Published in final edited form as:

Trends Cardiovasc Med. 2017 January ; 27(1): 1–11. doi:10.1016/j.tcm.2016.05.008.

Atrial fibrillation driver mechanisms: insight from the isolated human heart

Thomas A. Csepe¹, Brian J. Hansen¹, and Vadim V. Fedorov¹

Department of Physiology & Cell Biology, Davis Heart & Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, USA

Abstract

Although there have been great technological advances in the treatment of atrial fibrillation (AF), current therapies remain limited due to a narrow understanding of AF mechanisms in the human heart. This review will highlight our recent studies on explanted human hearts where we developed and employed a novel functional-structural mapping approach by integrating high-resolution simultaneous endo-epicardial and panoramic optical mapping with 3D gadolinium-enhanced MRI to define the spatiotemporal characteristics of AF drivers and their structural substrates. The results allow us to postulate that the primary mechanism of AF maintenance in human hearts is a limited number of localized intramural microanatomic reentrant AF drivers anchored to heart-specific 3D fibrotically-insulated myobundle tracks, which may remain hidden to clinical single surface electrode mapping. We suggest that *ex vivo* human heart studies, by using an integrated 3D functional and structural mapping approach, will help to reveal defining features of AF drivers as well as validate and improve clinical approaches to detect and target these AF drivers in patients with cardiac diseases.

Keywords

atrial fibrillation; reentrant driver; intramural microanatomic reentry; fibrosis; optical mapping; catheter ablation

Introduction

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia and a significant cause of hospitalization and morbidity, affecting more than 2.7 million Americans today and projected to increase to 16 million by 2050(1). Though there have been significant technological advances in this field, both pharmacological and ablation treatment options for AF remain limited. Several new ablation approaches focused on patient-specific AF mechanisms have been developed with varying outcomes(2). One of the major limitations of

Address for correspondence: Vadim V. Fedorov, PhD, 306 Hamilton Hall, 1645 Neil Avenue, Columbus OH 43210-1218, tel: 1-614-292-5154 fax: 1-614-292-4888 vadim.fedorov@osumc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

AF ablation is a limited understanding of the mechanisms underlying the disease, as recently reviewed by Wright and Narayan in this journal(2). Proposed mechanisms that sustain or drive AF include multiple replicating wavelets, focal activity, unstable drifting rotors, and localized stable reentry(3;4). Importantly, these conflicting hypotheses may stem from the atrial surface mapped, with endocardial (FIRM) mapping detecting mainly reentrant/rotor AF drivers(5) and multiple wavelets and breakthrough observed by epicardial mapping(6).

Recent results from our ex vivo human experiments(7–9) lead us to postulate that the primary mechanism of AF maintenance in human hearts is a limited number of localized intramural reentrant AF drivers anchored to heart-specific 3D fibrotically-insulated microanatomic tracks, which may remain hidden to clinical single-surface electrode mapping. Single-sided clinical mapping studies may lack the resolution necessary to resolve specific electro-anatomical substrates driving the “chaotic” activity throughout the complex intramural 3D atrial structures during AF(10). Thus, the optimal strategy for AF treatment for each patient remains unclear(10).

Importantly, high-resolution optical mapping, which may provide deeper insight on heart-specific AF mechanisms, is not currently possible to utilize clinically but is possible *ex vivo* using coronary-perfused human hearts. This review provides insights on AF mechanisms gained from experimental mapping studies of human hearts *ex vivo*, focused on defining patient specific AF driver mechanisms and how translating these findings may improve clinical strategies to identify and target these AF drivers in patients.

Controversy of AF mechanisms

AF driver mechanisms in human hearts are widely debated(3;4). Despite over a century of research, the mechanisms of initiation and maintenance of AF remain elusive(1;11), and the same proposed mechanisms have been debated for over 100 years. Although the idea of local sustaining sources, such as reentrant drivers, rotors, and/or discrete focal discharges, has been hypothesized, a lack of definitive evidence has concealed a conclusive answer in the human heart(6;10).

Almost 100 years ago, Lewis et al.(12) proposed that AF could be driven by a single reentry circuit with fibrillatory conduction propagating from it, later supported by electrode(13) and optical(14) mapping in animal models of acetylcholine-induced AF. Recently, several clinical groups(5;15) have identified different types of localized reentrant drivers in AF patients (Figure 1) by using 64-channel endocardial basket (Focal Impulse Rotor Mapping – FIRM) catheters and non-invasive body surface epicardial mapping (ECVUE)(16) in both paroxysmal and persistent AF. Importantly, both approaches(5;16) revealed localized reentrant AF drivers present in a patient specific manner across both left and right atria. Furthermore, these studies demonstrated that targeted ablation could successfully treat AF, which supports a localized driving mechanism for AF maintenance, rather than multiple random wavelets that could not be targeted. However, other groups using epicardial local high-density electrode mapping (Allessie(6) and Kalman(17)) and biatrial epicardial mapping(18) did not reveal localized reentrant drivers but rather observed stable or unstable breakthroughs or focal discharges. Concurrently, biatrial epicardial mapping(19) identified a

single discrete region of high frequency and reentrant activity in the majority of persistent and paroxysmal AF patients.

