# Pathophysiological insights into atrial fibrillation: Revisiting electrophysiological substrate, anatomical substrate, and possible insights from proteomics

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# Introduction

Atrial fibrillation (AF) is the most common supraventricular arrhythmia. In AF, the irregular cardiac rhythm is associated with intra-atrial blood stasis, subsequent thrombus formation and a three- to five-fold increase in the risk of stroke events.1

In the last 20 years, considerable progress has been made in understanding of the pathophysiological mechanisms leading to arrhythmogenesis, AF induced cardiac remodelling, and AF therapy. Yet, gaps in our knowledge exist and present therapies have many shortcomings. One major problem limiting long-term success of pharmacological and ablation therapies is the progressive nature of the arrhythmic substrate. Indeed, better understanding of the arrhythmic substrate and AF progression is vital for improving therapy.

This article provides a brief overview of AF-related electrophysiological substrate, anatomical substrate, their relationships, and possible new insights from proteomics. Such inter-relationships may provide new insights into AF pathophysiology, particularly into understanding the mechanisms of arrhythmogenesis and thromboembolism in this common arrhythmia.

# Electrophysiological substrates of AF

The rapid uncoordinated atrial activation during AF can be caused by rapidly discharging ectopic foci or by re-entrant activity. The ectopic foci that acts as the AF ‘trigger’ is generally a site of spontaneous action potential discharge commonly, but not exclusively localised in the pulmonary vein (PV, Figure 1).1,2

The PV has a sleeve of cardiomyocytes that extend out of the left atrium, with unique electrophysiological properties and anatomical structure (Figure 1). Indeed, rat PV cardiomyocytes display spontaneous Ca2+-waves that show increasing incidence and synchronization after higher rates of electrical stimulation.3 Such spontaneous Ca2+-release from the sarcoplasmic reticulum during diastole causing delayed afterdepolarizations increase the likelihood of ‘triggered activity’.2 Also, canine PV cardiomyocytes show less negative resting membrane potential and shorter action potential duration (APD) compared to left atrial myocytes due to smaller inward-rectifying K+-current (*I*K1), L-type Ca2+-current (*I*Ca, L) and larger slow and rapid delayed-rectifier K+-currents (*I*K, s and *I*K, r), making re-entry more likely.2 In addition, the PV sleeve has branching fibres with little lateral-coupling and abrupt changes in fibre-orientation that increase ectopic susceptibility and favours re-entry (Figure 1).2 Of note, the “pacemaker current”, *I*f responsible for automaticity in sinus node, is present in human left atrial myocytes and higher *I*f density in left atrial appendage (LAA) supports the hypothesis of *I*f as a contributor to abnormal automaticity there.2 AF can have significant heritability and the genes underlying AF risk appear in different biological pathways suggesting significant pathophysiological heterogeneity within the population.4

How can we explore in more depth and obtain deeper insights into the electromechanical substrate? Human induced pluripotent stem cell derived cardiomyocytes (hiPSCs-CMs) obtained from patient biopsies allow *in vitro* comparison of molecular and electrophysiological changes underlying inheritable forms of AF. In a recent study, Benzoni et al4 described the first iPSC-derived model of human AF, generated from 2/3 siblings that developed a drug-resistant form of AF. Fibroblasts derived from skin biopsies were differentiated into hiPSC-CMs for electrophysiological characterisation. AF hiPSC-CMs were beating at significantly higher rates due to increased levels of *I*f and *I*Ca, L, APD was prolonged, but sarcoplasmic reticulum Ca2+ handling remained unchanged. Catecholamine-mediated trigger for inducing AF is thought to involve increased atrial *I*Ca, L. In agreement with this, when stressed with isoproterenol (100 nM) and E4031 (300 nM; inhibits *I*Kr and prolongs APD), AF hiPSC-CMs presented significantly prolonged action potentials and a higher proportion displayed delayed afterdepolarizations and triggered action potentials and ectopic beats.4 HiPSC-CM technology has the potential to recapitulate AF patient phenotype, provide mechanistic insights and inform therapy, particularly in the setting of familial AF that is beset with genetic complexity.

