Atrial fibrillation driven by micro-anatomic intramural re-entry revealed by simultaneous sub-epicardial and sub-endocardial optical mapping in explanted human hearts

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Aims
The complex architecture of the human atria may create physical substrates for sustained re-entry to drive atrial fibrillation (AF). The existence of sustained, anatomically defined AF drivers in humans has been challenged partly due to the lack of simultaneous endocardial—epicardial (Endo—Epi) mapping coupled with high-resolution 3D structural imaging.

Methods and results
Coronary-perfused human right atria from explanted diseased hearts (n = 8, 43–72 years old) were optically mapped simultaneously by three high-resolution CMOS cameras (two aligned Endo—Epi views (330 μm² resolution) and one panoramic view). 3D gadolinium-enhanced magnetic resonance imaging (GE-MRI, 80 μm³ resolution) revealed the atrial wall structure varied in thickness (1.0 ± 0.7–6.8 ± 2.4 mm), transmural fiber angle differences, and interstitial fibrosis causing transmural activation delay from 23 ± 11 to 43 ± 22 ms at increased pacing rates. Sustained AF (>90 min) was induced by burst pacing during pinacidil (30–100 μM) perfusion. Dual-sided sub-Endo—sub-Epi optical mapping revealed that AF was driven by spatially and temporally stable intramural re-entry with 107 ± 50 ms cycle length and transmural activation delay from 23 ± 11 to 43 ± 22 ms at increased pacing rates. Sustained AF (>90 min) was induced by burst pacing during pinacidil (30–100 μM) perfusion. Dual-sided sub-Endo—sub-Epi optical mapping revealed that AF was driven by spatially and temporally stable intramural re-entry with 107 ± 50 ms cycle length and transmural activation delay of 67 ± 31 ms. Intramural re-entrant drivers were captured primarily by sub-Endo mapping, while sub-Epi mapping visualized re-entry or ‘breakthrough’ patterns. Re-entrant drivers were anchored on 3D micro-anatomic tracks (15.4 ± 2.2 × 6.0 ± 2.3 mm², 2.9 ± 0.9 mm depth) formed by atrial musculature characterized by increased transmural fiber angle differences and interstitial fibrosis. Targeted radiofrequency ablation of the tracks verified these re-entries as drivers of AF.

Conclusions
Integrated 3D structural—functional mapping of diseased human right atria ex vivo revealed that the complex atrial microstructure caused significant differences between Endo vs. Epi activation during pacing and sustained AF driven by intramural re-entry anchored to fibrosis-insulated atrial bundles.

Keywords
Atrial fibrillation • Intramural re-entry • Rotor • Optical mapping • Gadolinium-enhanced MRI • Fibrosis • Catheter radiofrequency ablation

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**Introduction**

Atrial fibrillation (AF) is the most common sustained arrhythmia and is associated with increased cardiovascular morbidity and mortality. Despite several decades of extensive research, gaps in understanding the mechanisms of AF still exist, making successful treatment particularly challenging. Electrophysiological and structural abnormalities have been shown to underlie AF development. However, the contribution of the atrial microstructure to both the development and persistence of AF in the diseased human atria has not been clearly elucidated.

Recently, several clinical studies have identified localized focal and re-entrant AF drivers in both the right and left human atria using either Epi or Endo electrode mapping protocols that can only identify AF driver mechanisms on the atrial surface. These technical shortcomings have thus far limited the advancement of AF research and treatment, a problem well stated by Allessie and de Groot: ‘Simultaneous endo-epicardial mapping is required to answer the question of whether AF is due to a rotor or to endo-epicardial dissociation’. Additionally, the structural substrate of human AF drivers is unexplored directly since the current resolution of clinical non-invasive methods, including late gadolinium-enhanced magnetic resonance imaging (GE-MRI), is insufficient to resolve the remodelled intramural atrial microstructure potentially responsible for AF maintenance. Ex vivo high-resolution mapping approaches in classical animal models of pharmacologically induced sustained AF have provided the hypothesis that localized small re-entrant circuits could drive AF; however, this mechanism has never been directly verified in human atria or linked to specific atrial transmural wall structure of the diseased human heart.

