. gov identifier)
GGA	HSP induction	Phase IV	Gastric ulcers Gastritis Gastric lesion	NCT01190657 NCT01547559 NCT01284647 NCT01397448
NYK9354	HSP induction	Pre-clinical	Atrial fibrillation	(Hoogstra-Berends et al., 2012)
Leupeptin ALLN MDL-28170 A-705239 A-705253	Calpain induction	Pre-clinical		reviewed in Cardiovascular Research (2012) 96, 23–31
Tubastatin	HDAC6	Pre-clinical	Arthritis Anti-inflammatory	(Vishwakarma et al., 2013)
ACY-1215	HDAC6	Phase I/II	Myeloma	NCT01323751 NCT01583283
Fasudil	ROCK	Phase III Phase II Phase II	Raynaud's phenomenon Vascular function study Atherosclerosis	NCT00498615 NCT00120718 NCT00670202
Ezetimibe		Phase IV	Glaucoma	NCT00560170
AR-12286		Phase II		NCT01936389
CCG-1423 Rhosin	Rho	Pre-clinical		(Evelyn, et al., 2007) (Shang, et al., 2012)

Promoting maintenance of proteostasis by revitalization of the PQC system may prevent the derailment of proteostasis and structural and functional remodeling in AF. Interestingly, the heat shock response as part of the PQC system can be pharmacologically boosted, and consequently cardiac remodeling may be prevented, halted or even be restored. Indeed, as depicted earlier, increasing HSP expression, by either pharmacologic compounds or molecular biological means, displays cardio-protective effects in various models for AF and in patients. HSP induction provided protection against loss of actin proteostasis by reducing RhoA-GTPase-induced remodeling [38] and against activation of calpain [38, 43, 44, 46, 52, 53]. Furthermore, in canine models for AF progression, treatment with geranylgeranylacetone (GGA) induced HSP expression and prevented AF initiation and progression by inhibition of the prolongation of the effective refractory period (ERP), shortening of APD and

reductions in L-type Ca^{2+} current and it revealed protective effects against atrial conduction abnormalities [44, 97].

Whether HSP induction also protects via HDAC inhibition is currently unknown. Of all HSP inducing compounds, GGA represents the most efficacious compound for the pharmacological induction of HSPs. GGA has already been applied clinically in Japan since 1984 as an antiulcer drug with no reported serious adverse reactions [98-102]. Due to the high LogP value for GGA, high dosages might be needed, therefore, GGA derivatives are developed with improve pharmaco-chemical properties [103] (Table 1). Induction of HSP is suggested to be the most promising therapeutic approach with pleiotropic protective effects.

HSPs as biomarkers

Following stress, HSPs get expressed intracellular, but can also be presented on the cell surface or released to the surroundings [104]. HSPs in serum may act as a biomarker to reveal the stage of AF. Elevated serum HSPD1 levels were found in patients with acute myocardial infarction and seemed to be predictive for post-AMI adverse events [105]. Elevated serum HSPA1A and HSPD1 have found to correlate to the severity of metabolic syndrome-associated factors in post-menopausal women [106]. HSPA1A and HSPD1 were found to positively associate with severity of cardiovascular disease [107-112]. Patients with coronary artery disease (CAD) have revealed antibodies to HSPB1 in serum [113], but a correlation between antibody titers to HSPB1 and the extent of CAD could not be found. Several studies have reported increased serum levels for HSPB1 several hours after myocardial infarction [114, 115]. In another study, anti-HSPB1 levels were found to be higher in patients with more advanced cardiac artery disease, making the authors to conclude that serum anti-HSPB1 titers may be associated with the presence and severity of cardiac artery disease [116]. Anti-HSPB1 titers measured in patients

with stroke were found significantly elevated [117]. These findings suggest that the measurement of HSP levels in serum, may be useful as biomarkers of disease initiation and progression.