The contradicting data may stem from the fact that clinical epicardial(4;18) or endocardial(5) electrode mapping can only visualize AF driver mechanisms on a single atrial surface but not through the 3D atrial wall that may obscure or distort specific AF mechanisms in the complex 3D anatomical structure(7). Moreover, not only does the choice of which surface to map affect AF driver interpretation, but different electrode densities, coverage, contact vs non-contact mapping, as well as dramatic differences in electrogram signal processing may provide different visualizations of intramural reentrant drivers. Indeed, recent endo-epicardial optical mapping studies of the explanted human right atria(7), which are capable of visualizing transmural conduction, suggest that contradicting clinical AF mechanisms may stem from intramural reentrant AF drivers in the complex human 3D atrial structure projecting differently on the endocardial and epicardial surfaces (Figure 1).

3D human atrial structure, fibrosis, and electrical conduction

Human atrial 3D myofiber anatomy governs the electrical activation and conduction patterns during both sinus rhythm and AF(8) but its role in AF is often underestimated(10;20–22). It is important to remind ourselves that myofiber orientation and natural anatomical bundle discontinuity, wall thickness variation, interstitial fibrosis, and fibrotic insulation of myobundles creates a complicated network of fine muscular ridges, which have a significant effect on the local conduction direction and speed, while also providing a local substrate for conduction blocks (e.g. longitudinal and transverse dissociation), especially during fast rhythm of AF(6;7). Detailed histological cadaver studies(23) and *ex vivo* 3D gadolinium-enhanced MRI (GE-MRI)(7) and micro-CT(8) of human atria have shown significant variations in atrial wall thickness, ranging from about 1 to 7mm. In addition to wall thickness, the natural complexity of the human atrial wall is significantly increased by heterogeneous patient-specific intramural fibrotic remodeling due to aging and cardiac diseases, including AF. The almost perpendicular myofiber orientations of the sup-epicardium and sub-endocardium(7;23), in combination with their separation by intramural fibrosis, leads to nearly two separate atrial layers that are connected by intramural bundles. The heart-specific uncoupling of atrial layers is exacerbated by AF progression and cardiac comorbidities and would lead to significant differences between local epicardial vs endocardial electrode recordings, especially during fast AF rhythm(6;20). Additionally, the substrate in which the mapping is performed may influence the relationship of activation between the endocardial and epicardial surfaces, as shown in a canine AF model by Everett et al(24). Thus, only simultaneous endo-epicardial electrophysiological mapping approaches directly integrated with high-resolution structural mapping can provide definitive answers on transmural conduction differences and resolve AF mechanisms(7;10;22).

Evolution of simultaneous endo-epicardial mapping: from contact electrode mapping to high-resolution optical mapping with near-infrared voltage-sensitive dye

Simultaneous endo-epicardial atrial mapping was first done by Zaitsev and others(25) in the canine model with 32×32 electrodes, showing that acetylcholine-induced spontaneous AF mechanisms are primarily reentry that was seen only from one side. Later, endo-epicardial 250×250 unipolar electrode(13;20) mapping also showed that sustained AF during high acetylcholine doses can be driven by a single localized reentrant or focal source in the canine right atria.

High-resolution optical mapping with voltage-sensitive dyes allow for resolution of conduction and repolarization at the cellular level and can accurately collect signals from a depth of the tissue (~0.5–1mm deep using conventional dye di-4-ANNEPS and ~1–4mm using near-infrared dye di-4-ANDBQBS)(7;26). Thus, near-infrared optical mapping(7) allows for more detailed spatial resolution of fibrillatory conduction compared to unipolar and bipolar contact electrode mapping that records extracellular surface activation with far-field influence from surrounding tissue(10;27).

The Jalife group's epicardial optical mapping with sub-millimeter resolution of acetylcholine-induced AF found the maintaining mechanism in the sheep model could be a single stable microreentrant driver(14). Simultaneous endo-epicardial optical mapping of the sheep left atria(28) observed drifting reentrant rotors seen endocardially and/or epicardially that form and meander around regions of sharp transitions in myocardial thickness. Further improvement was made by developing new near-infrared voltage-sensitive dye di-4-ANBDQBS and optical mapping with transillumination, which allowed the resolution of intramural conduction(29) and later in simultaneous endo-epicardial optical mapping of the canine atria(30).

All of these simultaneous endo-epicardial electrode and optical mapping animal studies suggest the true arrhythmia mechanism may remain hidden to mapping from only one side due to complex transmural conduction. Additionally, Verheule and others suggested that these differences between endocardial and epicardial activation should be greater in the aged, diseased, fibrotic human atria during persistent AF(22). However, while these studies demonstrate the utility of endo-epicardial mapping, no high-resolution simultaneous endo-epicardial optical mapping of AF in the human heart was performed until recently.

Transmural optical mapping of explanted human atria: Localized intramural reentrant AF drivers

Here at The Ohio State University, and at an increasing number of medical centers, researchers are gaining access to explanted human hearts directly from the operating room(7;8). To prevent ischemia during tissue collection, explanted hearts are cardioplegically-arrested and cooled to 4–7°C in the operating room following cross-clamping of the aorta and stored in cardioplegic solution at 4°C during transport, dissection

and cannulation. During mapping, both right and whole intact atria are coronary-perfused and superfused with 37°C oxygenated Tyrode's solution under constantly maintained pH (7.35) and pressure (55 mmHg) to preserve physiological conditions. Due to these precautions, we were able to maintain stable conduction and repolarization, as well as normal sinus rhythm in preparations with intact sinoatrial node, in the entire atrial human preparation for 6–8 hours(7;8;31;32) with all electrophysiological parameters in clinical range.