# Anatomical (and structural) substrate

The left and right atria possess structurally unique features that contribute to AF pathogenesis.1 For example, the *crista terminalis* and vena cavas in right atrium can provide focal triggers. Atrioventricular rings surrounding the tricuspid and mitral annulus express hyperpolarization-activated cyclic nucleotide–gated (HCN) channels responsible for the chief component of *I*f and can initiate ectopy.5

In contrast, the LA posterior wall and roof have complex subendocardial fibre orientation favouring conduction delay/block and re-entry that can worsen with fibrosis. The latter can remodel the atria into a discontinuous and branching structure susceptible to multiple wavelet re-entry. In persistent AF patients, AF re-entrant drivers persisted in regions with higher levels of fibrosis and AF in fibrotic substrates is perpetuated by re-entrant drivers that persist in fibrosis boundary zones.6

Nonetheless, the precise propagation and underlying processes of AF are not completely understood although investigations have shown various factors are associated, including many signalling systems, oxidative stress, atrial stretching, and ischemia.

Extracellular matrix remodelling involving fibrosis (Figure 1) is a key component of atrial structural remodelling and matrix metalloproteinases (MMPs) have been implicated.1,2 In the right and LAA tissue obtained from AF patients with mitral valve disease undergoing mitral valve surgery or coronary artery bypass graft surgery, MMP-1 and/or MMP-9 downregulation have been observed.1 Also, the molecular mechanisms responsible for atrial fibrosis include receptors systems for angiotensin II, platelet-derived growth factor, connective tissue growth factor, and transforming growth-factor β (TGF-β).2

**New insights from proteomics**

Mass spectrometry (MS) based proteomics has been optimised to study cardiac tissue and presents an unbiased approach to investigate thousands of proteins and provide a holistic picture of molecular changes in the tissue (Figure 1). The continually improving technology associated with -omics techniques coupled to powerful bioinformatic analysis is leading to even greater insight into mechanisms and pathways involved in AF providing greater understanding, potential therapeutic target as well as diagnostic biomarkers.

Plasma proteomic profiling of patient blood have confirmed previously known associations of N-terminal pro-B-type natriuretic peptide and ADAMTS13 with AF and importantly, identified other proteins including interleukin-6, T-cell immunoglobulin and mucin domain-1.7 Fibrotic tissue is thought to express different mitochondrial proteins; however, isolation and enrichment of mitochondria from highly fibrotic atrial samples can be difficult.

Instead, proteomic workflows involving hydrostatic pressure cycling-based lysis that employs rapid cycles of alternating high and ambient hydrostatic pressure to disrupt cell membranes, followed by label-free mass spectrometric analysis, may significantly enhance extraction efficiencies. Such MS analysis of mitochondrial-enriched fractions yielded ~700 proteins, 28 and 4 of the identified proteins were more abundant in AF and non-AF tissue, respectively.8 Four of the proteins abundant in AF: chaperonin containing TCP1 subunit 5, heat shock protein 27, destrin and four and a half LIM domain 2 were verified with reverse phase protein microarray analysis and found to have higher expression in AF tissue, thus validating the MS analysis.8 Proteomics therefore offers the possibility to identify parts of the molecular landscape that are unique to AF pathophysiology.

# Electro-anatomical substrate, thrombogenesis and proteomics in atrial fibrillation

The functional and structural changes in atrial myocardium and stasis of blood, especially in the LAA, generate a prothrombotic state in AF, leading to an increased risk of thromboembolism.1 Mechanisms for thrombogenesis in AF, blood based biomarkers and management implications have recently been reviewed.1

Proteomic techniques help better understand thrombi and thrombosis and provide a novel large-scale format for biomarker discovery with improved predictive parameters. For example, the proteomic profile of thrombi retrieved from acute ischemic stroke patients identified ~1600 proteins and provided a reference proteomic fingerprint of 341 proteins common to all subjects and involved in the interaction of fibronectin with 14-3-3 proteins, TGF-β signalling, and TCP complex network and linked to thrombus formation and in ischaemic setting.9

A study by Rao *et al*.10 investigated the protein composition of retrieved emboli from patients with differing stroke aetiologies and correlated the protein levels to serum predictors of atherosclerosis. In emboli retrieved from 20 consecutive acute stroke patients by thrombectomy during routine stroke care, 81 common proteins were found, including haemoglobin, fibrin, and actin. Proteomic analysis of stroke-causing thrombi was evaluated as a diagnostic tool to determine the stroke precipitator, i.e. whether atheroembolic originating from an unstable carotid plaque, or cardioembolic from a thrombus formed in the heart due to AF.

Knowledge of the source could help ascertain treatment options: surgery or stenting followed by an antiplatelet agent in the case of the former, and anticoagulation therapy for the latter.