In this ex vivo study, we tested the hypothesis that intramural re-entrant circuits are stabilized by micro-anatomic substrates within the right atrial wall and may maintain AF. For this purpose, we developed a novel approach to simultaneously map the sub-endocardial (sub-Endo) and sub-epicardial (sub-Epi) activation patterns and integrate these data with ex vivo GE-MRI 3D maps of the atrial micro-anatomic architecture in order to elucidate possible mechanisms sustaining AF in diseased human right atria.

**Translational perspective**

Our integrated 3D high-resolution structural-functional mapping of the diseased human heart ex vivo revealed significant transmural differences between endocardial and epicardial atrial activation during normal heart rate and especially during sustained atrial fibrillation (AF) were caused by the very complex transmural microstructure of the human atrial wall. Moreover, this study shows that sustained AF may be driven by localized microanatomic reentry anchored to regions of the highly fibrosis-insulated and twisted intramural atrial bundles. Targeting these microanatomic tracks of reentry with endocardial catheter ablation successfully disrupted AF and prevented its re-induction, suggesting the human 3D atrial architecture plays a key role in the maintenance of AF. Future studies to better understand the structural complexity of the human atrial micro-anatomy will offer more efficient and targeted treatment options to prevent and treat sustained AF.

**Materials and methods**

An expanded Materials and methods can be found in Supplementary material online, Supplementary Data.

**Optical mapping of coronary-perfused human right atrial preparations**

Explanted human hearts were obtained from The Ohio State University Cardiac Transplant team and LifeLine of Ohio (n = 8) in accordance with The Ohio State University Institutional Review Board. Patient-specific data can be found in Supplementary material online, Table S1. Human lateral right atrial (LRA) preparations were isolated and coronary-perfused as previously described. All preparations excluded regions of poor coronary perfusion/ischemia. Importantly, atrial repolarization and right atrial activation times were within the range of clinical measurements of patients with structural remodeling.

The LRA preparations were immobilized with 10 μM blebbistatin and stained with near-infrared dye di-4-ANBDQBS (10–40 μM). A dual-sided optical mapping system (Endo and Epi, 330 μm resolution, and panoramic, 940 μm resolution CMOS cameras (100 × 100 pixels), MiCAM Ultima-L, SciMedia Ltd, CA, USA) developed by our laboratory was used to obtain simultaneous sub-Endo and sub-Epi intramural optical action potentials (OAPs) and to calculate the transmural (Endo vs. Epi) activation delay. Excitation light simultaneously illuminated both surfaces to excite di-4-ANBDQBS such that each camera recorded intramural OAPs (1–4 mm deep), specifically weighted from the sub-Endo and sub-Epi layers, that would have been invisible without transillumination. In order to sustain AF in isolated denervated hearts ex vivo, adenosine triphosphate-regulated potassium channel opener pinacidil (30–100 μM) was used as previously described. Pinacidil mimics the action potential shortening that would be expected in fibrillating human atria in vivo where autonomic stimulation and metabolic stresses are present. Data were analysed as previously described. Atrial fibrillation cycle length (CL) was measured by the average dominant frequency (DF) of the tissue from the panoramic camera, while discrete islands of fastest DF were considered driver regions and denoted as the driver CL.

In LRA#4-8, we conducted targeted Endo radiofrequency ablation (RFA) of stable re-entrant patterns or stable pivot points where conduction made a U-turn in regions of highest DF during sustained AF with a non-irrigated RFA catheter (8Fr, 8 mm tip, Lg Crv Blazer II XP, Boston Scientific, MA, USA).

**Ex vivo gadolinium-enhanced magnetic resonance imaging and histology**

Gadolinium-enhanced magnetic resonance imaging and histological sections stained with Masson’s trichrome were used to define atrial anatomy, wall variation, myofiber orientation, and fibrosis. 3D myofiber orientations were estimated as previously described.