To unmask the critical role of transmural conduction in human AF mechanisms, our group recently studied diseased human right atria with both simultaneous high-resolution endo-epicardial and panoramic optical mapping (Figure 2). While two simultaneous and overlapped high-resolution (330 μ m²) endo/epicardial cameras captured transmural activation, a third panoramic camera (~1mm² resolution) covered the entire endocardium or epicardium and prevented omission of any arrhythmic events(7). Excitation light simultaneously illuminated both surfaces to excite near-infrared voltage-sensitive dye, such that cameras recorded intramural signals (~1–4mm deep) from almost the entire expanse of the atrial wall, which ranged in thickness in these hearts from ~1–7mm (Figure 2). Analysis of activation and conduction was determined by maximum upstroke of optical action potentials rather than phase mapping. Our reason for utilizing activation mapping was based on the fact that phase mapping is inherently biased towards detection of rotors(10).

Even during regular atrial rhythm, significant differences in activation patterns between atrial layers were revealed due to 3D structural features of these diseased human right atria (Figure 2). Fast atrial pacing caused local conduction discontinuity and transmural activation delay in regions of abrupt intramural fiber orientation changes and interstitial fibrotic layers that were in agreement with suggestions from Allesie's and others' studies(6;22). Transmural and longitudinal conduction blocks led to the development of sustained intramural reentrant drivers that maintained AF (Figure 3)(7). The reentrant AF drivers were defined as a localized stable source of electrical activity with the highest dominant frequency that maintained the fast fibrillatory conduction within the atria and were confirmed by targeted radio-frequency ablation (Figure 4).

How intramural reentrant drivers can explain discrepancies in AF maintenance mechanisms

Simultaneous endo-epicardial optical mapping(7) allowed us to uncover the mechanisms sustaining AF with a high level of certainty and demonstrated why intramural conduction may have elicited alternative hypotheses of the AF mechanisms from single-sided mapping (Figure 1). Importantly, sub-endocardial mapping visualized about 80% of the intramural reentry loop vs only 40% or less by simultaneous sub-epicardial mapping. Due to the transmural nature of reentrant AF driver circuits, the stable breakthrough region visualized by sub-epicardial mapping frequently represented a transmural pivoting point of the reentry circuit seen from sub-endocardium (Figure 3). The waves generated by such intramural microanatomic reentry propagate through the cardiac muscle and interact with anatomic and functional obstacles leading to fibrillatory conduction, fragmentation, and multiple wavelet

and breakthrough formation. In general, simultaneous endo-epicardial optical mapping revealed four distinct patterns of how intramural drivers can be visualized from a single atrial surface: (1) complete reentry circuits with two pivoting points; (2) incomplete reentry circuits where at least one pivoting point was clearly mapped and the reentry circuit was confirmed by mapping the opposite side; (3) stable breakthrough; and (4) unstable beat-to-beat variable breakthroughs. The latter was seen only by sub-epicardial mapping.

The specific path of reentrant driver activation through the atrial wall can be relatively tight, even a few millimeters when the circuit is viewed at an angle. This makes it difficult to find microanatomic reentry circuits even at the highest spatiotemporal resolution provided by transmural optical mapping and would be extremely challenging to define in a clinical setting during one sided electrode mapping. Thus, even one sided optical mapping with resolution >1mm may show only the location of the AF intramural microanatomic reentrant driver, which may appear more like a focal activity or breakthrough. Additionally, the microanatomic reentry may be observed as a rotor drifting or meandering as low-resolution atrial signals and phase mapping could be affected by the far-field influence of disorganized electrical activity outside the main reentrant path. To clarify, reentrant AF drivers discussed in this review refer to microanatomic reentry that we observed directly in the human heart and not rotors, which represent a different reentrant mechanism.

Single or multiple but limited drivers sustain human AF

The observation by transmural optical mapping of a single leading reentrant AF driver in right atrial preparations does not exclude the possibility of several simultaneous AF drivers coexisting in the whole intact atria (Figure 5A). In some hearts, targeted ablation of the single primary reentrant driver may unmask a secondary reentrant AF driver with a slower CL. Furthermore, recent high-resolution panoramic optical mapping studies of whole intact explanted human atria revealed two simultaneous, competing, localized reentrant drivers during sustained AF(8;33). As such, sustained AF in explanted human hearts may be sustained by one or more drivers that coexist in both left and right atria, which directly correlates with clinical biatrial mapping observations.

Studies using 64-electrode clinical FIRM mapping(5;15) observed ~2.3 AF drivers (rotors or focal sources) in each patient with 20%–30% in the right atrium. The number of drivers was higher in patients with persistent than paroxysmal AF. A body-surface ECVUE phase mapping study(16) identified a higher amount of drivers (~4) in persistent AF that increased with the duration of continuous AF from 2 to 6 driver regions. In general, both approaches revealed a limited number of spatially distinct, primarily reentrant, drivers that become more prevalent with progression/duration of AF, which suggest an important role of patient specific structural remodeling in the development and localization of AF drivers. However, it is still unclear how and what specific structural features leads to the localization and stability of reentrant AF drivers and determines their hierarchy.