**Conclusion**

Substantial advances in our understanding of AF-related electrophysiological substrate, anatomical substrate, and their close inter-relationships are evident in the literature. There are now possible new insights from proteomics in gaining insights into AF pathophysiology, particularly into understanding the mechanisms of arrhythmogenesis and thrombogenesis, linking electro-anatomical substrates.

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# Figure Legend

# Figure1: Illustration of mechanisms of atrial fibrillation initiation and proteomics workflow. Cardiac cellular remodelling involving sarcolemmal ion channels and proteins and sarcoplasmic reticulum Ca2+-cycling provide the basis for rapidly discharging ectopic foci causing atrial fibrillation (AF). Extracellular matrix deposition and tissue fibrosis can cause conduction slowing/block resulting in regions of fast and slow conduction and in turn enable ectopy (purple stars) and re-entry (blue arrows). Abnormal atrial automaticity, early and delayed afterdepolarizations are the chief determining factors for focal ectopic pacemaking. The pulmonary vein myocardial sleeve has abrupt changes in fibre orientation promoting conduction delays and blocks and favours ectopic foci (purple stars). Ectopic foci can occur outside the pulmonary vein in the *crista terminalis*, vena cava, AV ring and left atrial appendage. The rapid uncoordinated atrial activation causes stasis of blood, especially in the left atrial appendage and generates a prothrombotic state in AF, leading to an increased risk of thromboembolism. The thrombus travels in the arterial blood stream until reaching a narrow blood vessel in the brain, which it blocks causing ischaemic stroke. Mass Spectrometry based proteomic profiling of blood plasma, atrial tissue biopsy and retrieved thrombi offers immense potential for biomarker discovery, gaining mechanistic insights into atrial electrophysiological and structural remodelling, and thrombogenesis, which in turn can provide novel insights into AF management and anticoagulation therapy.

# Biographical sketch of the authors

**Robert Bentley:** Robert A. Bentley, BSc MRes is a current PhD student researching at the Liverpool Centre for Cardiovascular Sciences (LCCS) and the Institute of Life Course and Medical Sciences at the University of Liverpool.

He completed his undergraduate degree in Pharmacology at the University of Liverpool and progressed to postgraduate to study a Masters of Research focussing on drug safety science in particular, cardiotoxicity caused by the chemotherapeutic treatment doxorubicin in 2D, 3D and flow systems. He also gained insight into how doxorubicin-induced toxicity can be prevented by inducing the NRF-2 pathway to regulate antioxidants.

He then worked in an industrial setting at Teva Pharmaceuticals, which provided key insight into drug manufacturing, production, safety, and quality control.

His PhD project focusses on improving the understanding of the progression of the arrhythmia atrial fibrillation (AF), by utilising a proteomic approach.

**Dr. Sunil Jit R.J. Logantha:** Sunil Jit R.J. Logantha, BPharm (Hons), MMS, MSc, PhD is a Tenure Track Fellow at the Liverpool Centre for Cardiovascular Science and Department of Cardiovascular and Metabolic Medicine, University of Liverpool. After graduating with a first class Bachelor’s degree in Pharmacy and an MSc in Pharmacology and Biotechnology with distinction, Sunil developed an active interest in medical research. He was awarded the prestigious ORSAS scholarship and University Postgraduate Scholarship to pursue PhD at the University of Strathclyde, Glasgow. For his PhD, Sunil investigated electrical properties and Ca2+-signalling in pulmonary vein cardiomyocytes and was trained in cardiac tissue electrophysiology and Ca2+-imaging. He developed a keen interest in ectopic pacemaking and arrhythmogenesis and pursued this line of research with post-doctoral training in the Cardiac Conduction System group at the University of Manchester under the aegis of Prof. Mark Boyett and Dr. Halina Dobrzynski. Sunil’s research focussed on pacemaker tissues (sinus node, atrioventricular node, and Purkinje fibres) and subsidiary pacemakers and their role in arrhythmogenesis. Sunil specialises in integrating whole animal-, organ- and tissue-level electrophysiology (ECGs and action potentials) with data from multiple sources, including histology, electron microscopy, gene and protein expression analysis, and mathematical modelling, to investigate cardiac electrical remodelling in disease. In 2020, Sunil was appointed a Tenure Track Fellow at the University of Liverpool where he continues his research interests on the pulmonary vein and atrial fibrillation, and on role of thyroid hormones in cardiovascular disease.