**Statistical analysis**

Quantitative data are shown as mean ± SD. Analysis was done in SAS 9.2 (Cary, NC, USA) using repeat measurements ANOVA. A value of P < 0.05 was considered significant.
Results

Sub-Endo and sub-Epi conduction patterns and atrial architecture

All LRA demonstrated complex 3D atrial musculature (Figure 1A and B) consistent with previous anatomical observations. Figure 1C shows a transmural GE-MRI section along the path of preferential conduction through LRA#4, emphasizing the significant variation in LRA wall thickness. The LRA \( n = 8 \) structure ranged from 0.98 ± 0.67 to 6.75 ± 2.43 mm thick with branching free-running bundles (1.97 ± 0.49 mm thick) insulated by perimysial and interstitial fibrotic layers (seen as white layers in Figure 1C) causing discontinuity between the sub-Endo pectinate muscles (PMs) and the sub-Epi layers. Differences between sub-Endo and sub-Epi activation patterns along the line of preferential conduction mimicked the transmural variation in atrial wall structure (Figure 1B and D). Transmural activation delay was analysed using simultaneous sub-Endo vs. sub-Epi activations to establish the direction of transmural conduction (Figure 1C and D) seen by OAP upstrokes and dV/dts (Figure 1E).

Moreover, Figure 2 shows transmural activation delay directly correlated to atrial wall thickness along the path of preferential conduction \( r^2 = 0.81; P < 0.001 \). Complete analysis is provided for all four GE-MRI scanned LRA preps in Supplementary material online, Figure S1.

Figure 3 shows that the transmural activation delay in LRA#4 was significantly increased during pacing acceleration from 500 ms up to

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**Figure 1** Integrated functional and structural mapping of Epi–Endo discordance during atrial pacing in LRA#4. (A) Optical field of views (100 × 100 pixels) for CMOS Cameras #1 (black, Sub-Endo) and #3 (green, Panoramic). (B) Gadolinium-enhanced magnetic resonance imaging showing the complex 3D atrial pectinate muscle network. (C) Transmural gadolinium-enhanced magnetic resonance imaging section, intramural fibrosis displayed in white, along the path of preferential conduction. (D) Left to right: activation maps at 500 ms cycle length pacing from CMOS Camera #3 (green) with optical field of view for Cameras #1 and #2 (black); Camera #1 (sub-Endo); Camera #2 (sub-Epi); transmural activation delay (Endo delay in red, Epi delay in blue). Yellow and black arrows show preferential conduction on (A)–(D). (E) Optical action potentials and their dV/dts from locations 1–4 indicated on maps above (circled numbers). Cath, catheter; CL, cycle length; EG, electrogram recording; Endo, endocardium; Epi, epicardium; GE-MRI, gadolinium-enhanced magnetic resonance imaging; Inf, inferior right atrial free wall; LRA, lateral right atria; RAA, right atrial appendage; Sup, superior right atrial free wall; TA, tricuspid annulus.
the functional refractory period (FRP = 200 ms). Conduction patterns at FRP showed alternating beat-to-beat variability with the activation time of beat 1 to the next beat 2 prolonging as much as 30 ms due to conduction block and a 20 ms increase in transmural activation delay in LRA#4 (Figure 3A). Figure 3B shows evidence of the sub-Endo conduction blocks in PMs insulated by fibrosis (see also Figure 1C) where transmural activation delays increased dramatically, a consistent observation in all LRA (Figure 3C). On average, transmural activation delay increased from 24.1 ± 15.4 ms at 500 ms pacing to 42.9 ± 25.0 ms at the FRP (n = 8, P < 0.005).

The local sub-Endo conduction discontinuity/blocks, induced by fast pacing, were primarily seen between PMs and correlated with abrupt changes in intramural fiber orientation (Figure 4) and interstitial fibrotic layers (Figure 1C). Figure 4 shows sub-Endo fibers run nearly parallel in PMs, but are discontinuous between PMs; conversely, fiber orientation of the sub-Epi layers are smoother but run nearly perpendicular to sub-Endo fibers. Both the sub-Epi tissue layer and sub-Endo PMs exhibit strong unidirectional fiber orientations with dominant angles centered around −37.3 and 33.1°, respectively. However, no dominant fiber orientation patterns were observed in intramural layers. An average 65.9 ± 3.8° (n = 4) difference between sub-Epi and sub-Endo dominant myofiber orientations was observed in GE-MRI evaluated preparations.