Conclusion

AF naturally tends to progress from trigger dependent paroxysmal AF to a more substrate mediated (longstanding) persistent or permanent AF. Trigger focused treatments (endovascular or surgical PVI) might be successful in patients with paroxysmal AF, however this approach will not be sufficient for patients suffering from more advanced types of AF, who require substrate modification. Even treatments aimed at substrate modification, such as CFAE ablation, Cox maze III and ganglion ablation, are associated with AF recurrences. This implies insufficient understanding of the electrophysiological and structural changes which form a substrate underlying AF. Hence, as long as the electropathological substrate remains poorly understood, and the stage of electropathology cannot be evaluated, it is challenging to define the optimal approach per individual patient. Therefore, research is focused on the dissection of molecular mechanisms underlying electropathology. New findings indicate a role for derailment of cardiomyocyte proteostasis in AF progression and identified novel innovative targets for drug therapy. These targets are directed at the attenuation of electropathology and prevention of clinical AF progression. Since various drugs are already in clinical phase II/III for other indications, it seems worthwhile to test some in clinical AF.

Acknowledgements This work was supported by the LSH-Impulse grant (40-43100-98-008) and the Dutch Heart Foundation (2013T144, 2013T096 and 2011T046).

References

1. Einthoven W. Le télécardiogramme. Arch Int Physiol 1906;4:132-164.
2. Wilke T, et al. Incidence and prevalence of atrial fibrillation: an analysis based on 8.3 million patients. Europace 2013;15(4):486-493.
3. Krijthe BP, et al. Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. Eur Heart J 2013;34(35):2746-2751.
4. Aizer A, et al. Relation of Vigorous Exercise to Risk of Atrial Fibrillation. Am J Cardiol 2009;103(11):1572-1577.
5. Kirsh JA,et al. Prevalence of and risk factors for atrial fibrillation and intra-atrial reentrant tachycardia among patients with congenital heart disease. Am J Cardiol 2002; 90(3):338-40.
6. Patel NJ, et al. Contemporary Trends of Hospitalization for Atrial Fibrillation in the United States, 2000 Through 2010 Implications for Healthcare Planning. Circulation 2014;129(23):2371-2379.
7. Haissaguerre M, et al. Driver Domains in Persistent Atrial Fibrillation. Circulation 2014;130(7):530-538.
8. Konrad T, et al. Body surface potential mapping for mapping and treatment of persistent atrial fibrillation. Herzschr Elektrophys 2014;25:226-229.
9. Guillem MS, et al. Noninvasive Localization of Maximal Frequency Sites of Atrial Fibrillation by Body Surface Potential Mapping. Circ-Arrhythmia Elec 2013;6(2):294-301.
10. Rodrigo M, et al. Body surface localization of left and right atrial high-frequency rotors in atrial fibrillation patients: A clinical-computational study. Heart Rhythm 2014;11(9):1584-1591.
11. Moe GK. Atrial fibrillation as a self-sustaining arrhythmia independent of focal discharge. Am Heart J 1959;58(1):59-70.
12. Allessie MA. Experimental evaluation of Moe's multiple wavelet hypothesis of atrial fibrillation. Cardiac Electrophysiology and Arrhythmias 1985;265-276.
13. Allessie MA, et al. Circus Movement in Rabbit Atrial Muscle as a Mechanism of Tachycardia .3. Leading Circle Concept - New Model of Circus Movement in Cardiac Tissue without Involvement of an Anatomical Obstacle. Circ Res 1977;41(1):9-18.
14. Allessie MA, et al. The Wavelength of the Atrial Impulse and Re-Entrant Arrhythmias in the Conscious Dog. J Physiol-London 1985;366(Sep):P37-P37.
15. Konings KTS, et al. High-Density Mapping of Electrically-Induced Atrial-Fibrillation in Humans. Circulation 1994;89(4):1665-1680.
16. Mandapati R, et al. Stable microreentrant sources as a mechanism of atrial fibrillation in the isolated sheep heart. Circulation 2000;101(2):194-199.
17. Jalife J, et al. Mother rotors and fibrillatory conduction: a mechanism of atrial fibrillation. Cardiovasc Res 2002;54(2):204-216.
18. Waldo AL. Mechanisms of atrial fibrillation. J Cardiovasc Electr 2003;14(12):S267-S274.
19. Moe GK. A computer model of atrial fibrillation. Am Heart J 1964; 67(2):200-220.
20. Narayan SM, et al. Treatment of Atrial Fibrillation by the Ablation of Localized Sources CONFIRM (Conventional Ablation for Atrial Fibrillation With or Without Focal Impulse and Rotor Modulation) Trial. J Am Coll Cardiol 2012;60(7):628-636.
21. Lewis T. The nature of flutter and fibrillation of the auricle. Brit Med J 1921;1.
22. de Groot NMS, et al. Electropathological Substrate of Longstanding Persistent Atrial Fibrillation in Patients With Structural Heart Disease Epicardial Breakthrough. Circulation 2010;122(17):1674-1682.
23. Lee G, et al. Epicardial wave mapping in human long-lasting persistent atrial fibrillation: transient rotational circuits, complex wavefronts, and disorganized activity. Eur Heart J 2014;35(2):86-97.
24. Allessie MA, et al. Electropathological Substrate of Long-Standing Persistent Atrial Fibrillation in Patients With Structural Heart Disease. Circ-Arrhythmia Elec 2010;3(6):606-615.