Structural substrates or “Fingerprints” of microanatomic reentrant AF drivers

Fibrosis upregulation correlates with AF(1;11), but the pattern, content, and location of fibrosis that leads to stabilization of reentrant AF drivers is unclear in the human atria(7;34;35). The detailed microelectrode study(21) of isolated human atrial pectinate bundles found that fibrotic strands create a substrate for reentry within very small areas due to highly anisotropic conduction in diseased human atria, which does not occur in non-fibrotic atria. However, these were primarily 2D observations from individual myobundles, and it was not known if this structural substrate could be resolved *in vivo* in the complex 3D atrial wall.

Late gadolinium enhancement (LGE) magnetic resonance imaging (MRI) was originally proposed as a useful tool for fibrotic scars and has recently been applied for atrial fibrosis content analysis *in vivo*(35;36). Clinical LGE-MRI studies suggest that the majority of AF patients have high fibrotic structural remodeling due to diagnosed or subclinical cardiac diseases, AF progression, and ablation. Multiple *in vivo* studies have attempted to predict arrhythmogenic areas observed with functional mapping by utilizing LGE-MRI with limited success(35). It is important to note that given the in-plane resolution of current *in vivo* MRI studies (1.3–2mm), the intramural component of the AF driver track (~3mm) may only encompass 2–3 pixels at best, which may explain why a local structural substrate has not been identified in patients. Additionally, atrial fibrosis detection by clinical LGE-MRI was never histologically validated(37). Moreover, it is unclear exactly what type of fibrosis (insulating fibrosis, intramural interstitial fibrosis, or patchy fibrosis) is represented by different levels of LGE intensity. To overcome these methodological limitations, explanted human heart GE-MRI can be histologically validated and used to accurately characterize the fibrotic structural substrate of AF drivers.

Ex vivo transmural mapping integrated with high-resolution GE-MRI (80µm³) allows for the direct study of region-specific structural arrhythmogenic substrates of reentrant AF drivers(7). Our histologically-validated 3D GE-MRI of optically-mapped AF driver regions suggests that the combination of intramural fibrotic strands, misaligned endo-epicardial myofibers (myofiber discontinuity), and atrial wall thickness variation creates patient-specific anisotropic pathways composing microanatomic tracks of a limited number of localized reentrant AF drivers of varying number, size, and distribution in diseased human hearts (Figure 6). Specifically, reentrant AF drivers in our recent study were anchored on 3D fibrotically-insulated microanatomic tracks (~15×6mm surface projection and ~3mm depth). The tracks consisted of two limbs: a central myobundle (2–3mm thick) and either a neighbouring endocardial bundle or the epicardial layer, which were connected by small intramural bundles (~0.5mm thick) that created pivot points where the reentrant driver repetitively made a U-turn (Figure 6). These pectinate muscles were insulated by perimysial fibrosis (100–200 µm thick). This is the first quantitative analysis of 3D structural heterogeneities responsible for AF driver localization, and more data are needed to define the specific AF driver structural characteristics. Due to the higher resolution and histological validation of *ex vivo* techniques, *ex vivo* GE-MRI of human atria may help to improve

clinical LGE-MRI analysis of atrial fibrosis and myofiber structure to define the formula that dictates whether tissue can harbor a reentrant driver.

Validation of clinical mapping approaches in *ex vivo* human heart experiments to improve targeted AF ablation

New clinical mapping approaches (FIRM or ECVUE)(5;16) have been developed for patient-specific ablation treatment; however, these approaches have low spatial resolution (>7–8 mm) and may identify at best a large region ($\sim 1.5 \times 1.5$ – $2.5 \times 2.5 \text{ cm}^2$) of localized rotational activity. Thus, current ablation procedures require ablation of this entire >2cm² driver region and can lead to unnecessarily extensive damage as well as conflicting results of success. As transmural ablation of the entire AF driver region defined by clinical mapping may be difficult to complete, excitable gaps between ablation lesions may create additional substrates for atrial reentrant tachycardia, making successful AF treatment difficult to achieve without knowing the exact AF driver location and structure.

Despite the number of clinical studies on AF mechanisms, no direct experimental human data have confirmed the legitimacy of new clinically-mapped drivers (rotor or focal impulses) and clarified that they are not artifacts of limited electrode recordings and data processing(10;38). This is currently impossible in clinical procedures, but our group has the unique opportunity and required experience to test these types of clinical mapping systems *ex vivo* using the recently validated coronary-perfused human atria model(7;8;32). Combining clinical mapping techniques with high-resolution optical mapping in *ex vivo* human heart experiments could validate the accuracy of current *in vivo* techniques and refine the characteristics of AF drivers seen by these techniques. These types of experiments may shift clinical ablation practice paradigms from extensive ablation of unvalidated targets to a new mechanism-based, minimally damaging, targeted treatment of reentrant AF drivers. For example, if the sustaining AF mechanism is a drifting rotor or intermittent reentry, treatment would require the destruction of a large region. However, if a localized stable intramural reentry was driving AF, termination of AF could be achieved with a single or limited ablation of the microanatomic reentrant track, as was directly demonstrated in our *ex vivo* study (Figure 4).