Localized micro-anatomic tracks sustained intramural re-entry during atrial fibrillation

To sustain AF in explanted denervated hearts, we used pinacidil which induced action potential (APD80%) shortening on average from 282.9 ± 47 ms in Epi and 284.9 ± 45 ms in Endo to 158.7 ± 61 and 165.3 ± 64 ms during 500 ms CL pacing, respectively. In six LRAs (eight sustained AF episodes), AF was driven by localized stable re-entry with unstable ‘wave-break’ activity outside of the

### Figure 2

Correlation between transmural activation delay and atrial wall thickness. (A) Gadolinium-enhanced magnetic resonance imaging and atrial wall thickness of the optical field of view in LRA#4 with path of preferential conduction (purple arrow) from Figure 1. (B) Transmural activation delay of the same region from (A; Endo delay in red, Epi delay in blue) with purple arrow showing path of preferential conduction. (C) Graph of transmural delay (black solid line) and atrial wall thickness (black dotted line) vs. distance on path of preferential conduction (also displayed by purple arrow and optical action potentials 1–4) from (A) to (B). (D) Plot of transmural delay vs. atrial wall thickness in LRA#4. Red dashed line of best fit. Abbreviations as in Figure 1.
AF driver region. Dual-sided sub-Endo–sub-Epi optical mapping revealed that AF was driven by spatially and temporally stable intramural re-entry with 107 ± 50 ms CL and transmural activation delay of 67 ± 31 ms. The GE-MRI structure of a stable AF driver observed by sub-Endo mapping is provided in Figure 5. The 3D musculature of the AF driver region in Figure 5B reveals two parallel PMs converging at both ends. Figure 5C and D shows fibrillatory conduction emanating from a re-entrant circuit pinned to the two parallel PMs. The re-entrant AF driver in LRA#6 predominately resided in two Endo PMs connected through intramural myofiber pathways allowing conduction to pivot between the PMs. Sub-Endo activation revealed a complete re-entry circuit, whereas sub-Epi activation showed a stable breakthrough pattern caused by transmural conduction from the sub-Endo pivoting point (OAP#1).

Transmural GE-MRI and histological analysis revealed interstitial and perimysial fibrosis distributed in and around the re-entry track insulating PMs and the sub-Epi layer (Figure 6). Stable re-entrant AF drivers (15.4 ± 2.2 × 6.0 ± 2.3 mm² with 2.9 ± 0.9 mm depth, n = 5) were anchored on micro-anatomic tracks which consisted of two limbs: a central PM (2.0 ± 0.5 mm thick) and the sub-Epi layer, a neighbouring PM and/or the atrial vestibule (Figures 5 and 6). The limbs were connected by small intramural bundles (0.47 ± 0.21 mm thick) where the re-entrant driver repetitively made a U-turn. Perimysial fibrosis, 152 ± 49 μm thick, insulated PMs and intramural bundles from the surrounding atria and may stabilize re-entrant AF drivers. The structures of atrial regions that sustained a re-entrant driver were compared with regions where no driver was observed in the same LRA. Re-entrant driver regions were characterized by 58.6 ± 12° transmural fiber angle difference and 12.8 ± 1% interstitial fibrosis compared with 35.9 ± 21° (P = 0.052) and 10.3 ± 2% (P = 0.02) in non-driver regions. As such, unidirectional blocks in regions of re-entry circuits could be attributed to

Figure 3  Endo vs. Epi conduction alterations during fast atrial pacing in LRA#4. (A) Left to right: sub-Endo and sub-Epi activation maps during 200 ms cycle length pacing for beat 1 (top) and beat 2 (bottom); transmural activation delay maps for beats 1 and 2; optical action potentials and their dV/dt during beats 1 and 2 at locations 1 and 2 (circled numbers), showing conduction alterations. (B) Curves showing maximum transmural activation delays vs. pacing cycle length with beat-to-beat variation observed at cycle length <250 ms. (C) Graph showing significant increase in transmural activation delay during faster pacing in all lateral right atrials. Abbreviations as in Figure 1.
myocardial uncoupling caused by fibrosis, PM architecture, and abrupt changes in fiber orientation (Figures 2, 4, and 6; Supplementary material online, Figure S2).