25. Wijffels MCEF, et al. Atrial-Fibrillation Begets Atrial-Fibrillation - a Study in Awake Chronically Instrumented Goats. Circulation 1995;92(7):1954-1968.
26. Goette A, et al. Electrical remodeling in atrial fibrillation - Time course and mechanisms. Circulation 1996;94(11):2968-2974.
27. Ausma J, et al. Changes in ultrastructural calcium distribution in goat atria during atrial fibrillation. J Mol Cell Cardiol 2000;32(3):355-364.
28. Brundel BJJM, et al. Ion channel remodeling is related to intraoperative atrial effective refractory periods in patients with paroxysmal and persistent atrial fibrillation. Circulation 2001;103(5):684-690.
29. Qi XY, et al. Cellular signaling underlying atrial tachycardia remodeling of L-type calcium current. Circ Res 2008;103(8):845-U151.
30. Schotten U, et al. Electrical and contractile remodeling during the first days of atrial fibrillation go hand in hand. Circulation 2003;107(10):1433-1439.
31. Brundel BJJM, et al. Gene expression of proteins influencing the calcium homeostasis in patients with persistent and paroxysmal atrial fibrillation. Cardiovasc Res 1999;42(2):443-454.
32. Wang ZG. Role of redox state in modulation of ion channel function by fatty acids and phospholipids. Brit J Pharmacol 2003;139(4):681-683.
33. Dobrev D and Voigt N. Ion channel remodelling in atrial fibrillation. European Cardiology 2011;7(2):97-103.
34. Anderson ME. Calmodulin kinase and L-type calcium channels: A recipe for arrhythmias? Trends Cardiovas Med 2004;14(4):152-161.
35. Christ T, et al. L-type Ca2+ current downregulation in chronic human atrial fibrillation is associated with increased activity of protein phosphatases. Circulation 2004;110(17):2651-2657.
36. Greiser M, et al. Pharmacological evidence for altered src kinase regulation of I-Ca,I-L in patients with chronic atrial fibrillation. N-S Arch Pharmacol 2007;375(6):383-392.
37. Dobrev D and Nattel S. Calcium Handling Abnormalities in Atrial Fibrillation as a Target for Innovative Therapeutics. J Cardiovasc Pharm 2008;52(4):293-299.
38. Ke L, et al. HSPB1, HSPB6, HSPB7 and HSPB8 Protect against RhoA GTPase-Induced Remodeling in Tachypaced Atrial Myocytes. Plos One 2011;6(6).
39. Brundel BJJM, et al. Activation of proteolysis by calpains and structural changes in human paroxysmal and persistent atrial fibrillation. Cardiovasc Res 2002;54(2):380-389.
40. Sherman AJ, et al. Myofibrillar disruption in hypocontractile myocardium showing perfusion-contraction matches and mismatches. Am J Physiol-Heart C 2000;278(4):H1320-H1334.
41. Bito V, et al. Cellular mechanisms of contractile dysfunction in hibernating myocardium. Circ Res 2004;94(6):794-801.
42. Todd DM, et al. Repetitive 4-week periods of atrial electrical remodeling promote stability of atrial fibrillation - Time course of a second factor involved in the self-perpetuation of atrial fibrillation. Circulation 2004;109(11):1434-1439.
43. Zhang DL, et al. Activation of Histone Deacetylase-6 Induces Contractile Dysfunction Through Derailment of alpha-Tubulin Proteostasis in Experimental and Human Atrial Fibrillation. Circulation 2014;129(3):346-358.
44. Brundel BJJM, et al. Heat shock protein upregulation protects against pacing-induced myolysis in HL-1 atrial myocytes and in human atrial fibrillation. J Mol Cell Cardiol 2006;41(3):555-562.
45. Meijering RAM, et al. Loss of proteostatic control as a substrate for Atrial Fibrillation; a novel target for upstream therapy by Heat Shock Proteins. Frontiers in Cardiac Electrophysiology 2012;3(36).
46. Brundel BJJM, et al. Induction of heat shock response protects the heart against atrial fibrillation. Circ Res 2006;99(12):1394-1402.
47. Balch WE, et al. Adapting proteostasis for disease intervention. Science 2008;319(5865):916-919.