In preliminary *ex vivo* experiments, our lab has recently utilized optical mapping of both right atria and intact atria simultaneously with endocardial FIRMap 64-electrode baskets as well as modified epicardial and endocardial (8×8 electrode) flat FIRMap catheters(9). Following AF induction, the ability of FIRM to identify AF driver location was compared to the optical mapping identified AF driver location from both local high-resolution cameras as well as panoramic cameras. Based on our published endo-epi optical mapping(7) and preliminary data using both electrode and optical mapping(9), we perceive that endocardial mapping approach (both optical and electrodes) more accurately detects AF reentrant driver location than epicardial approach. However, current clinical endocardial mapping systems have limitations that must be improved upon, including limited resolution and panoramic coverage of atria. *Ex vivo* human atria experiments using both optical mapping with clinical mapping techniques and new high-density catheter array mapping may be used in the future

to test which electrode configurations (polarity, density) as well as signal processing and analysis, are ideal to visualize AF drivers. This may lead to more accurate identification of AF driver location with clinical techniques and thus provide more precise locations for targeted ablation. The new ablation strategies may be tested in *ex vivo* human heart experiments and optimized in computer modeling simulations of AF based on high-resolution functional, structural, and molecular mapping of *ex vivo* human atria, which would enhance the application of current computer models that are based on generic structural and electrophysiologic properties often derived from low resolution clinical studies or even animal studies(39;40).

Explanted human hearts also give us the unique opportunity to control autonomic influence and evaluate AF mechanisms at different levels of autonomic stimulation. *Ex vivo* atrial preparations are denervated and without the external influence of the autonomic nervous system and metabolic stresses that are known to play a critical role in AF(1;31). As such, autonomic or metabolic pharmacological stimulation with isoproterenol/adenosine, which are commonly used in clinical studies of AF(15;41), or pinacidil should be included to sustain AF even in hearts with a history of persistent AF to move closer to *in vivo* conditions(31). The pharmacological-induced *ex vivo* AF driver CLs may seem relatively fast (~7.6–11.0Hz)(7;8;33), but the averaged AF CL (~7Hz) is closer to the averaged AF CL (6–8Hz) reported in many clinical studies(19;42). Additionally, fast activity of 13.7Hz and 13.25Hz in discrete right atrial locations during chronic AF have been found in clinical studies(42) that have undertaken detailed catheter mapping (Figure 5B), which supports that our *ex vivo* results may represent clinical phenomena of AF and its utility in preclinical testing.

Conclusion and future directions

Ex vivo human atria experiments offer multiple advantages not achievable in current *in vivo* human atria studies. Only integration of dual-sided transmural *ex vivo* mapping of human atria with GE-MRI makes it possible to define the precise functional and structural features of 3D microanatomic reentrant AF drivers. New experiments should also define the exact molecular profile determining AF driver substrate to gain a more complete understanding of possible arrhythmogenic substrates in the human atria. Furthermore, *ex vivo* human atrial experiments may allow for new targeted ablation strategies to be tested without any risk to patients. The insights gained in AF driver functional, structural and molecular features, mechanisms, location and ablation from *ex vivo* human atria warrant a greater utilization of these experiments and their translational application will improve clinical strategies to identify and target these AF drivers in patients.

Acknowledgments

This work was supported by NIH HL115580.