Based on our results, we categorized four patterns of how intramural drivers were visualized by optical mapping from a single surface of the LRA shown in Figure 7A and Table 1: complete re-entry circuits with two pivoting points, incomplete re-entry circuits where at least one pivoting point was clearly mapped and the re-entry circuit was confirmed by mapping the opposite side,24 stable breakthrough, and unstable beat-to-beat variable breakthroughs. In three AF episodes, due to the transmurality of re-entrant circuits, the stable breakthrough region visualized by sub-Epi mapping

**Figure 4** Sub-endocardial conduction blocks and fiber orientation in LRA#4. (A) Panoramic sub-Endo activation map during 200 ms cycle length pacing showing lines of conduction block (black arrows) during beat 2. The white box indicates the location of the tissue section shown in (C). (B) Endo and Epi 3D fiber orientations generated from gadolinium-enhanced magnetic resonance imaging showing vertical orientations in blue and horizontal orientations in red. Conduction block (black lines) shown on Endo views. (C) Gadolinium-enhanced magnetic resonance imaging of 3D tissue section (indicated by white box in A and B) showing fiber angles for Sub-Epi(1), Intramural(2), and Sub-Endo(3) layers. (D) Fiber angle distributions in the Sub-Epi(1), Intramural(2), and Sub-Endo(3) myocardial layers (25 gadolinium-enhanced magnetic resonance imaging sections per region). Abbreviations as in Figure 1.
represented a transmural pivoting point of the re-entry circuit seen from sub-Endo mapping (Figure 6A). In all six LRA experiments, 77 ± 14% of the intramural re-entry loop was visualized by sub-Endo vs. 41 ± 32% by sub-Epi mapping (P = 0.01) (Table 1).

**Targeted ablation of micro-anatomic re-entrant drivers terminated atrial fibrillation**

We targeted the stable drivers in the region of highest DF and/or surrounding areas with RFA in five hearts (LRA#4–8). Atrial fibrillation was confirmed to be sustained by a primary driver before RFA was attempted. Targeted RFA (1.2 ± 0.4 min) eliminated the primary re-entrant driver; consequently, AF was terminated or the arrhythmia continued as AF with a new re-entrant driver in another location or as post-ablation atrial tachycardia (AT) with macro-re-entry around the ablation lesion. However, untargeted RFA (outside the re-entrant driver track) did not result in significant changes of AF frequency or morphology (Supplementary material online, Figure S3A–C). Gadolinium-enhanced MRI confirmed RFA lesion locations and transmurality (Supplementary material online, Figure S3D). Figure 5 outlines the ablation procedure for the re-entrant driver seen in Figure 7B: RFA#1 targeted the primary re-entrant driver track (Figure 5, left) and converted AF to macro-re-entrant AT around the RFA lesion (Figure 7B, middle). RFA#2 and 3 created a linear ablation

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**Figure 5** Sub-endocardial atrial fibrillation driver and its anatomic track in LRA#6. (A) 3D gadolinium-enhanced magnetic resonance imaging showing paths of fibrillatory conduction (red arrows) and re-entrant atrial fibrillation driver (black arrow). Driver region outlined in black. (B) Re-entrant atrial fibrillation driver (black arrow) along pectinate muscles, dotted lines show location of Sections 1, 2, and 3; gadolinium-enhanced magnetic resonance imaging of Sections 1, 2, and 3 show interstitial fibrosis (white) between myocardial layers. (C) Dominant frequency map of sustained atrial fibrillation; optical action potentials for locations (a) in driver region and (b) outside of driver region. (D) Left to right: activation maps during sustained atrial fibrillation for Camera #1 (sub-Endo), Camera #2 (sub-Epi), and corresponding transmural activation delay. (E) Optical action potentials 1–4 (circled numbers in A–D) along re-entrant driver for sub-Endo and sub-Epi. Abbreviations as in Figure 1.
and after each RFA in six hearts is plotted in Supplementary material online, Figure S4. Subsequent targeted ablation (4.0 ± 1.8 min) in all five LRAs terminated and prevented the reinduction of AF/AT. The total number of targeted and untargeted ablation lesions and RFA time can be found in Supplemental material online, Table S2.