48. Powers ET, Balch WE. Diversity in the origins of proteostasis networks - a driver for protein function in evolution. Nat Rev Mol Cell Bio 2013;14(4):237-248.
49. Wang XJ, Robbins J. Heart failure and protein quality control. Circ Res 2006;99(12):1315-1328.
50. Galli A. Proteotoxicity and cardiac dysfunction. New Engl J Med 2013;368(18):1754-1755.
51. Meijering RAM, Henning RH, Brundel B. Reviving the protein quality control system: Therapeutic target for cardiac disease in the elderly. Trends Cardiovas Med 2014;14:1050-1738.
52. Zhang DL, et al. Effects of different small HSPB members on contractile dysfunction and structural changes in a Drosophila melanogaster model for Atrial Fibrillation. J Mol Cell Cardiol 2011;51(3):381-389.
53. Ke L, et al. Calpain mediates cardiac troponin degradation and contractile dysfunction in atrial fibrillation. J Mol Cell Cardiol 2008;45(5):685-693.
54. Brown JH, et al. The Rac and Rho hall of fame - A decade of hypertrophic signaling hits. Circ Res 2006;98(6):730-742.
55. Sah VP, et al. Cardiac-specific overexpression of RhoA results in sinus and atrioventricular nodal dysfunction and contractile failure. Journal of Clinical Investigation 1999;103(12):1627-1634.
56. Adam O, et al. Role of Rac1 GTPase activation in atrial fibrillation. J Am Coll Cardiol 2007;50(4):359-367.
57. Reil JC, et al. Cardiac Rac1 overexpression in mice creates a substrate for atrial arrhythmias characterized by structural remodelling. Cardiovasc Res 2010;87(3):485-493.
58. Ogata T, et al. MURC, a muscle-restricted coiled-coil protein that modulates the Rho/ROCK pathway, induces cardiac dysfunction and conduction disturbance. Mol Cell Biol 2008;28(10):3424-3436.
59. Hubbert C, et al. HDAC6 is a microtubule-associated deacetylase. Nature 2002;417(6887):455-458.
60. Haggarty SJ, et al. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. P Natl Acad Sci USA 2003;100(8):4389-4394.
61. Matsuyama A, et al. In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. Embo J 2002;21(24):6820-6831.
62. Powers ET, et al. Biological and Chemical Approaches to Diseases of Proteostasis Deficiency. Annu Rev Biochem 2009;78:959-991.
63. St Rammos K, et al. Low preoperative HSP70 atrial myocardial levels correlate significantly with high incidence of postoperative atrial fibrillation after cardiac surgery. Cardiovasc Surg 2002;10(3):228-232.
64. Mandal K, et al. Association of high intracellular, but not serum, heat shock protein 70 with postoperative atrial fibrillation. Ann Thorac Surg 2005;79(3):865-871.
65. Cao HL, et al. Heat shock proteins in stabilization of spontaneously restored sinus rhythm in permanent atrial fibrillation patients after mitral valve surgery. Cell Stress Chaperon 2011;16(5):517-528.
66. Yang M, et al. Expression of heat shock proteins in myocardium of patients with atrial fibrillation. Cell Stress Chaperon 2007;12(2):142-150.
67. Willis MS, Patterson C. Proteotoxicity and Cardiac Dysfunction Reply. New Engl J Med 2013;368(18):1755-1755.
68. Liu J, et al. Further study on the role of HSP70 on Ca2+ homeostasis in rat ventricular myocytes subjected to simulated ischemia. Am J Physiol-Cell Ph 2006;290(2):C583-C591.
69. Kalmar B, Greensmith L. Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliver Rev 2009;61(4):310-318.
70. Sugiyama Y, et al. Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. J Biol Chem 2000;275(2):1095-1104.