Reference List

1. Nishida K, Nattel S. Atrial fibrillation compendium: historical context and detailed translational perspective on an important clinical problem. *Circ Res.* 2014; 114(9):1447–1452. [PubMed: 24763463]
2. Wright M, Narayan SM. Ablation of atrial fibrillation. *Trends Cardiovasc Med.* 2015; 25(5):409–419. [PubMed: 25572010]
3. Narayan SM, Jalife J. CrossTalk proposal: Rotors have been demonstrated to drive human atrial fibrillation. *J Physiol.* 2014; 592(15):3163–3166. [PubMed: 25085968]
4. Allesie M, de GN. CrossTalk opposing view: Rotors have not been demonstrated to be the drivers of atrial fibrillation. *J Physiol.* 2014; 592(15):3167–3170. [PubMed: 25085969]
5. Narayan SM, Krummen DE, Shivkumar K, Clopton P, Rappel WJ, Miller JM. 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. [PubMed: 22818076]
6. de Groot NM, Houben RP, Smeets JL, Boersma E, Schotten U, Schalij MJ, et al. Electropathological substrate of longstanding persistent atrial fibrillation in patients with structural heart disease: epicardial breakthrough. *Circulation.* 2010; 122(17):1674–1682. [PubMed: 20937979]
7. Hansen BJ, Zhao J, Csepe TA, Moore BT, Li N, Jayne LA, et al. Atrial fibrillation driven by microanatomic intramural re-entry revealed by simultaneous sub-epicardial and sub-endocardial optical mapping in explanted human hearts. *Eur Heart J.* 2015; 36(35):2390–2401. [PubMed: 26059724]
8. Zhao J, Hansen BJ, Csepe TA, Lim P, Wang Y, Williams M, et al. Intergration of High-Resolution Optical Mapping and 3-Dimensional Micro-Computed Tomographic Imaging to Resolve the Structure Basis of Atrial Conduction in the Human Heart. *Circulation Arrhythm Electrophysiol.* 2015; 8:1514–1517.
9. Hansen BJ, Briggs C, Moore BT, Csepe TA, Li N, Zhao J, et al. Human atrial fibrillation drivers seen simultaneously by focal impulse and rotor mapping and highresolution optical mapping. *Circulation.* 2015; 132:A18402.
10. Quintanilla JG, Perez-Villacastin J, Perez-Castellano N, Pandit SV, Berenfeld O, Jalife J, et al. Mechanistic Approaches to Detect, Target, and Ablate the Drivers of Atrial Fibrillation. *Circ Arrhythm Electrophysiol.* 2016; 9(1)
11. Heijman J, Voigt N, Nattel S, Dobrev D. Cellular and molecular electrophysiology of atrial fibrillation initiation, maintenance, and progression. *Circ Res.* 2014; 114(9):1483–1499. [PubMed: 24763466]
12. Lewis T, Drury AN, Iliescu CC. Further observations upon the state of rapid re-excitation of the auricles. *Heart.* 1921; 8:311–340.
13. Schuessler RB, Grayson TM, Bromberg BI, Cox JL, Boineau JP. Cholinergically mediated tachyarrhythmias induced by a single extrastimulus in the isolated canine right atrium. *Circ Res.* 1992; 71:1254–1267. [PubMed: 1394883]
14. Mandapati R, Skanes A, Chen J, Berenfeld O, Jalife J. Stable microreentrant sources as a mechanism of atrial fibrillation in the isolated sheep heart. *Circulation.* 2000; 101(2):194–199. [PubMed: 10637208]
15. Miller JM, Kowal RC, Swarup V, Daubert JP, Daoud EG, Day JD, et al. Initial independent outcomes from focal impulse and rotor modulation ablation for atrial fibrillation: multicenter FIRM registry. *J Cardiovasc Electrophysiol.* 2014; 25(9):921–929. [PubMed: 24948520]
16. Haissaguerre M, Hocini M, Denis A, Shah AJ, Komatsu Y, Yamashita S, et al. Driver domains in persistent atrial fibrillation. *Circulation.* 2014; 130(7):530–538. [PubMed: 25028391]
17. Lee G, Kumar S, Teh A, Madry A, Spence S, Larobina M, 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. [PubMed: 23935092]
18. Lee S, Sahadevan J, Khrestian CM, Cakulev I, Markowitz A, Waldo AL. Simultaneous Batrial High-Density (510–512 Electrodes) Epicardial Mapping of Persistent and Long-Standing

Persistent Atrial Fibrillation in Patients: New Insights Into the Mechanism of Its Maintenance. *Circulation*. 2015; 132(22):2108–2117. [PubMed: 26499963]