Figure 6 Atrial fibrosis and the intramural re-entry track in LRA#4. (A) Left to right: CMOS Camera #1 (sub-Endo), CMOS Camera #2 (sub-Epi), solid arrow and dotted arrow with star show observed path of re-entry and ‘breakthrough’ point, respectively; transmural activation delay with re-entrant pathway shown by a green arrow. (B) Gadolinium-enhanced magnetic resonance imaging showing fibrosis (pink) distribution within LRA#4 and location of re-entrant driver seen in (A) (black arrow). (C) 3D view of re-entry track with labelled histology section locations (blue dotted lines). Section 1 (D) and Section 2 (E) histology and gadolinium-enhanced magnetic resonance imaging showing interstitial and perimysial fibrosis. Cath EG, catheter electrogram recording. Abbreviations as in Figure 1.
Discussion

Localized atrial fibrillation re-entrant drivers in human hearts

Almost 100 years ago, Garrey and Lewis first hypothesized that re-entry could sustain and drive AF. Moreover, Lewis et al. proposed that AF could be driven by a single re-entry circuit with fibrillatory conduction propagating from it, later supported by electrode mapping by the Schuessler group and optical mapping studies by the Jalife group in animal models of acetylcholine-induced sustained AF. In patients, Cox et al. directly mapped a single stable re-entrant circuit in the right atria which maintained AF.

Recently, several clinical groups have explored the spatio-temporal characteristics of AF drivers using Endo contact basket and PentaRay catheters as well as non-invasive body surface (Epi) mapping to guide targeted ablation. Importantly, these approaches revealed localized AF drivers in left and right atria while the Allessie and Kalman groups’ Epi mapping studies revealed more wavebreaks than localized re-entrant activity. These apparent discrepancies between the two
sets of studies may be due to the substantial variation in the resolution of mapping, total mapped area, and patient populations. We suggest an additional factor may be that only a single surface was mapped and the contradicting AF mechanisms (localized drivers vs. multi-waveslets) may stem from intramural conduction in the human atria projecting differently on the Endo vs. Epi surfaces. Based on our results, clearer visualization of stable re-entrant AF drivers is achieved from sub-Endo mapping of the Endo PMs, while breakthrough type activity would more often be observed from sub-Epi (Figures 5–7), emphasizing the need for simultaneous sub-Epi and sub-Endo mapping combined with detailed 3D atrial microstructure imaging for a comprehensive view of the complex mechanism of AF maintenance. Additionally, unstable wavebreaks seen from sub-Epi did not portray the single stable re-entrant driver pattern seen simultaneously from sub-Endo (Figures 5 and 6), which corroborates as well as bridges results from major clinical mapping studies using either Epi or Endo mapping.

By proposing a way in which some of the discrepancies in the field may coexist, our research could represent a step towards resolving the puzzle of human AF. Although our current results clearly establish that a single localized micro-anatomic re-entry can sustain AF in the human heart ex vivo and support the localized driver or ‘Mother Ring’ hypothesis, we cannot dismiss the possibility of other well-known mechanisms of AF, such as multi-wavelets and focal activity.

**Simultaneous sub-Endo–sub-Epi mapping and 3D gadolinium-enhanced magnetic resonance imaging collectively resolved intramural atrial conduction patterns**

Simultaneous Epi–Endo electrode mapping of AF in animal models, first explored ex vivo by Zaitsev et al. and then Schuessler et al., revealed differences between Endo and Epi activation and suggested the existence of an intramural path for re-entrant AF drivers. The Eckstein et al. study in goat atria emphasized that intramural atrial remodelling could serve as an AF substrate in vivo. Yamazaki et al. observed drifting rotors seen from Endo or Epi in the left atria using optical mapping in sheep ex vivo. Although these studies demonstrated the feasibility of Endo–Epi mapping in other species, no high-resolution simultaneous Endo–Epi mapping of AF has been performed in human hearts until now.