71. Mounier N, Arrigo AP. Actin cytoskeleton and small heat shock proteins: how do they interact? Cell Stress Chaperon 2002;7(2):167-176.
72. Golenhofen N, et al. Comparison of the small heat shock proteins alpha B-crystallin, MKBP, HSP25, HSP20, and cvHSP in heart and skeletal muscle. Histochem Cell Biol 2004;122(5):415-425.
73. Salinthone S, et al. Small heat shock proteins in smooth muscle. Pharmacol Therapeut 2008;119(1):44-54.
74. Camm AJ, et al. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation. Eur Heart J 2012;33(21):2719-2747.
75. de Paola AAV, et al. Effectiveness and costs of chemical versus electrical cardioversion of atrial fibrillation. Int J Cardiol 2003;88(2-3):157-166.
76. Pisters R, et al. Clinical correlates of immediate success and outcome at 1-year follow-up of real-world cardioversion of atrial fibrillation: the Euro Heart Survey. Europace 2012;14(5):666-674.
77. Löwn B. New method for terminating cardiac arrhythmias. Use of synchronized capacitor discharge. JAMA 1962;182(5):548-555.
78. Camm AJ, et al. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation An update of the 2010 ESC Guidelines for the management of atrial fibrillation Developed with the special contribution of the European Heart Rhythm Association. Europace 2012;14(10):1385-1413.
79. Di Blase L, et al. Remote magnetic navigation - Human experience in pulmonary vein ablation. J Am Coll Cardiol 2007;50(9):868-874.
80. Luthje L, et al. Remote magnetic versus manual catheter navigation for circumferential pulmonary vein ablation in patients with atrial fibrillation. Clin Res Cardiol 2011;100(11):1003-1011.
81. Saliba W, et al. Atrial fibrillation ablation using a robotic catheter remote control system - Initial human experience and long-term follow-up results. J Am Coll Cardiol 2008;51(25):2407-2411.
82. Bordignon S, et al. Comparison of Balloon Catheter Ablation Technologies for Pulmonary Vein Isolation: The Laser Versus Cryo Study. J Cardiovasc Electr 2013;24(9):987-994.
83. Ganesan AN, et al. Long-term Outcomes of Catheter Ablation of Atrial Fibrillation: A Systematic Review and Meta-analysis. J Am Heart Assoc 2013;2(2).
84. Bhat T, et al. Major complications of cryoballoon catheter ablation for atrial fibrillation and their management. Expert Rev Anti-Infe 2014;12(9):1111-1118.
85. Yaksh A, et al. Atrial fibrillation: to map or not to map? Netherlands Heart Journal 2014;22(6):259-266.
86. Nademanee K, et al. A new approach for catheter ablation of atrial fibrillation: Mapping of the electrophysiologic substrate. J Am Coll Cardiol 2004;43(11):2044-2053.
87. Wu SH, et al. Benefits and risks of additional ablation of complex fractionated atrial electrograms for patients with atrial fibrillation: A systematic review and meta-analysis. Int J Cardiol 2013;169(1):35-43.
88. Verma A, et al. Selective Complex Fractionated Electrogram Targeting For Atrial Fibrillation Study (Select AF): A Multicenter, Randomized Trial. Circulation 2012;126(21).
89. Mikhaylov E, et al. Outcome of anatomic ganglionated plexi ablation to treat paroxysmal atrial fibrillation: a 3-year follow-up study. Europace 2011;13(3):362-370.
90. Pokushalov E, et al. Ganglionated plexi ablation for longstanding persistent atrial fibrillation. Europace 2010;12(3):342-346.
91. Evelyn CR, et al. CCG-1423: a small-molecule inhibitor of RhoA transcriptional signaling. Mol Cancer Ther 2007;6(8):2249-2260.
92. Shang X, et al. Rational Design of Small Molecule Inhibitors Targeting RhoA Subfamily Rho GTPases. Chem Biol 2012;19(6):699-710.
93. Inserte J, Hernando V, Garcia-Dorado D. Contribution of calpains to myocardial ischaemia/reperfusion injury. Cardiovasc Res 2012;96(1):23-31.