19. Schuessler RB, Kay MW, Melby SJ, Branham BH, Boineau JP, Damiano RJ Jr. Spatial and temporal stability of the dominant frequency of activation in human atrial fibrillation. *J Electrocardiol*. 2006; 39(4 Suppl):S7–S12. [PubMed: 16920139]
20. Schuessler RB, Kawamoto T, Hand DE, Mitsuno M, Bromberg BI, Cox JL, et al. Simultaneous epicardial and endocardial activation sequence mapping in the isolated canine right atrium. *Circulation*. 1993; 88(1):250–263. [PubMed: 8319340]
21. Spach MS, Dolber PC, Heidlage JF. Influence of the passive anisotropic properties on directional differences in propagation following modification of the sodium conductance in human atrial muscle. A model of reentry based on anisotropic discontinuous propagation. *Circ Res*. 1988; 62(4):811–832. [PubMed: 2450697]
22. Verheule S, Eckstein J, Linz D, Maesen B, Bidar E, Gharaviri A, et al. Role of endo-epicardial dissociation of electrical activity and transmural conduction in the development of persistent atrial fibrillation. *Prog Biophys Mol Biol*. 2014; 115(2–3):173–185. [PubMed: 25086270]
23. Sanchez-Quintana D, Pizarro G, Lopez-Minguez JR, Ho SY, Cabrera JA. Standardized review of atrial anatomy for cardiac electrophysiologists. *J Cardiovasc Transl Res*. 2013; 6(2):124–144. [PubMed: 23389853]
24. Everett TH, Wilson EE, Hulley GS, Olgin JE. Transmural characteristics of atrial fibrillation in canine models of structural and electrical atrial remodeling assessed by simultaneous epicardial and endocardial mapping. *Heart Rhythm*. 2010; 7(4):506–517. [PubMed: 20184976]
25. Zaitsev AV, Rozenshtaukh LV, Fast VG, Bobrovskii RV, Pertsov AM. [Study of spontaneous acetylcholine-dependent tachyarrhythmias using isolated specimens of the right canine atrium by bilateral mapping of the spread of excitation]. *Kardiologiya*. 1989; 29(6):80–85.
26. Fedorov VV, Ambrosi CM, Kostecki G, Hucker WJ, Glukhov AV, Wuskell JP, et al. Anatomic Localization and Autonomic Modulation of AV Junctional Rhythm in Failing Human Hearts. *Circ Arrhythm Electrophysiol*. 2011; 4(4):515–525. [PubMed: 21646375]
27. Herron TJ, Lee P, Jalife J. Optical imaging of voltage and calcium in cardiac cells & tissues. *Circ Res*. 2012; 110(4):609–623. [PubMed: 22343556]
28. Yamazaki M, Mironov S, Taravant C, Brec J, Vaquero LM, Bandaru K, et al. Heterogeneous atrial wall thickness and stretch promote scroll waves anchoring during atrial fibrillation. *Cardiovasc Res*. 2012; 94(1):48–57. [PubMed: 22227155]
29. Mitrea BG, Caldwell BJ, Pertsov AM. Imaging electrical excitation inside the myocardial wall. *Biomed Opt Express*. 2011; 2(3):620–633. [PubMed: 21412467]
30. Lou Q, Hansen BJ, Fedorenko O, Csepe TA, Kalyanasundaram A, Li N, et al. Upregulation of adenosine A1 receptors facilitates sinoatrial node dysfunction in chronic canine heart failure by exacerbating nodal conduction abnormalities revealed by novel dual-sided intramural optical mapping. *Circulation*. 2014; 130(4):315–324. [PubMed: 24838362]
31. Fedorov VV, Glukhov AV, Ambrosi CM, Kostecki G, Chang R, Janks D, et al. Effects of KATP channel openers diazoxide and pinacidil in coronary-perfused atria and ventricles from failing and non-failing human hearts. *J Mol Cell Cardiol*. 2011; 51(2):215–225. [PubMed: 21586291]
32. Csepe TA, Zhao J, Hansen BJ, Li N, Sul LV, Lim P, et al. Human sinoatrial node structure: 3D microanatomy of sinoatrial conduction pathways. *Prog Biophys Mol Biol*. 2016; 120(1–3):164–178. [PubMed: 26743207]
33. Hansen BJ, Csepe T, Kalyanasundaram A, Kamalov G, Zhao J, Janssen PM, et al. Leading Reentry Circuit(s) in the Right Atrial Free Wall Underlies Adenosine-Induced Atrial Fibrillation in Coronary Perfused Human Hearts. *Heart Rhythm*. 2013; 10(11):1745.
34. Kottkamp H. Fibrotic atrial cardiomyopathy: a specific disease/syndrome supplying substrates for atrial fibrillation, atrial tachycardia, sinus node disease, AV node disease, and thromboembolic complications. *J Cardiovasc Electrophysiol*. 2012; 23(7):797–799. [PubMed: 22554187]
35. Chrispin J, Gucuk IE, Zahid S, Prakosa A, Habibi M, Spragg D, et al. Lack of regional association between atrial late gadolinium enhancement on cardiac magnetic resonance and atrial fibrillation rotors. *Heart Rhythm*. 2016; 13(3):654–660. [PubMed: 26569460]

36. Oakes RS, Badger TJ, Kholmovski EG, Akoum N, Burgon NS, Fish EN, et al. Detection and quantification of left atrial structural remodeling with delayed-enhancement magnetic resonance imaging in patients with atrial fibrillation. *Circulation*. 2009; 119(13):1758–1767. [PubMed: 19307477]
37. Sramko M, Kautzner J. Atrial Fibrosis: An Illusion or a True Key to Successful Ablation of Atrial Fibrillation? *J Am Coll Cardiol*. 2015; 65(22):2465.
38. Allesie M, de GN. Rotors during AF: drivers or bystanders? *Eur Heart J*. 2014; 35(2):63–65. [PubMed: 24092245]
39. Zhao J, Butters TD, Zhang H, LeGrice IJ, Sands GB, Smaill BH. Image-based model of atrial anatomy and electrical activation: a computational platform for investigating atrial arrhythmia. *IEEE Trans Med Imaging*. 2013; 32(1):18–27. [PubMed: 23192521]
40. Trayanova NA. Mathematical approaches to understanding and imaging atrial fibrillation: significance for mechanisms and management. *Circ Res*. 2014; 114(9):1516–1531. [PubMed: 24763468]
41. Macle L, Khairy P, Weerasooriya R, Novak P, Verma A, Willems S, et al. Adenosine-guided pulmonary vein isolation for the treatment of paroxysmal atrial fibrillation: an international, multicentre, randomised superiority trial. *Lancet*. 2015; 386(9994):672–679. [PubMed: 26211828]
42. Sanders P, Berenfeld O, Hocini M, Jais P, Vaidyanathan R, Hsu LF, et al. Spectral analysis identifies sites of high-frequency activity maintaining atrial fibrillation in humans. *Circulation*. 2005; 112(6):789–797. [PubMed: 16061740]

A Human AF Driven by Intramural Reentry Anchored to 3D Microanatomic Track

(Hansen et al., EHJ 2015 [Ex Vivo Atria])

B Discrepancy in AF Mechanisms of Clinical Studies

Endo Mapping (FIRM)
Localized Stable Reentrant Drivers

(Narayan et al., JACC 2012)

Epi Mapping - Unstable
Breakthrough and Multi-Wavelets

(Allessie and de Groot, J Physiol 2014)

Figure 1. AF Driver Mechanism: Intramural Microanatomic Reentry Explains Mixed Results from Clinical Studies

A. Human AF driven by intramural reentry projected differently on epicardial and endocardial surfaces. **B.** Clinical studies showing the uncertainty of AF driver mechanisms: endocardial mapping shows localized stable reentrant drivers; epicardial mapping shows unstable breakthrough. *AF* – atrial fibrillation; *Endo* – endocardium; *Epi* – epicardium; *FIRM* – focal impulse and rotor modulation; *IVC/SVC* – inferior and superior vena cava; *RAA/LAA* – right and left atrial appendages. From Hansen et al.(7), Narayan et al(5) and Allessie and de Groot(4), with permission.