In this study, transillumination of near-infrared di-4-ANBDQSB allowed the collection of sub-surface intramural weighted OAPs from sub-Endo (1–4 mm deep) and sub-Epi (1–4 mm deep) from almost the entire expanse of the human LRA wall (0.98 ± 0.67–6.75 ± 2.43 mm thick) to resolve the complete pattern of AF driver propagation. Moreover, a complete re-entry circuit seen from sub-Endo may also be seen from sub-Epi depending on atrial thickness and Epi fat. Importantly, the intramural re-entry pathways would not have been seen by Endo and Epi surface electrode mapping.

**Discontinuity of human atrial musculature aggravated by fibrosis creates structural substrates for re-entrant atrial fibrillation drivers**

Atrial fibrillation-sustaining mechanisms are facilitated by electrical and structural remodelling, including increased fibrosis, in AF patients with and without cardiac comorbidity. Although late GE-MRI has been used to evaluate left atrial AF structural substrates in patients, current clinical resolution (>1 mm3) only captures large fibrotic scars and cannot resolve the interstitial fibrosis distribution within the atrial walls; furthermore, detailed myofiber orientations of the human atria have not been fully captured in vivo.

Sub-Endo–sub-Epi optical recordings in our study were correlated with high-resolution GE-MRI in order to clearly define the structural substrates of functionally mapped AF drivers. We found that architectural discontinuities between atrial PMs and small intramural bundles led to both longitudinal and intramural conduction blocks, especially during fast atrial pacing (Figures 1–6). This observation is in agreement with Epi–Endo electrode mapping studies in

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**Table 1** Characteristics of atrial fibrillation drivers in the human lateral right atrial during sustained atrial fibrillation episodes

<table>
<thead>
<tr>
<th>LRA#</th>
<th>Driver location</th>
<th>Driver no.</th>
<th>Driver CL (ms)</th>
<th>AF CL (ms)</th>
<th>% Re-entry visualized</th>
<th>Max transmural delay (ms)</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>Superior LRA</td>
<td>Primary driver</td>
<td>124</td>
<td>159</td>
<td>69 ± 6%</td>
<td>AF 79</td>
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<tr>
<td>4</td>
<td>Inferior LRA</td>
<td>Primary driver</td>
<td>57</td>
<td>88</td>
<td>64 ± 2%</td>
<td>AF 48</td>
</tr>
<tr>
<td>5</td>
<td>Superior LRA</td>
<td>Secondary driver</td>
<td>68</td>
<td>135</td>
<td>89 ± 5%</td>
<td>AF 22</td>
</tr>
<tr>
<td>6</td>
<td>Middle LRA</td>
<td>Secondary driver</td>
<td>94</td>
<td>313</td>
<td>54 ± 7%</td>
<td>AF 50</td>
</tr>
<tr>
<td>7</td>
<td>Superior vestibule</td>
<td>Primary driver</td>
<td>210</td>
<td>249</td>
<td>72 ± 15%</td>
<td>AF 101</td>
</tr>
<tr>
<td>8</td>
<td>Middle LRA</td>
<td>Primary driver</td>
<td>110</td>
<td>111</td>
<td>88 ± 4%</td>
<td>AF 92</td>
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Average: 107 ± 50

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Average: 107 ± 50

% Re-entry visualized indicates the percent of the re-entrant driver cycle length (ms) resolved by sub-Endo or sub-Epi mapping along the intramural re-entry circuit, averaged over five consecutive beats. *P = 0.01 (see text for details).
animal models that correlated functional mapping to transmural atrial structure and propose that the atrial wall contains at least two distinct layers with opposing fiber orientation that may support re-entrant activity.\textsuperscript{33,34}

Furthermore, increased fibrosis due to cardiac diseases (Supplementary material online, Table S1) could have worsened the architectural discontinuity, thereby creating anisotropic conduction blocks and source-sink mismatches\textsuperscript{6} and eventually a structural substrate for localized micro-anatomic re-entrant AF drivers (Figures 4 – 6). Indeed, GE-MRI analysis of the atrial wall found that AF-sustaining regions had an increase in the total percent of fibrosis and an increase in transmural fiber angle discontinuity compared with non-driver regions in the same heart. Structural analysis revealed that AF drivers travelled primarily through tracks of intramural insertions of fibrotic PMs to the RA base and small intramural branching connections to the sub-Epi wall (Figures 5 and 6). The formula that dictates whether tissue can harbour a re-entrant driver is complex and we suggest that it is a combination of local myofiber orientations, wall thickness variation, and fibrosis distribution, as well as functional and molecular differences not directly studied here.