94. Butler KV, et al. Rational Design and Simple Chemistry Yield a Superior, Neuroprotective HDAC6 Inhibitor, Tubastatin A. J Am Chem Soc 2010;132(31):10842-10846.
95. d'Ydewalle C, et al. HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1-induced Charcot-Marie-Tooth disease. Nat Med 2011;17(8):968-U986.
96. Santo L, et al. Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma. Blood 2012;119(11):2579-2589.
97. Sakabe M, et al. Effects of a heat shock protein inducer on the atrial fibrillation substrate caused by acute atrial ischaemia. Cardiovasc Res 2008;78(1):63-70.
98. Murakami M, et al. Anti-Ulcer Effect of Geranylgeranylacetone, a New Acyclic Polyisoprenoid on Experimentally Induced Gastric and Duodenal-Ulcers in Rats. Arzneimittel-Forsch 1981;31-1(5):799-804.
99. Unoshima M, et al. Enhancement of MxA expression and phosphorylation of PKR during influenza virus infection. Antimicrob Agents Ch 2003;47(9):2914-2921.
100. Katsuno M, et al. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. P Natl Acad Sci USA 2005;102(46):16801-16806.
101. Yanaka A, et al. Geranylgeranylacetone protects the human gastric mucosa from diclofenac-induced injury via induction of heat shock protein 70. Digestion 2007;75(2-3):148-155.
102. Fujimura N, et al. Geranylgeranylacetone, Heat Shock Protein 90/AMP-Activated Protein Kinase/Endothelial Nitric Oxide Synthase/Nitric Oxide Pathway, and Endothelial Function in Humans. Arterioscl Throm Vas 2012;32(1):153-U350.
103. Hoogstra-Berends F, et al. Heat Shock Protein-Inducing Compounds as Therapeutics to Restore Proteostasis in Atrial Fibrillation. Trends Cardiovas Med 2012;22(3):62-68.
104. van Oosten-Hawle P, Morimoto RI. Organismal proteostasis: role of cell-nonautonomous regulation and transcellular chaperone signaling. Gene Dev 2014;28(14):1533-1543.
105. Novo G, et al. Hsp60 and heme oxygenase-1 (Hsp32) in acute myocardial infarction. Transl Res 2011;157(5):285-292.
106. Giannessi D, et al. Circulating heat shock proteins and inflammatory markers in patients with idiopathic left ventricular dysfunction: their relationships with myocardial and microvascular impairment. Cell Stress Chaperon 2007;12(3):265-274.
107. Zhu JH, et al. Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease - Evidence for an autoimmune component of atherogenesis. Circulation 2001;103(8):1071-1075.
108. Metzler B, et al. Epitope specificity of anti-heat shock protein 65/60 serum antibodies in atherosclerosis. Arterioscl Throm Vas 1997;17(3):536-541.
109. Burian K, et al. Independent and joint effects of antibodies to human heat-shock protein 60 and Chlamydia pneumoniae infection in the development of coronary atherosclerosis. Circulation 2001;103(11):1503-1508.
110. Xu Q, et al. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis: clinical significance determined in a follow-up study. Circulation 1999;100(11):1169-1174.
111. Hoppichler F, et al. Changes of serum antibodies to heat-shock protein 65 in coronary heart disease and acute myocardial infarction. Atherosclerosis 1996;126(2):333-338.
112. Birnie D, et al. Anti-heat shock protein 65 titres in acute myocardial infarction. Lancet 1994;344(8934):1443.
113. Lavoie JN, et al. Modulation of cellular thermoresistance and actin filament stability accompanies phosphorylation-induced changes in the oligomeric structure of heat shock protein 27. Mol Cell Biol 1995;15(1):505-516.
114. Vander Heide RS. Increased expression of HSP27 protects canine myocytes from simulated ischemia-reperfusion injury. Am J Physiol-Heart C 2002;282(3):H935-H941.
115. Knowlton AA. The Role of Heat-Shock Proteins in the Heart. J Mol Cell Cardiol 1995;27(1):121-131.

116. Pourghadamyari H, et al. Serum antibody titers against heat shock protein 27 are associated with the severity of coronary artery disease. Cell Stress Chaperon 2011;16(3):309-316.
117. Azarpazhooh MR, et al. Serum high-sensitivity C-reactive protein and heat shock protein 27 antibody titers in patients with stroke and 6-month prognosis. Angiology 2010;61(6):607-612.