Sunil has 20 peer-reviewed publications to his credit and has been invited to review manuscripts for British Journal of Pharmacology, Frontiers in Pharmacology, Experimental Physiology and Circulation: Arrhythmia and Electrophysiology. He is a full member of The Physiological Society and his research has benefitted from British Heart Foundation grant funding.

**Parveen Sharma:** Parveen Sharma is a Senior Lecturer in the department of Cardiovascular and Metabolic Medicine at the University of Liverpool. She graduated with a degree in Medical Biochemistry from the University of Birmingham and then went onto study for a PhD at Birmingham investigating the interactions between the cardiac sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) and its regulating protein phospholamban. Dr Sharma then moved to the University of Toronto, Canada and obtained a fellowship from the Heart and Stroke Foundation of Canada to investigate mutations in the Ryanodine Receptor responsible for malignant hyperthermia with Prof David MacLennan. She then became a Research Associate with Prof Anthony Gramolini at the University of Toronto investigating cardiovascular pathophysiology and cardiomyopathy. Dr Sharma then returned UK and took up a lectureship at the University of Liverpool in the Department of Pharmacology. Her group are interested in drug induced cardiotoxicity with an emphasis on investigating cardioprotection from oncology drugs. She has benefited from funding from the MRC and North West Cancer. She has led and contributed to, over 40 publications and has served as a reviewer for several journals.

**Richard Rainbow:** Richard D Rainbow is a Senior Lecturer in the department of Cardiovascular and Metabolic Medicine at the University of Liverpool. Dr Rainbow graduated with a Medical Biochemistry Degree from the University of Leicester and went on to study for a PhD investigating the cardiac ATP-sensitive Potassium Channel with Prof Nick Standen and Dr Noel Davies at the University of Leicester. Following a post-doctoral role in Leicester investigating the regulation of potassium channels by protein kinases, he moved to the University of Strathclyde to research the calcium store arrangement in vascular smooth muscle with Prof John McCarron. Dr Rainbow returned to Leicester in 2008 as a tenure track fellow investigating mechanisms of cardioprotection and toxicity. Dr Rainbow runs a research group that continues to investigate mechanisms of protection and toxicity with particular expertise in ion channel physiology, calcium homeostasis and the effects of metabolic dysregulation on cardiovascular function. His group recently moved to the University of Liverpool and continue to benefit from research funding from the British Heart Foundation and from MRC confidence in concept awards to both Leicester and Liverpool. He has contributed to, or led, over 30 publications and has also contributed to book chapters.

**Gregory Y.H. Lip:** Professor Lip, MD, is Price-Evans Chair of Cardiovascular Medicine, at the University of Liverpool, UK – and Director of the Liverpool Centre for Cardiovascular Science at the University of Liverpool and Liverpool Heart & Chest Hospital. He is also Distinguished Professor at Aalborg University, Denmark; Adjunct Professor at Yonsei University and Seoul National University, Seoul, Korea. He also holds Visiting or Honorary Professorships in various other Universities in UK, Serbia (Belgrade), China (Beijing, Nanjing, Guangzhou), Thailand (Chiangmai) and Taiwan (Taipei).

Half of his time is spent as a clinical cardiologist, including outpatient clinics (leading atrial fibrillation and hypertension specialist services) and acute cardiology.

Professor Lip has had a major interest into the epidemiology of atrial fibrillation (AF), as well as the pathophysiology of thromboembolism in this arrhythmia. Furthermore, he has been researching stroke and bleeding risk factors, and improvements in clinical risk stratification. The CHA2DS2-VASc and HAS-BLED scores - for assessing stroke and bleeding risk, respectively – were first proposed and independently validated following his research, and are now incorporated into international guidelines. In 2014, Professor Lip was ranked by Expertscape as the world's leading expert in the understanding and treatment of AF [http://bit.ly/2apB1Dt], a position still maintained in 2020 (<https://bit.ly/3eP3qR4>).

Professor Lip was on the writing committee for various international guidelines, including the American College of Chest Physicians (ACCP) Antithrombotic Therapy Guidelines for Atrial Fibrillation, as well as various guidelines and/or position statements from the European Society of Cardiology (ESC) or EHRA. He was recently Chair of the new 2018 ACCP Guidelines on antithrombotic therapy for AF.

Professor Lip has acted as senior/section editor for major international textbooks and at senior editorial level for major international journals, including Thrombosis & Haemostasis (Editor-in-Chief, Clinical Studies); Europace (Associate Editor); and Circulation (Guest Editor).