Targeted ablation of re-entrant drivers disrupts atrial fibrillation

Current catheter ablation procedures have proven to be relatively successful in paroxysmal AF treatment, but are much less effective in the management of persistent AF, particularly in the setting of multifactorial heart diseases.\textsuperscript{12} Narayan et al.\textsuperscript{10} recently used Focal Impulse and Rotor Modulation to terminate AF by targeting drivers while Haissaguerre et al.\textsuperscript{8} used body surface mapping to ablate high AF driver activity regions in persistent AF. However, both mapping approaches lack a mechanistic explanation for their reported success, in part due to limited spatial resolution.\textsuperscript{11}

In our ex vivo, pinacidil-induced AF right atrial preparations, we found that targeting the structural substrate/anatomical tracks of the re-entrant driver with RFA could disrupt the sustained re-entry circuit and thus terminate sustained AF, which supports the critical dependence of AF maintenance on the leading re-entrant driver in this model. Consequently, secondary post-RFA drivers and macro-re-entrant AT were commonly induced after termination of the primary driver, suggesting that more than one region in the atria is capable of sustaining AF.

Limitations

All observations on AF mechanisms in our study were made in ex vivo preparations from diseased hearts that may not directly translate to AF mechanisms seen in vivo. Moreover, the diversity of medical histories and the number of hearts studied limit the extrapolation of our findings to wider AF populations. Although disease-induced structural remodelling of our atrial preparations may play a role in the maintenance of AF, the lack of control non-diseased atria, and the diverse history of our atria in the study prevent us from attributing the presence of re-entrant drivers directly to the pathological alterations in the atria. Based on optical mapping restrictions, we studied AF drivers in LRA; however, up to 30% of drivers in persistent AF have been found clinically in the RA.\textsuperscript{7,8,30}

Our observation of a single leading re-entrant AF driver in our LRA preparations does not exclude the possibility of several simultaneous AF drivers coexisting in the whole intact atria. Furthermore, targeted ablation was used in this study to confirm the driving nature of the re-entry circuits and requires further study to confirm the success rate of targeting such regions during an ablation procedure in the treatment of AF.

Since denervated and mechanically arrested ex vivo atrial preparations lack the influence of the autonomic nervous system and metabolic stresses that are known to play a critical role in AF,\textsuperscript{3,14} pinacidil was included in our protocols to sustain AF.\textsuperscript{14} Our experimental model, by shortening APD, may approximate chronic AF\textsuperscript{17} in hearts with structural remodelling rather than paroxysmal AF.\textsuperscript{38}

The driver CL reported in this study (Table 1) is relatively fast (107 ± 50 ms, ≈ 9 Hz), but our averaged AF CL (144 ± 71 ms, ≈ 7 Hz) is closer to the averaged AF CL (≈150 ms, 6–8 Hz) reported in clinical studies.\textsuperscript{20,21,25} Additionally, fast activity of 13.720 and 13.25 Hz\textsuperscript{21} in discrete right atrial locations during chronic AF have been found in clinical studies which have undertaken detailed catheter mapping.

Conclusions

Our integrative study revealed for the first time that in the diseased human heart ex vivo, the atria’s complex microstructure caused significant differences between Endo and Epi activation during pacing and sustained AF. Fibrotically insulated PMs and intramural myocardial bundles in the LRA create a micro-anatomic track for a spatially and temporally stable intramural re-entry that drives AF, suggesting the critical importance of human 3D atrial architecture for the maintenance of AF.

Supplementary material

Supplementary material is available at European Heart Journal online.

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References

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