Implications of the cardiomyocyte stress response on protein homeostasis in atrial fibrillation
Wiersma, Marit

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Chapter 1

Diagnosis and therapy of Atrial Fibrillation: the past the present and the future.

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ABSTRACT
Atrial fibrillation (AF) is the most common age-related cardiac arrhythmia. It is a progressive disease, which makes treatment difficult. 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, guidance 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 aging population. However, an increasing prevalence is seen in young adults, especially in endurance athletes4 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 studies7-10 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 Moe11 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\textsuperscript{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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{20}

Electropathology associated with persistence of atrial fibrillation

High-resolution wavemapping studies\textsuperscript{22} of AF in patients with valvular heart disease and longlasting 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). The coupling interval was longer than the dominant AF cycle length, and unipolar electrograms at the epicardial origin of these waves exhibited R-waves.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.

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 1.25 X 1.25cm 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.

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.
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. 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\textsuperscript{2+}-current channels, which causes the shortening of action potential duration (APD) and electrical remodeling, thereby providing a further substrate for AF.\textsuperscript{26-30} Also, several other ion channel currents are affected, either on the expression level or phosphorylation and redox status.\textsuperscript{31-33} In addition, various kinases and phosphatases become activated and regulate the function of ion channels and other downstream target proteins, for example transcription factors, various calcium handling proteins (such as RyR2, Sarcoplasmic Reticulum Ca\textsuperscript{2+}-ATPase (SERCA) or Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger) and the actin cytoskeleton.\textsuperscript{34-38}

When AF persists beyond a few days, irreversible structural remodeling occurs, especially hibernation\textsuperscript{39} (Figure 2). Various research groups\textsuperscript{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\textsuperscript{40} a ‘second factor’ underlies the persistence of AF, having a time course comparable to AF-induced structural changes (hibernation/myolysis) in the atrial cardiomyocytes.\textsuperscript{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.\textsuperscript{38,39,43-46}

**Derailed proteostasis: novel concept of cardiomyocyte remodeling**

Proteostasis is defined as the homeostasis in protein synthesis, folding, assembly, trafficking, guidance by chaperones and clearance by protein degradation systems.\textsuperscript{47-50} Healthy proteostasis is controlled by an exquisitely regulated network of molecular components and cellular pathways, the protein quality control (PQC) system.\textsuperscript{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.\textsuperscript{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, HSP27, cvHSP, HSP20 and HSP22 are important members of the PQC system and attenuate derailment of proteostasis in AF by assisting in the refolding of unfolded proteins,\textsuperscript{38,51} prevention of AF-induced damage to contractile proteins\textsuperscript{44,52} and attenuation of protein breakdown.\textsuperscript{43} In this way, HSPs normalize the proteostasis and protect the cardiomyocyte against remodeling and AF progression.

![AF-induced cardiomyocyte remodeling diagram](image)

**Figure 2 Overview of AF-induced cardiomyocyte remodeling.** AF induces time-related progressive remodeling. First, AF causes a stressful cellular Ca\textsuperscript{2+} overload, which results in a direct inhibition of the L-type Ca\textsuperscript{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\textsuperscript{2+} overload but at the expense of creating a substrate for persistent AF. When AF persists, derailment of proteostasis occurs, which results 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 cardioprotective HSPs are of therapeutic interest for future treatment of clinical AF.
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 the 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, but also in human permanent AF. 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), cardiomyocyte dysfunction and AF progression. 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. 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 and trigger the initiation of AF. Recent studies revealed that in AF, RhoA-GTPase becomes 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. Furthermore, it was recently found that histone deacetylases (HDACs), such as HDAC6, are implicated in AF-induced cardiomyocyte remodeling. HDACs affect cardiomyocyte proteostasis by epigenetically regulating protein expression and modulating various cytoplasmic proteins, including α-tubulin, a structural protein from the microtubule network. 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. 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. HSPs are generally under the control of heat shock transcription factor 1 (HSF1) and represent important chaperones in proteostatic control. During excessive stress situations, such as AF, HSP levels were found to become exhausted. This finding suggests that upregulation of HSP levels might normalize proteostasis and improve cardiomyocyte function in AF. In clinical studies, induced HSP levels are associated with protection against AF initiation and progression. HSP70 atrial expression levels were found to correlate with reduced incidence of post-operative AF in patients in sinus rhythm undergoing cardiac surgery. In another clinical study, a potent Heat Shock Response (HSR) and high HSP27 levels have been associated with restoration of normal sinus rhythm in patients with permanent AF after mitral valve surgery. Higher atrial HSP27 levels were found to be related to shorter AF duration and less myolysis when comparing paroxysmal versus persistent AF and sinus rhythm. These findings suggest that HSPs become activated after AF episodes and exhaust in time in a stress-related manner. 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.

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 the \(\text{Na}^+/\text{Ca}^{2+}\)-exchanger, suggesting that HSPs attenuate AF progression by protecting against (tachypacing-induced) changes in calcium-handling proteins. Several HSPs (including HSP27) were shown to reduce oxidative stress, thereby potentially preventing or restoring the redox status of the ion channels and preventing damage to the actin...
cytoskeleton. This protective effect of HSP27 was found via direct binding to actin filaments and indirectly by preserving the redox status.\textsuperscript{43,44,70-73} Reducing oxidative stress preserves proteostasis and electrophysiological and contractile function of the cardiomyocyte in AF. Moreover, HSPs prevent calpain activation\textsuperscript{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.\textsuperscript{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%\textsuperscript{75,76} for patients with paroxysmal or persistent AF and is highest when performed in AF <48 hours.\textsuperscript{76} Immediate (prior to discharge) AF recurrences were observed in 3% and AF relapsed in 30-40% of patients within one year with continuation of anti-arrhythmic drugs.\textsuperscript{76} When CCV is unsuccessful, ECV is next treatment in line. Immediate restoration of sinus rhythm is achieved in 88-97%.\textsuperscript{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
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by fluoroscopy or electro-anatomical mapping systems, or robotically using remote (non-) magnetic navigation systems.\textsuperscript{79-81} Despite the promising acute success rates, one year AF free survival is approximately 40-50% and redo ablations are frequently performed.\textsuperscript{79-82} This data is confirmed in a large meta-analysis by Ganesan et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{85} In addition, an arrhythmogenic substrate may also develop over time as a result of a progressive cardiomyopathy.

\begin{figure}[h]
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\caption{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.}
\end{figure}

Different ablation approaches targeting the assumed substrate of AF have therefore been developed in the past years,\textsuperscript{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). Wu et al. concluded in a meta-analysis that CFAE ablation could reduce the recurrence of atrial tachycardia in patients with nonparoxysmal 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 and 74% in CFAE ablation additional to PVI. 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. When performed additionally to (repeat) PVI in patients with persistent AF, 16 months success rate rises to 59%. 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 provides the explanation why, in case the endo- and epicardial layers are electrically dissociated, ablative therapy is not successful anymore.

**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.
**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.

![Figure 5 Atrial epicardial mapping](image)

**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 and 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.
(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).\textsuperscript{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.

**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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{93}
HDAC6 inhibition, by tubacin, conserves α-tubulin proteostasis and prevents its degradation by calpain, thereby protecting 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, other promising HDAC6 inhibitors, such as tubastatin A and ACY-1215, have been recently developed (Table 1). Interestingly, tubastatin A showed to protect against tachypacing-induced cardiac remodeling in a canine model for AF, supporting the use of HDAC6 inhibitors as a novel therapeutic approach in AF.

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 cardioprotective effects in various models for AF and in patients. HSP induction provided protection against loss of actin proteostasis by reducing RhoA-GTPase-induced remodeling and against activation of calpain.

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 Ca²⁺-current and it revealed protective effects against atrial conduction abnormalities.

Whether HPS induction also protects via HDAC inhibition is currently unknown. Of all HSP inducing compounds, GGA represents the most promising compound for the pharmacological induction of HSPs in AF. GGA has already been applied clinically in Japan since 1984 as an anti-ulcer drug with no reported serious adverse reactions. Due to high LogP value for GGA, high dosages might be needed, therefore, GGA derivatives are developed with improved pharmacochemical properties (Table 1).

Induction of HSPs 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. HSPs in serum may act as a biomarker to reveal the stage of AF. Elevated serum HSP60 levels were found in patients with acute myocardial infarction (AMI) and seemed to be predictive for post-AMI adverse
Table 1 Novel therapeutic targets

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<td>ROCK</td>
<td>III</td>
<td>Raynaud’s Phenomenon</td>
<td>NCT00498615, NCT00120718, NCT00670202</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td></td>
<td>II</td>
<td>Vascular function study</td>
<td>NCT00560170, NCT01936389</td>
</tr>
<tr>
<td>AR-12286</td>
<td></td>
<td>II</td>
<td>Atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>CCG-1423</td>
<td>Rho</td>
<td>Pre-clinical</td>
<td></td>
<td>(Evelyn et al., 2007) (Shang et al., 2012)</td>
</tr>
<tr>
<td>Rhosin</td>
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Elevated serum HSP70 and HSP60 were found to correlate to the severity of metabolic syndrome-associated factors in postmenopausal women. HSP60 and HSP70 were found to positively associate with severity of cardiovascular disease. Patients with coronary artery disease (CAD) have antibodies to HSP27 in serum, but a correlation between antibody titers to HSP27 and the extent of CAD could not be found. Several studies have reported increased serum levels for HSP27 several hours after myocardial infarction. In another study, anti-HSP27 levels were found to be higher in patients with more advanced cardiac artery disease, making the authors to conclude that serum anti-HSP27 titers may be associated with the presence and severity of cardiac artery disease. Anti-HSP27 titers measured in patients with stroke were found significantly elevated. 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 electrophathological substrate remains poorly understood,
and the stage of electrophathology 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 electrophathology. 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
electrophathology and prevention of clinical AF progression. Since various drugs are
already on in clinical phase II/III for other indications, it seems worthwhile to test some
in clinical AF.

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References


68. Liu J, Kam KW, Borchert GH, Kravtsov GM, Ballard HJ, Wong TM. Further study on the role


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