A Panoramic and Transmural Optical Mapping of Human Atria

B Integration of Simultaneous Sub-Endo/Sub-Epi Activation Maps with GE-MRI (43 y.o. Female, HTN/CAD/DM)

C Max Endo vs Epi Activation Delay in Human RA (43 y.o. Female, HTN/CAD/DM)

D Max Transmural Delay in Human RA (n=8)

Figure 2. Dual-Sided and Panoramic Mapping Shows Transmural Activation Delay due to Atrial Structure

A. Schematic of dual-sided and panoramic optical mapping of the human atria. **B.** (1st row left to right) Sub-Endo and sub-Epi activation maps during 500ms pacing; Transmural activation delay map. (2nd row left to right) 3D GE-MRI showing locations 1–4 on tissue; GE-MRI section showing atrial thickness along preferential conduction. **C.** Curves showing maximum transmural activation delays vs. pacing cycle length with beat-to-beat variation observed at cycle length <250ms. **D.** Graph showing significant increase in transmural activation delay during faster pacing and AF in the RA. *Abbreviations as in Figure 1. GE-MRI – gadolinium enhanced MRI; RA – right atrium. From Hansen et al.(7), with permission.*

A Simultaneous Sup-Endo/Sub-Epi Activation Maps During AF (43 y.o. Female, HTN/CAD/DM)

B 3D GE-MRI

C 3D Microanatomic Reentry Track

D Transmural Activation Delay, ms

E Reentrant Drivers Projected Differently on Epi and Endo Surfaces

Figure 3. Microanatomic tracks of Reentrant AF Drivers Resolved by Dual-Sided Optical Mapping and GE-MRI

A. Activation maps from panoramic (Left), sub-Endo (Middle), and sub-Epi (Right) cameras. Solid arrow and star show path of reentry and “breakthrough” point, respectively.
B. GE-MRI showing fibrosis (white) distribution and location of reentrant AF driver (white arrow).
C. 3D view of reentry track. **D.** Transmural activation delay map with reentrant pathway shown by green arrow. **E.** Locations of AF drivers from experiments in 6 RA. Individual atria are distinguished by color and drivers with the same color and region on

Endo and Epi correspond to two visualizations of the same driver. *Abbreviations as in Figure 1. TA – tricuspid annulus. From Hansen et al.(7), with permission.*

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4. Radio Frequency Ablation of Microanatomic Reentrant Track Confirms AF Driver Regions

Left: Activation map showing reentrant AF driver before targeted ablation. Middle: AF converted to macroreentrant atrial tachycardia (AT) after RFA. Right: Arrhythmia terminated and prevented by targeted RFA from the reentrant track to anatomical border. *Abbreviations as in Figure 3. RFA – radio frequency ablation. From Hansen et al.(7), with permission.*

A *Ex vivo* DF Map of AF in Intact Human Atria (Zhao et al., Circ A&E 2015)

B *In vivo* DF Map of AF (Sanders et al., Circ 2005)

Figure 5. Similarity of DF and AF Driver Patterns *In Vivo* and *Ex vivo*

A. Left: *Ex vivo* DF map during sustained AF and frequency power at 7.6Hz showing stable regions in intact human atria. Right: 3D micro-CT shows underlying atrial structure with main anatomic regions highlighted and AF driver locations shown by white arrows. Power frequency chart and optical action potentials (OAPs) from the left atrial roof, floor, and lateral right atrium denoted as 1 to 3, along with atrial ECG. **B.** *In vivo* dominant frequency map from consequent electrode recordings in patients with paroxysmal (Top) and permanent (Bottom) AF. Abbreviations as in Figure 1. LIPV/LSPV/RIPV/RSPV – left, right, superior, inferior pulmonary veins; PLA – posterior left atria; SAN – Sinoatrial node. From Zhao et al.(8) and Sanders(42) with permission.

Figure 6. Structural Features of AF Reentrant Driver Regions

A. Endo and Epi 3D fiber orientations generated from GE-MRI imaging showing relative vertical orientations in blue and horizontal orientations in red. AF driver denoted by black arrow on Endo view, while Star denotes breakthrough visualization from Epi. **B.** (Top) Difference between fiber orientation of sub-Epi (blue) and sub-Endo (red) layers in the driver region is greater than in non-driver regions. Large red and blue arrows represent global average fiber angle of the epi and endo, respectively, in these specific driver and non-driver regions. (Bottom) Interstitial fibrosis (red) distribution for driver vs non-driver

regions. AF driver shown by white arrow. *Abbreviations as in Figure 3. From Hansen et al. (7), with permission.*

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript