Novel Personalised Determinants of Atrial Substrate in Atrial Fibrillation

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Abstract

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia encountered in clinical practice in developed countries, with a rising incidence reaching epidemic proportions. Beyond adverse impacts on quality of life, AF is associated with significant morbidity, heart failure, stroke and a markedly higher risk of mortality. Current understanding incorporates contributions from focal triggers and remodelled atrial substrate, however the precise interactions between these mechanisms remain incompletely understood. The paradigm of AF management over the last decade has evolved to a more lifestyle directed and holistic approach rather than basic pharmacological rate versus rhythm control measures. However, the AF disease process is multifactorial and the optimal treatment of particularly persistent forms of AF continues to be elusive.

This thesis aims to evaluate novel mechanisms and influences on electroanatomic atrial substrate contributing to AF which may form the basis for emerging strategies in personalised AF therapy. Initially, we assess the genetic predisposition to AF by effects on atrial substrate and post AF ablation outcomes. We explore the impact of novel pacing strategies and sex-differences on atrial substrate in patients with AF. Finally, we define the influence of AF on sinus node and crista terminalis characteristics.

Chapter 1 delineates the role of genetics in AF, a rapidly progressive area in cardiovascular medicine. We then explore the evolving understanding of pathogenesis of the AF mechanism with an emphasis on the impact and importance of atrial substrate, sex-differences and sinus node remodelling.
Chapter 2 investigates the impact of genetic susceptibility in patients with AF to electrical and structural remodelling and outcomes. Chapter 2 is a prospective cohort study of 102 patients undergoing AF ablation who undergo genetic sequencing for a 4q25 single nucleotide variant (SNV) and high-density electroanatomical mapping of their left atria. The genetic aspects were completed under the supervision of Prof Diane Fatkin’s Inherited Heart Diseases Laboratory at the Victor Chang Cardiac Research Institute. We document long-term outcomes 2 years post ablation utilising high intensity monitoring including insertable loop recorders and regular Holter monitors. We compare carriers and non-carriers to determine whether there are differences in electrophysiological and conduction properties between groups. We conclude that the 4q25 variants is associated with adverse atrial remodelling characterised by greater conduction heterogeneity and presence of complex fractionated signals with poorer long-term outcomes.

Chapter 3 and 4 examine the impact of pacing strategies on electroanatomic atrial substrate in patients with AF. Chapter 3 describes rate-dependent conduction differences in maps created at different cycle-lengths in 56 patients with a history of AF. It observes globally greater atrial substrate at a faster cycle-length across multiple electrophysiologic parameters including voltage, conduction velocity and complex signals. Chapter 4 then evaluates the impact of direction-dependent conduction in 17 patients with AF when pacing from the pulmonary vein. It concludes a highly regional increase in atrial substrate posteriorly. Together, these data suggest the dynamic nature of atrial substrate maps with marked variation according to changes in pacing rate and direction.
Chapter 5 focusses on the comparison of electrophysiologic properties underpinning sex-based differences in AF as despite having a lower incidence of AF compared with men, women carry higher risks of stroke and adverse AF-related outcomes. We perform a cross-sectional electroanatomic mapping study of 93 patients with AF and 45 control patients with SVT. Interestingly, in both patients with and without AF, women have a greater degree of atrial substrate when compared with men. Coupled with these substrate differences, we demonstrate that women had higher single and multi-procedure arrhythmia-recurrence following AF ablation.

Chapter 6 presents an ultra high-density mapping study investigating the characteristics of the sinus node and anatomically-determined regions of right atrial substrate in relation to AF. We conduct a mapping study on 25 patients with AF and 25 age-matched controls undergoing SVT ablation. Key findings included more significant sinus node remodelling in patients with AF and persistent forms of AF in particular characterised by progressive caudal shifts in sinus node activation, loss of multicentricity, lower sinus node voltage and greater ‘latent’ substrate at the crista terminalis.

Chapter 7 concludes the thesis by summarising the pertinent translational findings and implications for the clinical outlook of each study. Moreover, the future directions of novel mechanisms of AF may help pave the way for personalised AF strategies to better treat AF.
Declaration

This thesis is the sole work of the author and the material contained herein has not been previously published or written by another person except where due reference has been made in the text. The work was performed by the candidate in Melbourne at the Cardiology Departments of the Royal Melbourne Hospital, the Baker Heart and Diabetes Institute, and the Alfred Hospital’s Heart Centre for the express purpose of this thesis and no part thereof has previously been presented for the award of a degree at this or any other university.

I certify that the writing of this thesis, the results, interpretations, opinions and suggestions are entirely my own work. This thesis does not exceed the length of 100,000 words exclusive of table, figures, appendices and bibliography.

Geoffrey R. Wong

[Electronically approved]
Preface

This work was performed in collaboration with a number of institutions including:

1. The Royal Melbourne Hospital (Department of Cardiology)
2. The Alfred Hospital (Department of Cardiology)
3. The University of Melbourne (Department of Medicine)
4. Victor Chang Cardiac Research Institute (Department of Molecular Genetics)

All manuscripts and publications emanating from this thesis were undertaken with the candidate as the principal author. Responsibilities included: protocol design, ethics and governance application, participant recruitment and follow-up, data collection, statistical analysis, and manuscript preparation. PhD supervisors performed limited revision and editing of submitted manuscripts only. Additional co-authors listed in publications assisted in one or more of the above aspects, in addition to critical revision of manuscripts, consistent with co-authorship requirements for the respective journals.

Chapter 2 required critical input from the second listed co-author predominantly for patient recruitment and data collection, who was afforded co-principal status in the accepted manuscript, although my contribution was >50%. However no co-authors had any role in drafting the manuscripts and chapters contained herein (excluding critical revision) and those works do not form components of any other submitted body of work.

Financial support for this PhD was provided by:

2. Cardiac Society of Australia & New Zealand Research Scholarship – 2016

A full list of publications and published abstracts emanating from this body of work is provided in the section entitled ‘Peer reviewed publications’.
Peer-reviewed publications

Chapter 1
Published manuscript


Chapter 2
Submitted manuscript

❖ Wong GR et al. Genetic susceptibility to atrial fibrillation is associated with left atrial remodelling and worse long-term post-ablation outcomes. (under review by European Heart Journal as of October 2019)

Published abstracts

❖ Wong GR et al. Genetic susceptibility to atrial fibrillation at the 4q25 locus is associated with left atrial remodelling (Finalist – Ralph Reader Young Investigator Award; Cardiac Society of Australia & New Zealand 2019, Adelaide)

❖ Wong GR et al. Genetic susceptibility to atrial fibrillation is associated with left atrial remodelling and adverse long-term post-ablation outcomes. (Finalist – Samuel L. Levine Young Investigator Award; American Heart Association 2019, Philadelphia)
Chapter 3

Published abstracts

- **Wong GR et al.** Regional Atrial Substrate Abnormalities in Persistent AF with High Density Mapping: Implications for Substrate Ablation (Heart Rhythm Society 2017, Chicago)

- **Wong GR et al.** Incremental Pacing Identifies Target Atrial Substrate in Atrial Fibrillation (Asia Pacific Heart Rhythm Society 2018, Taipei)

- **Wong GR et al.** Incremental Pacing Identifies Target Atrial Substrate in Paroxysmal and Persistent Atrial Fibrillation (Cardiac Society of Australia and New Zealand 2018, Brisbane)

Chapter 4

Published manuscript


Published abstract

- **Wong GR et al.** Rate & Direction-Dependent Changes in Atrial Substrate with High-Density Mapping of AF (Heart Rhythm Society 2019, San Francisco)
Chapter 5
Submitted manuscript

- **Wong GR** et al. *Gender related differences in atrial remodelling in patients with atrial fibrillation: Relationship to ablation outcomes* (under review by Heart Rhythm Journal as of October 2019)

Published abstracts

- **Wong GR** et al. *Greater Atrial Remodelling in Females using High Density Electro-anatomic Mapping* (Runner-up & Finalist – Best moderated abstract award, Asia Pacific Heart Rhythm Society 2018, Taipei)

- **Wong GR** et al. *Greater Atrial Remodelling in Females using High Density Electro-anatomic Mapping* (Heart Rhythm Society 2018, Boston)

- **Wong GR** et al. *Greater Atrial Remodelling in Females using High Density Electro-anatomic Mapping* (Cardiac Society of Australia & New Zealand 2018, Brisbane)

Chapter 6
Submitted manuscript

- **Wong GR** et al. *Sinus Node Remodelling in Atrial Fibrillation: Insights from High-density Mapping* (under review by Heart Rhythm as of October 2019)
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This PhD represents the culmination of one of the most difficult yet rewarding challenges of my life. Seemingly insurmountable at times, it is with the utmost gratitude that I acknowledge the help and support from many individuals and institutions without which this thesis would not be possible.

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To my parents, my late father Philip and my mother Ruth, I am forever in admiration of and inspired by your selfless desire to provide unconditionally for your children. I cherish the life lessons and the never-ending prayers. None of this would be possible without you both, and your core values of work ethic, diligence and dedication. These have been strongly instilled in me and my only hope is that I have made you both proud. To my brothers, Jonas, your strong will in the face of adversity is a constant source of inspiration for me, and Davy, your independent resourcefulness is always welcome.
Finally, to my wife Megan, your enduring love and patience knows no bounds. Your sensible, pragmatic and practical approach to all situations is always a breath of fresh air and perfectly complementary. Thank you for always listening and for your unending support through this entire journey, all whilst completing tremendously difficult radiology examinations and planning the wedding of a lifetime. Megan, I dedicate this thesis to you.
For Ruth & Megan

In loving memory of Philip
Abbreviations

AAD  Anti Arrhythmic Drug
ACT  Activated Clotting Time
AF   Atrial Fibrillation
AP   Anteroposterior
APD  Action Potential Duration
BIFA Box-Isolation of Fibrotic Areas
BMI  Body Mass Index
CA   Catheter ablation
CI   Confidence interval
CMR  Cardiac Magnetic Resonance
CFAE Complex Fractionated Atrial Electrogram
CRP  C-Reactive protein
CoV  Coefficient of Variation
CS   Coronary Sinus
CSNRT Corrected Sinus Node Recovery Time
CT   Computed Tomography
CV   Conduction Velocity
CP   Complex Points
DNA  Deoxyribonucleic Acid
DP   Double Potential
EAS  Earliest Activation Site
ERP  Effective Refractory Period
FP   Fractionated Potential

XIV
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<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>rLVZ</td>
<td>Relative Low Voltage Zone</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>SBO</td>
<td>Sinus Break-Out</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SNV</td>
<td>Single Nucleotide Variant</td>
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<tr>
<td>TIA</td>
<td>Transient Ischaemic Attack</td>
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<td>TOE</td>
<td>Transoesophageal Echocardiogram</td>
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Chapter 1:

Literature Review

Introduction

Atrial fibrillation (AF) is the most frequently encountered cardiac rhythm disorder in clinical practice. Its prevalence is overall approximately 1%, but progressively increases with age, with a prevalence of up to 10% in individuals above 80 years of age. An individual’s lifetime risk of developing AF reaches almost 25% in those aged over 40 (1,2). AF can significantly impair quality of life through development of symptoms including palpitations, lethargy and breathlessness, and is independently associated with poorer outcomes owing to increased risks of heart failure, ischaemic stroke and death (3,4).

A large section of this PhD is devoted to novel personalised determinants of atrial substrate and outcomes in patients with AF to help better understand the management of this complex condition in context of the electrical changes in the heart. These include the genetic link with AF, strategies to help optimise substrate-based ablation approaches, sex- differences in AF and high-density mapping of right atrial structures such as the sinus node and crista terminalis involved in the AF-remodelling process.

Epidemiology of atrial fibrillation

AF is a highly variable condition characterised by distinct phenotypes on a clinical spectrum that often begins with clinical risk factors (5,6) that can lead to marked electrical and structural remodelling in the left atrium (LA) and predispose to the development of AF. The phenotypes of AF range from a paroxysmal form of self-
terminating episodes lasting less than 7 days, to persistent AF (lasting longer than 7 days), longstanding persistent and finally permanent AF when sinus rhythm is no longer achievable. The clinical progression of AF has been well established, with a 25% risk of moving from a paroxysmal to persistent phenotype over a 5 year duration (7,8). Moreover, the incidence of AF is growing at an exponential rate with a 60% increase over the last 20 years (9), and expected to increase 2.5-fold in the next 30 years, particularly in context of ageing populations worldwide (1). Anchoring this evolution is a dynamic relationship between electrical ectopic triggers of AF and atrial remodelling. During the early stages of paroxysmal AF, electrical triggers play a predominant role. However, as AF progresses, electrophysiological changes in the atria occur in addition to triggers. This atrial myocardial remodelling provides the substrate that promotes the progression and perpetuation of AF.

**Morbidity, mortality and economic impact**

Atrial fibrillation results in significant morbidity and is associated with a 3-fold increased risk of progressing to heart failure(10), a 5-fold risk of ischaemic stroke (11), and 2-fold risk of cognitive decline and onset of dementia (12). After adjustment for multiple risk factors including age, AF is associated with a 1.5 and 1.9 fold-increased risk in mortality for both men and women respectively (3).

Atrial fibrillation represents a significant burden on healthcare both in Australia and worldwide, accounting for one third of hospital admissions for cardiac arrhythmias (13). The huge cost of AF health expenditure primarily relates to hospitalisation (52% of costs), with the average annual AF-related cost estimated as being up to €3000/year/patient (14).
Clinical atrial fibrillation risk factors

Numerous non-modifiable and modifiable clinical risk factors can predispose to the development of AF. At a population health level, the Framingham Heart study has been a rich source of data. Non-modifiable risk factors for AF development in the Framingham Heart Study include age (for every decade increase in age, odds ratio [OR] 2.1 [men], 2.2 [women]) and male sex (OR 1.5) (6). Modifiable cardiovascular risk factors contribute to greater than half of newly diagnosed AF cases (15). Independent predictors of incident AF include diabetes mellitus, hypertension, valvular heart disease, myocardial infarction, and heart failure (6). Structural cardiac remodelling with parameters such as increased left atrial dimensions (Hazard Ratio [HR] 1.39), reduced left ventricular fractional shortening (HR 1.34 per 5% decrease) and LV wall thickness (HR 1.28 per 4mm increase) have been found to confer heightened risks of incident AF (5,16). Progressive diastolic impairment is also predictive of new-onset AF (17).

More recent risk factors shown to predispose to the development of AF include obesity (18), obstructive sleep apnoea (19), aortic stiffness (20) and alcohol (21). Body Mass Index (BMI) appears to be a strong contributor to AF risk with each unit increase associated with a 4% increase in AF, even after adjusting for traditional cardiovascular conditions (22). Often co-morbid in obese patients, sleep apnoea is an independent predictor for AF development (OR 2.2) (23). Pericardial fat on computed tomography (CT) has also been shown to be a multivariable predictor of AF (OR 1.28 for each standard deviation increase in epicardial adipose tissue) in the Framingham Offspring Study (24).
Infective and inflammatory states have been implicated in triggering AF. In a study of over 60,000 US Medicare patients in hospital with sepsis, the overall incidence of AF was 25.5% with the majority having a prior history of AF (18.3%) and the remaining cases being new AF (7.2%) (25). In the Cardiovascular Health Study, patients with elevated C-reactive protein (CRP) levels at baseline had a greater risk of future AF episodes compared to those with baseline CRP within normal limits (26).

Additional risk factors for AF include chronic kidney injury (adjusted HR 2.2) (27), obstructive airways disease (OR 1.3 [FEV1 60-80%], OR 1.8 [FEV1<60%]) (28), hyperthyroidism (adjusted HR 1.98; 95% confidence interval [CI], 1.29-3.03) (29), and elite athletic training (30). Post-operative AF after cardiothoracic surgery also frequently occurs in up to 30% of patients after lung resection or coronary artery bypass surgeries and 60% following concomitant bypass graft and valvular operations (31).

**Genetics and atrial fibrillation**

Personalised medicine is a burgeoning and rapidly progressive field, particularly in cardiovascular healthcare and research. In addition to the role played by environmental and clinical factors in the aetiopathogenesis of AF, it is increasingly recognised that genetic factors are also involved. At present, however, relatively little is known about what these genetic factors are and how they cause AF. Individualised patient-centred management has recently been a pivotal focus of improving the care of patients with AF (32). Whereas many studies have focussed on modifiable lifestyle strategies (33), there is emerging data to support the potential utility of patient-specific factors such as genetics to help guide risk scores or patient-tailored therapeutic targets (34). Early studies demonstrated that a family history of AF was associated with a 40-70%
increased risk of AF in offspring (35,36) and a Danish twin study found greater than 60% of AF heterogeneity was potentially explained by genetic influences (37). Rare variants sufficient to cause disease have been identified in families with AF (38). However, familial forms of AF are thought to be relatively uncommon overall. Numerous common variants that affect vulnerability to AF have been identified by genome wide association study (GWAS), with recent data reported in >500,000 subjects (39).

**Rare Single Gene Mutations**

Early work of the role of genetics in AF have largely been obtained from studies of early-onset (<65 years) lone AF or familial AF with Mendelian inheritance patterns. Single gene mutations that have a large functional effect size have been identified in a small number of families with AF and these mutations are thought to have a primary role in disease pathogenesis (40). The majority of these disease-causing mutations have been identified in genes and transcription factors encoding cardiac potassium and sodium channel sub-units impacting on various ion-channel components involved in action potential generation. Over fifty rare and highly penetrant mutations associated with AF have been detected (34). However, study of these monogenic genes is limited to linkage and segregation analysis in large families and not directly applicable to the general population.

**Stretch-sensitive Gene Mutations**

It is likely that in many people with AF there are multiple contributing factors that may variably include genetic susceptibility, increasing age, atrial dilatation and co-morbidities such as hypertension. These factors do not necessarily act independently.
and there may be significant functional interactions. Atrial stretch is one such environmental factor which might modify the functional effects of genetic variants. Fatkin’s group identified a novel missense mutation within the \textit{KCNQ1} gene, which encodes the alpha-subunit of the cardiac $I_{Ks}$ channel, in a large family with both AF and a high prevalence of hypertension associated with atrial dilatation (41). Although hypertension was present in a majority of individuals in the older generation, only those family members who had both the \textit{KCNQ1} variant \textit{and} hypertension-associated atrial dilatation developed AF. These findings were confirmed by patch-clamp studies of transfected cells that showed no effects of the mutant \textit{KCNQ1} on $I_{Ks}$ under baseline conditions, but a gain-of-function effect when cells were stretched. These seminal observations suggest that in populations of patients with hypertension and other conditions that result in atrial dilation, that there may be subgroups of individuals who are genetically-predisposed to develop AF.

\textit{Common Variants in AF}

In contrast to rare highly-penetrant variants with large effects sizes, common variants with smaller effect sizes in multifactorial common disease can be studied by GWAS. GWAS’ performed in large groups of unrelated individuals with AF have identified a number of chromosomal loci in which groups of single nucleotide variants (SNVs) are associated with an increased susceptibility to AF. The first seminal GWAS by Gudbjartsson and colleagues in 2007 studied an Icelandic population of 50,000 patients and identified a significant SNV in an intergenic region on chromosome 4q25 (42). Since then, international collaborative efforts in pooled initiatives such as the AFGen Consortium have helped to dramatically expand the knowledge in this area with ever increasing population genetic studies of AF. These have continued to identify new
variants and loci implicated in AF (43,44). A recent meta-analysis of almost up to half a million patients including those with and without AF identified twelve novel genetic loci potentially important in AF (45). To date, over 30 GWAS loci significantly associated with AF have been reported. With growing numbers of patient data available in GWAS of AF, the 4q25 locus remains the most significantly associated with AF, robustly replicated variant in studies of hundreds of thousands of patients (45,46).

The majority of SNV loci occur in non-coding regions of the genome known as ‘genetic deserts’. Intergenic regions or ‘introns’ were once thought to not play a significant role compared with gene coding regions or ‘exons’ however current thinking is that this is not necessarily the case (34). Despite not being in gene-coding regions, the SNV’s in ‘genetic deserts’ are presumed to play a role in regulatory elements of downstream factors including transcription factor binding sites with important function altering roles in promoter or repressor expression regulating target genes. Further, SNV’s exist within haplotypes and may co-localise or ‘tag’ other SNV’s which have more direct roles on regulator expression (47). The complexity of loci identified from GWAS in AF is such that downstream target genes may be located nearby on the same chromosome or remotely on an entirely different chromosome. This poses a challenge for the identification of downstream genetic targets of variant-related expression and the current target genes of AF-associated loci such as 4q25 have yet to be discovered. However, the prospect of linking genetics to AF is accompanied by significant implications for AF mechanisms, novel downstream targets, personalised medicine and risk stratification.
Chromosome 4q25 and Atrial Fibrillation

Several distinct haplotypes located within chromosome 4q25 have been independently associated with AF risk with the strongest association consistently being found for the rs2200733 haplotype. Additionally, ischaemic stroke has been demonstrated to correlate with LA fibrosis (48) and 4q25 SNV’s (49). An explanation for the links between this locus and AF has remained elusive. The only intermediate phenotype reported to date has been subtle PR prolongation but this was only seen in homozygous SNV carriers, with no changes seen in the relatively greater number of heterozygous individuals (50).

Although these associations have been robustly replicated in tens of thousands of people, the underlying mechanisms linking these SNVs to AF have not yet been identified (46).

PITX2 and Atrial Substrate

The 4q25 locus is located in a region of the genome that is devoid of genes, and it has been hypothesised that this region contains a regulatory element that affects gene expression. The closest gene is paired-like homeodomain transcription factor 2 (PITX2), a cardiac transcription factor implicated in heart development. Although no target gene of the 4q25 variant has been identified, several lines of evidence support PITX2 being a good candidate gene for AF and it has been widely implicated owing to its proximity to the 4q25 locus (51-54). PITX2 is located 150 kilobases upstream and the PITX2c isoform has been implicated as a key determinant of cardiac left-right asymmetry (LRA) (53) and ion channel regulation (55). Knockout mice studies have shown that deficiency of PITX2 leads to altered electrophysiological and structural
properties of the atria and pulmonary vein which might be expected to contribute to a substrate for AF (51-54). Two studies have identified potential enhancer sequences in the 4q25 locus that interact with the PITX2c promoter (56,57). In mice and humans, PITX2 expression is greater in the left atrium than the right atrium (58). However, despite PITX2’s role in cardiac development and possible candidate gene, there are currently no data that show convincing evidence that the RS2200733 haplotype is associated with altered PITX2c expression in the adult human LA. In a large group of patients undergoing thorascopic AF ablation, SNV status did not appear to correlate with PITX2 mRNA expression in specimens sampled from human left atrial appendages (59).

*PITX2 and Atrial Conduction*

Proposed effects of PITX2 deficiency, based on data from PITX2 knockout mice, include defective development of the pulmonary vein myocardial sleeve or failure of sinus node-related genes suppression in the atrium. Fibrotic changes in side-to-side cell electrical coupling, connexin expression and resting membrane changes which may form the substrate for re-entry necessary for maintenance of AF (60). Supporting this notion, PITX2 has been implicated in structural changes in intercalated discs and the regulation of multiple downstream targets including gap junction proteins and ion channels (61). Translational work from Syeda and colleagues elegantly demonstrated that PITX2 plays an integral role in regulating atrial myocyte membrane potential with flecainide-mediated sodium channel blockade being more effective in PITX2 deficient mice and human models (59). Moreover, PITX2 may be regionally expressed given its role in left-right asymmetry (53) and studies in humans with mitral valve disease have
shown relative enrichment of $PITX2$ at the pulmonary vein-left atrial (PV-LA) junction (62).

**Transcription factors and AF**

Effects on ion channels, myocardial structural components and transcription factors may lead to electrical and structural changes which contribute to AF. Several genetic studies have demonstrated associations between AF and transcription factors including $GATA-4/5/6$, $HAND2$, $NKX2-5$, $NKX2-6$ and $TBX5$ (63). These transcription factors potentially interact with $PITX2$ in AF pathogenesis and are necessary in the differentiation of cardio-myocyte precursor cells with mutations leading to congenital heart defects. Recent evidence suggests that the developmental effects of these various transcription factors are also expressed in the adult heart and stress-induced hypertrophic remodelling (64). Altered transcriptional activity has been shown in luciferase assays of rare variants such as $SHOX2$. However, the precise mechanisms linking SNV’s and atrial arrhythmogenesis still requires clarification. The lack of correlation between downstream candidate gene expression and variant allele copies may be a reflection of disparate temporal associations from a developmental template to transient promoter or enhancer related expression in mature hearts. It is also important to note that functional sequelae of genetic mutations are likely to be dependent on both intrinsic and extrinsic factors which may impact genetic variant expression and downstream protein function (65). Complicating the genetic landscape further, is that the AF disease process itself may have effects on transcriptional expression (66) in addition to novel epigenetic mechanisms that may mediate gene expression in the adult via DNA methylation and histone modification (67,68).
Genetics in AF management

The clinical yield of panel testing and screening of AF associated genes is unclear. Current guidelines do not recommend genetic screening in the routine assessment of familial AF (69). However, with the rapid progression and decreasing cost of genetic sequencing technologies, these guidelines may change in the future. Further evaluation and characterisation of overlap phenotypes such as SCN5a-related sick sinus syndrome and Brugada syndrome will facilitate a greater understanding of specific genes which may be tested on a case-by-case basis. As the incidence of monogenic familial AF is relatively low in the population, genetic risk scores (GRS) which aggregate common SNV’s within an individual have been shown to have incremental predictive efficacy over clinical AF risk factors (70). In this study, Lubitz and colleagues demonstrated strong GRS associations with ischaemic stroke and AF. GRS studies have also been shown to predict other AF-associated cardiovascular diseases including hypertension (71), coronary artery disease (72) and obesity (73). However, the clinical utility of these scoring systems and specific therapies to inhibit downstream target genes still requires further investigation.

Mechanisms of atrial fibrillation

The precise mechanism of atrial fibrillation has not yet been fully elucidated. Despite this, three early theories include multiple random propagating wavelets, rapidly firing ectopic foci, and reentrant activity with fibrillatory conduction (74).

Multiple wavelet hypothesis

The multiple wavelet hypothesis is the most widely accepted working explanation of the AF mechanism. In late 1950s and early 1960s, Moe first theorised that AF is formed
by a critical number of wavefronts propagating into refractory atrial tissue leading to fractionation and multiple wandering daughter wavefronts. These daughter wavefronts independently propagate in a random manner, colliding to create new wavefronts or terminate others in a heterogeneous atrial substrate (75,76). The heterogeneity in shortened atrial refractoriness, increased atrial mass and slowed conduction velocity was shown in Moe’s computer modelling of atrial tissue to facilitate AF perpetuation (75). In a canine model, when clamping the left atrial appendage after AF induction would lead to sudden AF cessation in the appendage while arrhythmia continued in the remainder of the left atrium. Moe et al posited that the LAA atrial mass was insufficient to support the minimum number of wavelets required to sustain AF, consistent with the findings of Garrey over a hundred years earlier (77). Allessie and co-workers validated these models suggested that four to six wavelets were necessary to maintain AF with greater numbers reducing the likelihood of spontaneous termination due to random wavefront collision extinguish all wavelets (78).

In the early 1990s Cox and colleagues developed a surgical model of intra-operative atrial activation mapping of AF in both canines and humans (79). This group found non-linear conduction around zones of functional bi-directional block with multiple wave-fronts occurred in AF. Subsequently, Cox et al developed a successful and currently utilised operative approach to treat AF designed to compartmentalise the atria by surgical incisions and render the atrial incapable of sustaining AF (79). A recent high density epicardial mapping study of patients with persistent AF undergoing cardiac surgery, showed that AF was associated with multiple propagating wave-fronts (56.2 ± 32%) or disorganised activity (24.2 ± 30.3%), with a minority of maps (<6%) having a single stable activation pattern (80).
**Focal triggers**

The AF mechanism has been described as the interplay between initiating ectopic triggers and the electrophysiological substrate that perpetuates the arrhythmia. Haissaguerre and colleagues performed the seminal work which demonstrated that the majority of AF triggers originated from the pulmonary veins, and the outcomes of targeting these triggers on the maintenance of sinus rhythm (81-83). This pioneering study evaluated 45 patients with paroxysmal AF by placing a multi-electrode catheter inside the pulmonary veins and found that the ectopic foci which triggered AF were earliest in the pulmonary veins in 94% of patients. These triggers could be eliminated by focal ablation in the pulmonary veins which cured AF in up to 60% of patients (83).

Although the mechanism of focal PV firing has not been established, there are several possible explanations. These include abrupt changes in fibre orientation (84), conduction delay at the proximal PVs (85), heterogeneous PV pacemaker cells/action potential properties, and shorter refractory period compared to non-PV tissue (86). Taken together, these properties represent substrate for re-entry across the pulmonary venous-left atrial (PV-LA) junction, leading to non-uniform anisotropy and development of AF (74).

Possible alternative mechanisms of AF triggers include PV automaticity and triggered activity. Rabbit and canine models of PV electrophysiology found that rapid pacing and thyroid stimulation was associated with early and late afterdepolarisations consistent with triggered activity and PV pacemaker cell activity which enhanced automaticity (87-89). These focal triggers were validated in a canine PV model of rapid pacing induced persistent AF, not seen in the control group (90).
Endocardial and epicardial mapping studies performed at high density have expanded the understanding of electrophysiological substrate associated with AF drivers arising from the PV-LA junction. A study using multi-electrode high resolution basket catheters in the left atrium by Kumagai and colleagues showed that the PV-LA endocardium exhibited heterogeneity of atrial refractoriness, anisotropy, and re-entrant tachycardia (91). In an epicardial mapping study, Prof Kalman’s group showed that a majority of patients with AF possessed three patterns of conduction slowing or conduction block at the PV-LA junction, potentially creating a substrate for the development of AF (92). Further, non-pulmonary vein triggers of AF have been shown to occur in up to a third of patients across the left atrial posterior wall, coronary sinus, left atrial appendage, crista terminalis, superior vena cava, ligament of Marshall and peri-annular locations (74).

Localised re-entrant activity

Localised re-entrant activity associated with AF characterised by re-entry around a fixed anatomical obstacle was initially proposed by Lewis in the 1920s (93). Subsequently, Allessie and co-workers proposed the leading circle theory of functional re-entry around a refractory core (94) and spiral waves (95) were reported. Deterministic mechanisms due to stable electrical rotors have been shown to be the driving source of functional re-entry comprised of 2-dimensional reverberations which radiate high speed spiral waves into adjacent tissue from a phase singularity (96). In basic literature, Winfree first described a rotor as a stable cyclic activation pattern of rotation and diffusion around a central core, also known as a phase singularity (97,98). The phase singularity is defined as the point at which the depolarizing head of the wave-front meets the repolarizing tail, without electrical excitation or an excitable gap (99).
As rotor cores are characterised by functional rather than anatomic re-entry, rotors have been shown to precess within a defined area (100). This movement or precession may potentially play a role in the apparent disorganised re-entrant activity in fibrillatory conduction in AF. The ionic basis of rotors may relate to potassium channels as shown in a study of transfected monolayers of cardiomyocytes, which showed that $I_{KS}$ currents play an important role in promoting rotors (101). Transgenic mice expressing an up-regulation in $I_{K1}$ expression exhibited rapid and stable rotors not observed in control mice (102).

Rotor spiral waves have also been found to exhibit significant heterogeneity in electrophysiologic measures of refractory period, conduction velocity and wavelength owing to the source-sink imbalance generated by the convex wave-front (103). The spiral wave core contains the greatest source-sink imbalance and shortest wave-length as a function of reduced conduction velocities and reduced action potential duration (104). Rotors localized to the PV-left atrial junction potentially act as the source of repetitive fibrillatory wave-fronts which collide and fuse to maintain AF (98).

In an ovine model, Kalifa and colleagues showed that the formation of rotors may be modulated by left atrial volume loading. This study found that the dominant frequency of activity at the PV-LA junction was higher with increased intra-atrial pressures $>10\text{cm H}_2\text{O}$, with a significant correlation between LA pressure and the number of rotors propagating from the PV antral region (105).
Importance of the posterior wall

Substantial evidence exists to suggest that the posterior LA may be an important contributor to the AF mechanism (106-110). Oral et al found that the site of the shortest tachycardia-cycle length alternated from the PV to the posterior wall between rapid firing of PV tachycardia during spontaneous and pacing-induced AF (111). Following disconnection after pulmonary vein isolation (PVI), AF inducibility was reduced and PV tachycardias during AF terminated suggesting that there was an association between the PV and posterior wall. Subsequently, Ndrepepa and co-workers mapped AF with a multi-electrode basket catheter and demonstrated that the posterior wall had shorter cycle lengths than the pulmonary veins consistent with passive organised PV proximal-distal activation patterns (112). In keeping with this, Kumagai et al postulated that PV rapid firing resulted in comparable cycle lengths in the other PVs facilitated by conduction across the posterior wall of the left atrium (113). Dominant frequency (DF), the distribution of spatial-temporal excitation frequencies measured in AF using spectral electrogram techniques, has been shown to be highest at the posterior wall and PV-LA junction (106,114). Kalifa and colleagues showed that the highest posterior LA dominant frequency was associated with wavefront fractionation around these sites suggesting that function re-entry in the posterior LA may play a role in the perpetuation of AF (115). Mandapati and colleagues identified a left to right gradient in dominant frequency in a Langendorff-perfused sheep heart model utilising optical mapping and bipolar electrogram analysis, and concluded that the posterior LA was the site of highest dominant frequency in 80% of cases with transient rotor like characteristics (114). In keeping with these findings, Sanders et al showed a similar left-to-right gradient in dominant frequency in persistent AF patients who were more likely to have
a dominant frequency outside of the PV-LA junction compared to paroxysmal AF patients (106).

Regarding atrial substrate, the posterior wall has also been shown to have a higher proportion of low voltage zones (LVZs) in patients with AF when compared to the anterior wall (116) which may have impacts on long term outcome (117).

The normal anatomical structure of the left atrium across the posterior wall may serve as a predisposing factor to AF through abrupt changes in fibre orientation leading to both anatomical and function block sufficient to facilitate re-entry. In an elegant anatomical pathology and endocardial mapping study, Markides and colleagues studied patients with paroxysmal AF and demonstrated a cranio-caudal line of functional conduction block across the posterior LA traversing from the roof, between the superior and inferior PVs and passing septally below the fossa ovalis towards the septal mitral annulus (109). This line of block correlated with the lateral margin of the septo-pulmonary bundle, a site of abrupt change in subendocardial fibre orientation and wall thickness in the posterior LA, in a post-mortem analysis of 10 human hearts taken from patients without AF. The functional block demarcated by the septo-pulmonary bundle was shown to be involved in the initiation of PV ectopic induced AF by the development of macro re-entry or the formation of daughter wave-fronts by wave-front breakup across this line (109). In an epicardial mapping study of the posterior LA in 23 patients undergoing surgery, Roberts-Thomson et al identified consistent lines of block in the posterior LA in a similar position. The extent of functional block across this line was more extensive in the presence of atrial stretch conditions due to mitral regurgitation, left ventricular systolic dysfunction, and atrial fibrillation (107).
proportion of patients who developed complete functional block in the posterior wall were found to have circuitous propagation around this line which may be important in the re-entrant AF mechanism.

Multiple studies in patients undergoing AF ablation have shown significant data to support the role of the posterior wall in the AF mechanism. In a surgical cohort, Todd et al demonstrated that the isolated posterior wall was capable of maintaining AF which was not seen in the other LA regions suggesting the posterior wall is a site vulnerable to arrhythmia (118). Posterior wall isolation (PWI) has shown incremental benefits in addition to PVI with reduced recurrence rates and increased AF-free survival (119,120). However, results are conflicting (121) and may reflect a heterogeneity in patient selection. More recently, a meta-analysis from Sanders’ group demonstrated that inclusion of posterior wall isolation in addition to PVI is associated with acute procedural PWI success of 94%, and a 62% 12-month freedom from arrhythmia (122). Randomised trials to evaluate the utility of PWI as an adjunctive strategy are currently underway (ANZCTR: ACTRN12616001436460).

**Electrical atrial remodelling**

Atrial remodelling has been well established as being important in pathogenesis of AF with effects on electrical, cellular, structural or mechanical remodelling. Wijffels and Allessie first coined the widely used phrase “AF begets AF” which relates to AF-induced remodelling providing an explanation of AF disease progression from paroxysmal to longer phenotypes of AF including persistent and permanent AF. This progression is significantly enhanced in conjunction with traditional and novel AF risk
factors including heart failure, increasing age, hypertension, valvular disease, sinus node dysfunction, pulmonary hypertension, alcohol and obesity (8,123).

**Alterations to atrial refractoriness and conduction**

Electrical remodelling is often the initial phase in the course of atrial fibrillation. Extensive work in rapid pacing animal models significantly expanded the understanding and role of electrical remodelling in AF. The seminal work by Wijffels and Allessie was performed in chronically instrumented goats that underwent AF induction by rapid atrial stimulation with multiple pacing electrodes directly sutured to both left and right epicardial atrial surfaces (124). It was found that as the duration of pacing-induced AF increased, the duration of sustained AF following pacing cessation also prolonged. These changes were thought to reflect electrical remodelling which contributed to a self-sustaining process of impaired rate adaptation, shortened atrial refractoriness and shortened atrial cycle length. Interestingly, upon reversion to sinus rhythm after up to one month of sustained AF, parameters of electrophysiological remodelling were reversed within one week. These pioneering findings were confirmed by Morillo and co-workers in a canine model of rapid atrial pacing, where the pacing-induced reductions in effective refractory periods (ERPs) positively correlated with AF inducibility (125). Subsequent work by Nattel and co-workers provided further support of electrical remodelling in a canine model of AF which demonstrated similar ERP reductions, decreased rate adaptation and increased AF duration (126). Nattel postulated that changes in action potential duration and refractoriness induced by chronic atrial pacing related to changes in Ito and Ica channels. Another report demonstrated consistent findings of ERP reductions and AF inducibility following six weeks of atrial flutter (127). Coupled with changes in refractoriness, Nattel’s group
showed increased spatial heterogeneity of ERP which predicted inducibility and duration of AF (128). Inter-regional variability in ERP heterogeneity and recovery of electrical remodelling may lead to differential refractoriness with some regions refractory and others able to conduct, thereby meeting the necessary conditions for re-entry (129).

Studies in humans have shown findings consistent with animal data confirming the effects of acute electrical remodelling in the context of sustained atrial arrhythmias. Multiple studies have demonstrated that atrial conduction times and refractoriness are shorter in patients with AF compared to controls post cardioversion to sinus rhythm (130-132). Stiles et al evaluated atrial electrophysiological parameters in patients with paroxysmal AF at least 7 days remote from any episode of AF. They reported a prolongation in both conduction time and conduction velocity (CV) in patients with lone AF compared to control patients, associated with atrial dilatation and reduction in bipolar voltage. Similar to prior animal work, these findings are suggestive of the presence of a “second factor” influencing remodelling beyond AF itself (133). Teh and colleagues showed progressive electrophysiological remodelling in both paroxysmal and persistent AF patients compared to controls, with persistent AF patients having the slowest CV and atrial activation times (134). This study also showed a stepwise reduction in ERPs, bipolar voltage and fractionated electrograms in patients with persistent AF when compared to paroxysmal AF and reference patients.

Alterations to sinus node function

Anatomically, the sinus complex is located at the superior aspect of the crista terminalis containing specialised pacemaker cells with the ability to spontaneously depolarise
Functionally, the sinus node spreads diffusely over a wider area, found to be up to 7.5 x 1.5 cm in an epicardial mapping study of control patients with Wolff-Parkinson-White syndrome (WPW) (136). This region incorporated the junction of the right atrial appendage and superior vena cava anteriorly and the crista terminalis posteriorly. Elvan et al utilised a canine rapid pacing AF model to demonstrate lengthening of sinus node recovery time following 2-6 weeks of AF with incomplete recovery post cardioversion and 1 week in sinus rhythm (137). Human data has shown comparable inhibition of sinus node function caused by 10-15 minutes of rapid atrial pacing in patients with normal atria, and identified significantly prolonged sinus node recovery times (138). These changes in sinus node function have also been seen following cardioversion from chronic AF, lasting up to 24 hours post reversion (139) and chronic atrial flutter (140). The sinus node site of earliest activation shifted more caudally in the context of rapid atrial pacing in both controls and flutter patients. However, compared to control patients, remodelled atria in patients with chronic atrial flutter resulted in diminished pacing-induced shifts suggestive of a restricted sinus node complex (141).

**Alterations the autonomic nervous system**

Current evidence suggests that the autonomic nervous system is an important contributor to the development of AF and potentially vulnerable to remodelling because of AF itself. In a canine rapid pacing-induced AF model, Jayachandran and co-workers tagged sympathetic nerve terminals with C-11 hydroxyephedrine (HED) and imaged the sympathetic nervous system by positron-emission tomography. Compared with reference dogs without AF, they found that AF was associated with increased sympathetic nerve terminal density, higher concentration of norepinephrine in atrial appendage samples and greater sympathetic variability which correlated with
changes in atrial refractoriness. Taken together, these changes provide a potential mechanism by which the sympathetic nervous system may promote AF (142). Histological studies in further canine AF models have also found staining evidence of sympathetic nerve growth in left and right atrial tissue (143). These animal findings have been validated in human studies of patients undergoing cardiac surgery. Gould and colleagues sampled atrial appendages taken from 24 matched patients with and without AF and showed that tyrosine hydroxylase and norepinephrine staining of sympathetic nerves was significantly increased in patients with AF (144).

**Structural atrial remodelling**

Beyond electrical remodelling, structural remodelling is postulated to be the important ‘second’ factor contributing to AF progression in studies of atrial substrate. Changes in atrial structure have been associated with AF at a macroscopic level (increased left atrial dimensions) and microscopic level in the form of ultrastructural changes. Structural remodelling may play a crucial role in increasing the vulnerability of the atrium to develop and perpetuate AF, particularly in the context of clinical AF risk factors.

**Atrial dilatation**

Atrial dilatation often occurs together with AF (145,146) and age (147), hypertension (148,149) and valvular heart disease (150). Left atrial dilatation is an independent risk factor for developing AF and for every 5mm increment in LA size, the risk of incident AF has been shown to increase by almost 40% (16). Increased left atrial size is also predictive of progression to persistent and chronic AF in patients newly diagnosed with paroxysmal forms of AF after up to 4 years follow-up (151). This association was bi-
directional with persistent phenotypes of AF also associated with enlarged left atria. Atrial dilatation may mechanistically predispose to AF by increased atrial mass providing a greater surface area with a higher likelihood of sustaining multiple wavelets without spontaneously terminating (75).

*Interstitial and cellular changes associated with atrial fibrillation*

In contrast to electrical remodelling which displays reversible properties following reversion to sinus rhythm, atrial structural remodelling exhibits a more prolonged recovery period that is also often incomplete. Todd et al identified time dependent adverse atrial electrical remodelling in a rapid pacing goat model of AF (152). Although electrical remodelling was reversible following termination of AF, longer durations of AF correlated with progressive reductions in the amount of burst pacing required to initiate AF and increased frequency of AF induction. These findings represented an important initial breakthrough in raising suspicions that a second factor such as structural remodelling, played an important role in AF inducibility.

Multiple animal and human studies have reported cellular changes associated with atrial fibrillation. Mechanisms of atrial arrhythmia-related changes to myocytes include hypertrophic cellular changes, mitochondrial size changes, loss of myofibrils, sarcoplasmic reticulum disarray, glycogen accumulation, lysosome accumulation and increased extracellular matrix deposition (145,153-155). Infiltrative inflammatory changes have been reported in structurally abnormal hearts in patients with significant mitral valve disease and persistent AF (156). Given the complex interplay and relative contributions of co-morbid AF risk factors to substrate, the impact of AF on structural remodelling can be difficult to quantify. However, even lone AF in the absence of co-
morbid cardiovascular diseases is associated with substrate progression and atrial myocyte irregularities including hypertrophic and vacuolar degeneration and infiltrative inflammatory changes (145). Amyloid deposits in Congo red stained right atrial appendage samples were found in 16% of patients undergoing cardiac surgery and showed correlations with multiple factors including age, p-wave duration, female sex, valvular disease and AF (157). In this study, on Cox regression analysis, only AF remained a significant multivariable predictor of amyloid deposition. Greater amounts of amyloid deposition have also been found in up to 46% of subjects with valvular disease and persistent AF, compared with 12% of matched reference patients also with structural heart disease and heart failure, but without AF (158).

**Atrial fibrosis and structural remodelling**

Interstitial fibrosis, an important marker of structural and electrical remodelling, may cause anisotropic conduction (74), which may provide the necessary substrate to facilitate re-entry and AF (159). Collagen proteins form major components of the extracellular matrix and in the heart, type I and III collagens are most prevalent (160). Rapid pacing induced heart failure in a canine model by Nattel’s group, demonstrated evidence of atrial fibrosis and scarring associated with conduction heterogeneity and conduction slowing and block, giving rise to the phrase ‘remodelling of a different sort’ (161). Burstein et al showed significant abnormalities in fibroblast function and up-regulation of extracellular matrix gene expression in cardiomyocytes following rapid pacing (162). The atria have shown markedly greater susceptibility to fibrotic changes in response to rapid ventricular pacing. Hanna and colleagues reported significantly greater left atrial fibrosis (10±1%) after five weeks of rapid ventricular pacing compared with the left ventricle (0.4±0.1%, p < 0.01) (163).
Atrial fibrosis has often been found in human heart samples taken during cardiac surgery, from patients with pre-existing AF. Although evidence in the literature is mixed, atrial fibrosis has also been demonstrated in patients without AF. Histologically, fibrosis occurs in response to tissue injury characterised by areas of increased collagen deposition and interstitial matrix expansion. Increased collagen I expression has been shown in explanted hearts taken from patients who underwent heart transplant with a history of AF, compared with reference cases without AF (164,165). These changes occurred concomitantly with an upregulation in atrial metalloproteinase (MMP)-2 and down-regulation of tissue inhibitor of metalloproteinase (TIMP)-2 (164). Additionally, collagen I volume fraction (CVF-1) correlated with atrial size and TIMP-2:MMP2 ratio, and in persistent AF patients the CVF-I:CVFII ratio correlated with AF duration and number of episodes of AF recurrence. However, overall collagen III was not significantly different in these studies. Frustacci and colleagues examined right atrial tissue samples in patients undergoing cardiac surgery with lone AF and reference controls with WPW. They found that patients with paroxysmal AF all had a degree of histologic change whilst patients in the reference WPW group did not (145). Changes included significant atrial myocyte hypertrophy with vacuolar degeneration (n=2), necrotic lymphocytic infiltrate (n=8), diffuse fibrosis (n=5) and patchy fibrotic change (n=2). Atrial fibrotic change was demonstrated by Goette and co-workers in right atrial appendages from 259 patients (mean fibrotic volume 15.8±4.3%) but in the absence of a history of AF (166). The amount of fibrotic tissue significantly correlated with both p-wave duration and age. Interestingly, a composite of p-wave duration and fibrotic volume was capable of predicting post-operative AF which was progressively higher in incidence across three grades of atrial fibrosis.
**Gap junctions and atrial conduction**

Normal patterns of myocardial conduction critically depend on gap junctions to facilitate the robust and uniform intercellular transfer of electrical current (167,168). Gap junctions are comprised of transmembrane channels which serve to join cytoplasmic compartments between surrounding myocytes (168). Gap junction proteins known as connexins, are expressed as three major subtypes in the myocardium: connexin40, connexin43 and connexin45. In the atria, both connexin40 and connexin43 are seen, whilst in the ventricle, connexin43 is the predominant subtype (169). Connexin45 is expressed the least of the three subtypes in both atrial and ventricular tissue (168). However, the body of evidence on gap junction remodelling in the atria in the literature appears mixed. Animal studies have reported a spectrum of findings from increased connexin43 expression and gap junction lateralisation (170) to a greater heterogeneity of connexin40 distribution but without any changes in connexin43 expression (167,171,172). The increase in connexin40 heterogeneity strongly correlated with increased AF stability and structural (myolysis) changes in atrial myocytes (172). These findings are consistent with work by Ausma and co-workers who showed that connexin40 expression in the context of prolonged pacing-induced AF subsequently returned to baseline after reversion to sinus rhythm, suggesting a degree of reversibility (171). A connexin40 knockout mice model demonstrated multiple conduction disturbances in sino-atrial, inter-atrial and atrio-ventricular conduction coupled with increased AF susceptibility suggesting the importance of gap junction proteins in arrhythmogenesis (173).

Human studies on the role of gap junction remodelling in AF have also been controversial. Findings have ranged between increased (174-176) or decreased
connexin40 expression, and variable or no significant differences in connexin43 expression. However, these studies have frequently showed that connexin expression of the various subtypes is greater laterally, and heterogeneous in the context of AF. This lateralisation and variability of connexin expression potentially leads to abnormal anisotropic conduction and increased conduction heterogeneity fulfilling criteria for re-entry and maintenance of AF. Genetic polymorphisms in connexin40 have been shown to confer increased susceptibility to AF. Further studies of connexin40 target-genes have also implicated gap junction proteins as being potentially important in the development of AF. Gollob and colleagues showed somatic missense mutations in GJA5, the coding gene of connexin40, selectively in cardiac tissue in 27% of AF patients. Additionally, highly penetrant monogenic heterozygous mutations in connexin40 genes have also been co-segregated in linkage analysis in a large family with familial AF.

Structural remodelling and electrophysiological remodelling

Fibrosis can cause disruption of side-to-side electrical coupling between cells, interfering with uniform wave-front propagation and facilitating re-entry. Lines of conduction slowing of block may be attributed to fibrosis-mediated zig-zagging of transverse conduction, resulting in greater wave-front complexity. These disruptions in transverse conduction may be due to collagenous septa between myofibrils and the formation of lines of block (<0.08m/sec) that may facilitate micro re-entry in small areas of atrial tissue. A number of studies have evaluated the consequence of structural remodelling in goats. Ultra-structural changes have been shown to occur with AF even in the absence of interstitial fibrosis. Ausma et al evaluated chronically
instrumented goats subjected to up to 23 weeks of AF and identified that electrical remodelling occurred acutely after AF onset and stabilised after 2 weeks, whilst structural remodelling was seen only after 1 week and stabilised at 8-16 weeks (154,155,184). Microscopic structural changes included:

- increase in myocyte size (up to 195%)
- loss of myofibrils
- perinuclear myolysis accompanied by contractile protein loss in sarcomere-free zones and peripheral sarcomere remnants
- sarcoplasmic reticulum fragmentation
- accumulation of glycogen and sarcomere replacement
- distortion of mitochondrial shape and size
- nuclear chromatin dispersion

Interestingly, these structural changes were observed in the absence of interstitial changes or cellular degeneration. The authors postulated that this was due to an adaptive response to fibrillatory conduction via dedifferentiation of myocytes as opposed to a degenerative process. Subsequent studies in favour of this theory demonstrated changes in structural protein expression profile similar to foetal myocytes to components including cardiotin, titin, intercalated disc desmin and alpha-smooth muscle actin re-expression (155).

Despite significant evidence linking structural remodelling with AF, the association between atrial fibrosis and electrical remodelling is variable. Studies have showed a degree of significant correlation (161,185), while others have reported electrophysiological remodelling in the absence of fibrosis (186). Utilising a transgenic murine model, Verheule et al investigated the effects of TGF-β1 over-expression in the
atria and identified greater AF inducibility in 14/29 of the transgenic group, compared to none in the wild type group (p<0.01). This was accompanied by decreased right atrial conduction velocity in TGF-β1 over-expressed mice. A prevailing theory is that conduction heterogeneity related to fibrotic change may give rise to lines of functional block that may facilitate re-entry. Conversely, Allessie and co-workers demonstrated adverse electrophysiological changes in chronically instrumented goats even without fibrotic or gap junction remodelling (186). In this study, goats underwent baseline ablation to induce 4 weeks of chronic atrioventricular block which resulted in substrate changes in the form of significant right atrial dilatation and increased AF duration after induction (5 seconds to 6 minutes). They also found greater regional conduction slowing compared with reference goats (3.7±1.0 vs 0.9±0.5%, p<0.05), and atrial hypertrophy but without atrial fibrosis or connexin alteration.

**Mechanisms of cellular remodelling**

Mechanisms of cellular remodelling contributing to atrial structural remodelling include augmentation of pro-fibrotic pathways, inflammation, atrial ischaemia, apoptosis, and oxidative stress. Neurohormonal actions of the renin-angiotensin-aldosterone (RAA) pathway and transforming growth factor-beta (TGF-β1) are established causes of cardiac fibrosis.

*Renin-angiotensin system*

Alternation of the renin-angiotensin system has been shown in patients with AF in multiple studies. Serum angiotensin converting enzyme (ACE) has been shown to be greater in patients with AF compared to controls, accompanied by modulations in angiotensin II (AT2) receptor expression (187,188). Stretch-induced angiotensin II
release (189) and angiotensin I receptor activation (190) may create a positive feedback loop of persisting structural remodelling in response to atrial dilatation. Downstream effects include angiotensin II-mediated apoptosis (191) and aldosterone secretion which has been shown to be a pro-fibrotic hormone (192). Additionally, ACE stimulates the production of TGF-β1 which is also capable of promoting fibrosis by upregulating collagen synthesis (193). Up-regulation of regional atrial fibrosis in a transgenic TGF-β1 mouse model resulted in substrate that predisposed to AF independent of other cellular mechanisms (185). Consistent with these findings, administration of spironolactone, an aldosterone receptor antagonist, resulted in attenuation of the extent of atrial fibrosis in a rapid pacing heart failure model (194).

Clinical trials have shown that inhibition of the RAA pathway may result in cardio-protective effects on remodelling. Studies of ACE inhibitor use in heart failure with reduced ejection fraction (HFREF) (195) and hypertension (196) have found a potential reduction in the incidence of AF. A meta-analysis has suggested that these benefits are particularly pronounced in patients with HFREF or left ventricular hypertrophy (197). Inhibition of the RAA pathway by angiotensin II receptor blockers (ARB) may beneficial in maintaining sinus rhythm in conjunction with amiodarone post cardioversion (198) and in patients with symptomatic HFREF (199). The efficacy of RAA pathway blockade on AF has been attributed in part, to decreased atrial fibrosis and reversed structural remodelling (200,201). This may be mediated by reduced angiotensin II mediated activation of Erk1/Erk2 protein kinases which have been implicated in pro-fibrotic pathways (187). Cardio-protective and anti-fibrotic benefits of reduced electrical remodelling have been shown in rapid pacing canine models (202). Conversely, electrophysiological changes in atrial refractoriness and conduction
were not seen in spite of haemodynamic effects in the context of patients receiving angiotensin II infusions (203).

**Atrial fibrosis**

Despite the precise mechanisms involved in atrial fibrosis being incompletely defined, pro-fibrotic factors beyond the RAA pathway involved in AF include Transforming Growth Factor β (TGF-β), platelet derived growth factor (PDGF) and connective tissue growth factor (CTGF)(204). Further, matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) also appear to contribute to the formation of fibrotic atrial substrate.

**TGF-β**

Regulated by the RAA pathway, TGF-β stimulates collagen formation by fibroblasts, and has been shown to be up-regulated in several AF clinical risk factors such as valvular disease, cardiomyopathy, and ischaemic heart disease (205). Nattel’s group demonstrated that the activated isoform of TGF-β is selectively higher in the atrium whilst remaining relatively unchanged in the ventricle in a chronic heart failure pacing model (163). Associated TGF-β atrial changes included increased fibrosis, angiotensin II levels, inflammation, apoptosis and mitogen activated protein (MAP) kinases. TGF-β expression has been utilised to help predict the degree of structural remodelling and recurrence risk in patients following AF ablation. Kim and colleagues assessed baseline plasma TGF-β and tissue inhibitor of matrix metalloproteinase (TIMP)-1 levels in 242 AF ablation patients. They found that these tissue factors significantly correlated with structural remodelling on CT and electro-anatomical mapping (206). Higher TGF-β and TIMP-1 levels were associated with larger left atrial dimensions and lower mean
LA voltages. In a surgical Cox-Maze study of 86 persistent AF patients, baseline TGF-β significantly correlated with LA fibrosis and 12-month arrhythmia recurrence suggesting the importance of this factor in the pathogenesis of AF (207).

Connective tissue growth factor (CTGF)
An important downstream target of AT2 and TGF-β in the pro-fibrotic cascade is connective tissue growth factor (CTGF). The presence of tachycardia has been shown to cause AT2 release, which then results in TGF-β expression by fibroblasts, and subsequently CTGF expression and collagen upregulation (208,209). Surgical right atrial appendage specimens identified relatively greater CTGF levels in 10 patients with AF compared to 10 reference patients without AF (210). Adam et al identified almost twice the amount of fibrosis in atrial tissue, with a 2.5-fold increase in collagen cross linking and CTGF upregulation in atrial tissue in patients with AF compared to those without a history AF (211). Utilising a canine rapid atrial pacing model with an ARB (olmesartan), Kiryu et al identified significant increases in CTGF and collagen I and III compared to controls, in the absence of change in TGF-β (212). Dogs that were also administered olmesartan demonstrated suppression of pacing-induced changes leading the authors to postulate that CTGF upregulation is intimately related to AT2 given changes occurred independent to TGF-β expression.

Matrix metalloproteinases
Matrix metalloproteinases (MMPs) promote extracellular matrix (ECM) breakdown, whilst tissue inhibitors of matrix metalloproteinases (TIMPs) serve to inhibit MMPs. Studies have shown increased MMP and decreased TIMP levels in multiple cardiac disease states including AF, cardiomyopathy and ischaemic heart disease (205). Atrial
remodelling in rapid atrial pacing canine models was accompanied by a 50% increase in MMP-9 and corresponding 50% decrease in TIMP-4 (213). A similar porcine model of AF found increased MMP-9, whilst TIMP-1 and TIMP-3 were also increased (214). Wang and co-workers identified that atrial dilatation was associated with increased MMP 3, 7 and 9, and TIMP 1, 2, 3, and 4 levels in right atrial biopsies in patients with AF compared to those without AF (215). In atrial samples taken from explanted hearts from patients with documented AF, MMP-2 expression was progressively higher and TIMP-2 lower, according to AF severity and compared with donor controls (164). The investigators found that the collagen volume fraction increased with a greater left atrial dimension and lower TIMP-2:MMP-2 ratio. In a study of 13 patients with AF and 25 patients without AF, Zhu et al utilised polymerase chain reaction and ELISA in atrial tissue samples to quantify MMP-9 and TIMP-1 expression. Patients with AF demonstrated greater activated MMP-9 levels particularly in the perivascular and sub-epicardial zones (216). A subsequent study from this group in 24 patients with AF and 12 reference controls found similar increases in MMP-9 in the setting of AF, coupled with decreased TIMP-1, and significant correlation with LA size and AF duration (217).

Inflammation

Inflammation and inflammatory mediators are implicated in the pathophysiology of AF via extra-cellular matrix deposition and collagen formation (205). Initial evidence supporting the role of inflammation in AF demonstrated that patients with myocarditis develop atrial arrhythmias (218) and that AF patients had inflammatory fibrosis and necrosis not present in those without AF (145). Confirming these findings, Chen et al identified greater inflammatory changes in AF compared with sinus rhythm in patients
undergoing valvular surgery with a global pattern of change (219). A study performed by Yamashita et al in patients undergoing cardiac surgery involved immunohistochemical analysis of appendages from patients with AF. This group showed a greater extent of (CD 45+) inflammatory infiltrate in AF patients across the atrial endocardium and sub-endocardium, was largely macrophage (CD 68+) rather than T cell (CD 3+) origin. Accompanying macrophage-related infiltrative changes included differences in adhesion molecules: intra-cellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and expression of pro-fibrotic growth factors such as tumour necrosis factor (TNF)-β and interleukin (IL)-6 (220).

C-reactive protein, complement (C3, C4), TNFα and interleukins such as IL-6 and IL-8 are well-established markers of inflammation. IL-1, IL-6 and TNFα have been known to upregulate MMP leading to reduced collagen deposition and formation, and increased collagen breakdown (205). In the presence of prolonged duration of AF, elevated IL-6 is associated with structural remodelling as measured by atrial dimension (221). AF has been found to positively correlate with CRP levels with a progressive stepwise increase in controls, then paroxysmal, and finally persistent AF (222). An observational study found that elevated baseline CRP levels were associated with a greater risk of incident AF (26). Although the evidence in the literature is conflicting, findings linking CRP levels and AF have been replicated in several other studies (223-226). Watanabe and colleagues showed that high-sensitivity was capable of predicting success of cardioversion and maintenance of sinus rhythm (226). A meta-analysis has since confirmed this association suggesting that inflammation may be important in AF (227-229). However, Conway et al showed that inflammatory markers such as interleukins (IL-8) and CRP were not elevated in the setting of AF after multivariable
adjustment (230). In support of this, Letsas et al studied predictors of recurrence post AF ablation and demonstrated that CRP was a univariate predictor of AF recurrence, with multivariate predictors being white cell count, hypertension and left atrial size (231). Ellinor et al also did not show any differences in CRP between lone AF patients and reference controls (232). Taken together, these studies suggest that CRP and IL-8 may be more reflective of a systemic inflammatory process or a marker of comorbid cardiovascular disease, rather than being independently associated with AF.

Atrial fibrillation in the setting of cardiac surgery is a common occurrence in up to 40-50% patients (233). Inflammation post bypass surgery is associated with post-operative AF as measured by elevated white cell count, CRP, and elevated complement (C3 and C4) (234-236). Supporting the role of inflammation in AF, a randomised study of intravenous hydrocortisone in patients post cardiac surgery has been shown to reduce the incidence of AF to 30% compared with 48% in untreated controls (p=0.04) (237). Similar findings have been identified following AF ablation with patients treated with corticosteroids experiencing lower AF recurrence in 15% compared with 29% in controls (p=0.03) (238). More nuanced studies of the precise mechanisms of the role of inflammation in AF are required to explain these randomised clinical observations.

**Atrial Ischaemia**

Several lines of evidence have linked atrial ischaemia with the pathophysiology of AF. Sinno et al selectively occluded arterial branches supplying different atrial regions in a canine model and found a significantly longer AF duration induced by rapid pacing within 3 hours of ischaemia compared to baseline. Regional analysis identified severe conduction slowing within and around zones of ischaemia which correlated with
underlying necrosis on histopathology and were thought to be a substrate for re-entry (239). Nadolol and diltiazem attenuated ischaemia-related AF duration prolongation and conduction delay whilst class I antiarrhythmics such as flecainide did not (240). Nattel’s group further evaluated the mechanisms of chronic atrial ischaemia and the development of AF (241). In this study, 40 dogs with right atrial branch occlusion underwent macroscopic assessment, optimal mapping and patch clamp studies compared with reference dogs. The atrial ischaemia group were found to have increased triggered activity at fibrotic scar border zones, elevated sodium calcium exchange current, spontaneous calcium release, greater border zone conduction heterogeneity, higher burden of atrial ectopy and induced-AF duration. Yamazaki et al found similar infarct border zone conduction slowing findings in an ovine model of left atrial ischaemia. Accompanying this was an increase in the dominant frequency of the non-infarct zone, and an interplay between AF promoting focal triggers and rotor activity at the peri-infarct zone (242). Sanders and co-workers evaluated an ischaemic ovine model by occlusion of the left anterior descending (LAD) and left circumflex (LCx) arteries compared with a sham control group. The investigators found that ischaemic sheep had significantly worse left ventricular function, higher LA pressure and reduced atrial refractoriness compared to the sham group (243). Specifically, the LCx infarct model had increased atrial infarction, reduced atrial conduction velocity, increased heterogeneity of conduction, increased AF inducibility and prolongation in AF duration compared to the LAD infarct group. In a chronic myocardial infarction (MI) model, Kettlewell et al found that altered calcium (Ca2+) handling was associated with atrial action potential duration alternans and greater frequency of spontaneous depolarisations (244).
In a study of 157 patients undergoing coronary bypass surgery compared to 191 controls in sinus rhythm, Koletsis et al. identified that predictors of AF included ischaemia markers such as prolonged cardiopulmonary bypass time, reduced peri-operative myocardial ischaemia index, lower post-operative oxygen saturations, greater inotrope administration and peri-operative myocardial infarction (245). Myocardial infarction may be associated with higher AF incidence in a large study of 5983 patients whereby 19% of inpatients had AF, which also correlated with higher overall mortality, and mortality related to cardiac disease, compared to sinus rhythm controls (246). A systematic review and meta-analysis which included 43 studies involving over 270 000 patients, identified that although AF was a predictor of mortality, this was no longer the case following adjustment for confounders on a sensitivity analysis (247). A study of 1395 patients found that typical clinical AF risk factors were predictors of in-hospital AF post myocardial infarction. These included increased age, female sex, LV dysfunction, reduced thrombolysis, transmural anterior infarction, hypertension, heart failure, diabetes, pulmonary disease and stroke (248). Additionally, De Jong and colleagues found that right coronary artery disease was an independent predictor of post-operative AF in 162 patients following cardiac surgery, potentially related to the right coronary artery supplying the atrial circulation downstream (249). More recently, the RIPPAF randomised study in 146 patients with paroxysmal AF showed the novel utility of remote ischaemic preconditioning in reducing AF inducibility (OR 0.35, 95% 0.17-0.71, p=0.003), sustainability of AF (OR 0.36, 95% 0.16-0.81, p=0.01) and dispersion of atrial ERPs (p=0.021).
Oxidative stress has been implicated as a potential contributor to the pathogenesis of AF through reactive oxygen species (ROS) triggering modulation of cardiac myocytes and fibroblasts. Several surrogate markers of oxidative stress have been used with variable results. Carnes and co-workers identified that a reduction in atrial refractoriness was associated with reduced tissue ascorbate levels and that ascorbate administration attenuated these differences in a rapid pacing canine model (250). Pre-treatment with ascorbate in patients undergoing cardiac surgery reduced post-operative AF (250). A study in a rapid pacing AF model in pigs identified upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase leading to increased left atrial superoxide production in left atrial appendage samples. Corradi et al found that a marker of oxidative stress, heme oxygenase-1, was elevated in association with atrial fibrosis and myolysis in the setting of AF and mitral valve disease (251). In a group of patients undergoing AF ablation, Shimano et al evaluated derivatives of reactive oxidative metabolites (DROM) and identified that DROM levels were greater in chronic AF compared to paroxysmal AF patients and predicted higher AF recurrence (252). However, this study also showed mixed results with DROM not correlating with structural remodelling and left atrial diameter (252). Kim and colleagues studied oxidative markers in plasma and right atrial appendage samples in patients undergoing bypass surgery (253). They identified that NADPH-oxidase activity was a significant multivariate predictor of post-cardiac surgery AF (odds ratio 2.41, 95% CI 1.71-3.40, p<0.001) (253). The anti-inflammatory and anti-oxidant effects of statin therapy have been studied in the context of AF prevention. Simvastatin reduced the impact of rapid ventricular pacing on atrial refractoriness and calcium channel down-regulation in a canine model (254). Attenuation in pacing-induced
conduction changes, atrial fibrosis, and AF duration was also seen in the context of simvastatin administration. Human data is conflicting with some observational work showing reduced post-cardioversion AF recurrence due to statin therapy (255) not seen in randomised studies (256-258). Whilst a recent meta-analysis showed no overall impact of statins on post ablation recurrence, a sub-group analysis of randomised controlled trials did show a signal for statins to reduce post-ablation recurrence (259). Atorvastatin in post cardioversion patients reduced inflammatory markers but did not significantly impact oxidative stress markers or AF recurrence (257).

**Atrial ionic remodelling**

Transmembrane ion channels play a crucial role in cardiomyocyte action potential generation. The action potential comprises of four key phases: phase 0 represents rapid depolarisation once the resting membrane threshold has been reached, phase 1 involves early repolarisation, phase 2 is a balanced counter-current leading to a plateau, then a late repolarisation phase 3, and return to resting state in phase 4. Changes in the ionic signalling may affect action potential duration (APD) and refractory period properties which modulate the threshold of the atria to developing re-entry (260).

**Calcium currents**

L-type calcium currents (I_{CaL}) are activated by transmembrane depolarisation and enable Ca2+ movement into myocytes and act during phase 2 (plateau) of the action potential. AF with rapid ventricular response is associated with more pronounced Ca2+ into cells which leads to intra-cellular calcium loading. Subsequently, atrial myocytes respond by an initial adaptive down-regulation of I_{CaL}. However after sustained stimulation, reduced APD can ensue and contribute to the perpetuation of AF. Yue at
al identified that a decrease in $I_{\text{CAL}}$ and $I_{\text{to}}$ channels was associated with reduced atrial ERPs and rate adaptation which may facilitate AF induction in a rapid pacing canine model (126). The same group performed PCR and Western Blot to show that down-regulation of the $\alpha$-subunit resulted in decreased expression of both $I_{\text{CAL}}$ and $I_{\text{to}}$ channels (261). Van Wagoner et al studied patients undergoing cardiac surgery and identified that patients who developed post-operative AF had increased $I_{\text{CAL}}$ expression suggesting that calcium overload may be involved in the AF mechanism (262). However, persistent AF was associated with reduced $I_{\text{CAL}}$ expression suggesting that longer term AF may result in an adaptive response and feedback loop to chronic calcium overload. A study in cardiac surgery patients found that persistent AF had transcriptional down-regulation of $I_{\text{CAL}}$ mRNA, not present in paroxysmal AF patients (263). Down-regulation of $I_{\text{CAL}}$ may be explained by mechanisms such as post-transcriptional alteration by phosphatases and calpains-mediated proteolysis (264,265).

To investigate this further in vitro, Carnes et al identified that down-regulation of $I_{\text{CAL}}$ and oxidative stress occurred due to increased S-nitrosylation and decreased atrial glutathione with normalisation of $I_{\text{CAL}}$ with glutathione repletion (266).

**Sodium currents**

Voltage-gated sodium channels are important determinants of the initial depolarisation phase of the action potential. The $I_{\text{Na}}$ current is a significant contributor to myocyte wavefront propagation and conduction velocity (178). In a canine model, Gaspo and co-workers identified that rapid pacing resulted in reduced $I_{\text{Na}}$ density which persisted up to 42 days and correlated with induced-AF duration ($R^2=0.57$, $P<0.001$) (267). These changes in $I_{\text{Na}}$ density were accompanied by conduction slowing implicating sodium channels in the AF substrate and re-entry mechanism. Whilst another canine
study validated these findings (268), human data have failed to show any differences in I_{Na} density in patients with persistent AF (269). Studies of transcriptional modulation of sodium channels are conflicting. Yue and colleagues identified reduced alpha-subunit and protein levels downstream of I_{Na} encoding mRNA (261). Conversely, a number of animal (270) and human studies (263,271) have not shown any differences in I_{Na} related mRNA expression. Despite the important role of sodium channels in action potential conduction, due to mixed and relatively sparse data, the precise role of I_{Na} current in AF remains incompletely defined.

**Potassium current**

Potassium channels have been implicated in a number of heart rhythm disturbances. The baseline resting transmembrane potential in non-nodal cardiomyocytes lies between -80 to -90mV and is determined by the relatively higher resting permeability of inward-rectifier potassium channels: I_{K1} and I_{Kach} (to a lesser extent). Outward I_{K} currents are primarily responsible for cellular repolarisation. The rapid outward I_{to} current results in the fast phase 1 repolarisation phase, whilst the ultra-rapid delayed rectifier I_{Kur} (absent in ventricular myocytes) together with the I_{Kr} and I_{ks} (outward rapid and slow-rectifier) currents are major components of phase 3 rapid depolarisation and action potential termination.

Chronic AF has been shown to result in increased inward-rectifier potassium current (I_{K1}) activity in both animal (272) and human studies (269,273,274) which leads to a relatively higher potassium conductance and negative resting membrane potential (275). The stimulation of cholinergic receptors by drug administration or vagal nerve activity serves to up-regulate I_{Kach} activity. Reduced I_{Kach} expression leads to a
blunted response to cholinergic stimulation in the context of AF (263,275,276). Arrhythmogenesis is thought to be the result of an abbreviated action potential duration due to up-regulated $I_{K_{ACh}}$ remodelling in response to atrial arrhythmia independent of muscarinic receptor agonists (178). Both $I_{K_1}$ and $I_{K_{ACh}}$ upregulation and inward-rectifier current remodelling appear to be significant contributors to the AF mechanism by effects on action potential shortening (178,277). Additionally, several animal (278) and human (269,273,276) studies have identified reduced $I_o$ activity induced by rapid atrial pacing occurring acutely from 24 hours and maximally up to 6 weeks later (178). However, the impact of these changes on the genesis of AF is incompletely defined. Variable evidence exists on $I_{Kur}$ remodelling due to AF with studies ranging widely from no change (126), increased activity (269) to reduced activity (279). More recently, attention has turned to the modulation of atrial-specific $K^+$ channels which are not expressed in the ventricle and as such do not carry ventricular pro-arrhythmic effects (280). Novel atrial-selective potassium channels including K2P, SK and KV1.1 channels have been identified and selective inhibitors of potassium channels are being investigated in early phase studies (281). In contrast, a recent randomised study identified that dual multi-channel blockade may provide synergistic effects on AF burden reduction at low-moderate doses in patients with paroxysmal AF (282).

**Mechanical remodelling associated with atrial stretch**

Atrial dilatation is a well-established independent risk factor predisposing to AF through mechano-electrical remodelling also known as contraction excitation feedback (16). Given that AF itself can lead to the development of atrial dilatation, a positive feedback loop may ensue whereby further stretch leads to greater AF burden further sustaining the AF mechanism (151). Stretch conditions are known to cause acute effects
on electrical remodelling and chronic effects more likely to impact on structural remodelling.

**Acute atrial stretch**

Studies in animal and human models have identified that acute atrial stretch is associated with variable alteration to atrial refractoriness, conduction velocity slowing, and increased susceptibility to AF.

Zipes and colleagues utilised a canine model of simultaneous atrioventricular (AV) pacing (283) and demonstrated that acute haemodynamic stretch due to fluid loading (284) resulted in biatrial ERP prolongation and increased heterogeneity of ERPs. Conversely, Wijffels group found no significant change in refractory periods with acute stretch using fluid loading in goats (285). Allessie and colleagues utilized 5 Langendorff-perfused rabbit hearts with varying controlled ligation of the caval and pulmonary veins to induce stretch (286). This study demonstrated shortened ERP at high (but not low) atrial pressures associated with monophasic APD shortening and greater AF inducibility. Interestingly, these changes in response to acute stretch were reversible within 3 minutes of reduction in atrial pressure. In a subsequent rabbit heart model, Allessie’s group identified that increasing atrial pressure resulted in conduction velocity slowing (31% reduction; 14cm H$_2$O) and greater conduction block (from 1.6% to 6.6%; 14cm H$_2$O) using a high density epicardial mapping plaque (287). Similarly, Eijsbouts et al also utilised isolated rabbit hearts and showed that increasing atrial pressure correlated with conduction slowing and greater regions of conduction block dependent on direction of pacing and anatomic structures such as the crista terminalis in the right atrium (287).
Analogous to animal models, human studies have also reported variable ERP changes with atrial stretch. Calkins et al identified that sequential AV pacing resulted in shortening in atrial ERP, which also occurred in context of autonomic blockade (288). Conversely, Klein et al reported an increase in ERP in response to sequential AV pacing (289). In another study of right atrial electroanatomic mapping before and after simultaneous AV pacing, Ravelli et al showed that a 23% increase in atrial volume was associated with a 16% decrease in conduction velocity, a 54% increase in regional conduction slowing or block, and 60% of patients developed AF (290). More recently, Walters et al evaluated the impact of acute atrial stretch using an intravenous fluid bolus in 10 cardiac surgery patients and mapped the pulmonary vein-left atrial junction with triangular high-density epicardial plaques (291). The investigators demonstrated that a 2.5mmHg mean rise in right atrial pressure resulted in significant conduction slowing and increased complex signals. The PV-LA changes in response to acute stretch are thought to increase the susceptibility to re-entry important in the AF mechanism.

**Chronic atrial stretch**

Whilst studies of acute stretch have demonstrated mixed results, models of chronic atrial stretch have yielded findings that are more consistent. Boyden et al investigated canine models of right atrial enlargement due to surgically-induced tricuspid regurgitation (292) and left atrial enlargement due to mitral valve fibrosis (293). These studies of both left and right atria found greater connective tissue and myocyte changes because of chronic atrial stretch. Morrillo et al performed high rate atrial pacing for six weeks in a canine model with resultant increases in atrial size, AF inducibility and ERP shortening associated with more and larger mitochondria and sarcoplasmic reticulum disruption on electron microscopy (125). Using a rapid ventricular and atrial pacing
heart failure canine model, Nattel’s group identified significant atrial remodelling associated with greater inducibility of AF in both pacing groups (161). Although the rapid ventricular pacing group did not have alterations in ERP, there was significantly increased conduction heterogeneity due to greater focal regions of conduction slowing and fibrosis on histopathology. Verhuele performed surgically-induced mitral regurgitation to achieve chronic atrial dilatation in dogs (294). This study identified a three-fold increase in atrial volume that was significantly associated with increased AF inducibility and histologically greater fibrosis despite prolongation in ERP. The investigators proposed that susceptibility to AF was due to histopathologic interstitial fibrosis and inflammatory change indicative of structural remodelling. Moreover, it is possible that different chronic stretch models induced different forms of atrial remodelling given the differential ERP responses to rapid atrial pacing (shorter) compared to mitral regurgitation related stretch (longer).

Human studies have provided further evidence in support of structural remodelling and atrial ERP changes due to chronic atrial stretch. Chen and colleagues evaluated patients with and without paroxysmal atrial flutter and found significant prolongation of ERP in those with atrial dilatation (295). Sparks and co-workers assessed the impact of chronic single right ventricular lead pacing (VVI) compared with dual-chamber pacing and showed that the VVI group experienced a significant increase in atrial size with longer atrial refractoriness, slower conduction velocity and evidence of sinus node dysfunction (296). Subsequently, clinical electroanatomic mapping studies in patients with chronic stretch due to heart failure, atrial septal defect and valvular disease have demonstrated greater electrical and structural remodelling (127,297,298). These conditions are AF clinical risk factors and strongly associated with the perpetuation of
AF. Thus, overall it appears that structural remodelling is important in AF development in chronic atrial stretch despite studies demonstrating atrial wavelength prolongation rather than shortening. Atrial dilatation may facilitate a larger atrial mass and greater number of propagating re-entrant wavelets, conduction slowing and complex fractionated electrograms indicative of abnormal regions that may sustain AF.

**Clinical determinants of atrial substrate**

Rapid pacing models in both animals and humans have revealed important insights into tachycardia-induced changes in the atria which contribute to the AF mechanism. These studies however, do not specifically address the underlying atrial substrate which may be present in multiple risk factors which increase the likelihood of AF initiation and perpetuation. These traditional risk factors include ageing, heart failure, mitral valve disease, sinus node dysfunction and hypertension, and novel risk factors such as alcohol, obesity and obstructive sleep apnoea. Multi-modality assessments of the impact of these AF risk factors have significantly enhanced the understanding of how these conditions may promote AF.

**Ageing**

The incidence of AF rises with age to approach over 20% over 80 years (2). Animal models have demonstrated that interstitial fibrosis and electrical remodelling correlates with increased age and susceptibility to AF (299,300). Hayashi and colleagues studied 2-3 month old, compared with 22-24 month old, Langendorf perfused rat hearts and showed that older rats exhibited AF inducibility, conduction slowing and structural remodelling characterised by larger atrial dimensions and fibrosis (299). Similarly, a study of canines found increased conduction heterogeneity, spatial variability, APD
shortening and adaption in older dogs with AF and sinus rhythm compared with younger adult dogs (300).

In humans, advancing age has also been shown to be associated with atrial fibrosis and electrical uncoupling of side-to-side cellular connections (147). Histologically, these changes in ageing resulted in disruptions in uniform propagation to a more pronounced zig-zag pattern. The group then described age-related remodelling in atrial microarchitecture associated with sodium current repolarisation gradients resulting in increased heterogeneity and small re-entrant circuits during single premature extra-stimulus pacing (301). Kistler et al significantly expanded understanding on electrophysiological and electro-anatomic right atrial data in three groups of patients aged < 30 years, 31-59 years and > 60 years (302). Investigators demonstrated that ageing was associated with ERP prolongation, conduction slowing, markedly greater complex electrograms clustered around the crista terminalis and increased low voltage regions and voltage heterogeneity. A retrospective analysis of patients undergoing AF ablation found reduced left atrial but not right atrial bipolar voltage in patients above 50 years who also experienced higher AF recurrence (303). More recently, a study of left and right atrial tissue taken from healthy explanted donor hearts and patients with mitral valve disease showed age-related electrical remodelling, lower L-type calcium channels and gene expression dysregulation (miR-328) (304). These microstructural and ultrastructural changes in ageing are purported to lead to abnormal conduction and higher propensity to development of AF.
**Heart Failure**

Heart failure is a well-established risk factor for AF with Framingham heart data finding a 4.5-fold increase in males and almost 6-fold increase in females for AF risk on multivariate analysis (6). A seminal study by Li and Nattel showed that ventricular pacing-induced heart failure resulted in atrial remodelling characterised by greater and more enduring structural abnormalities (161). Power and colleagues also showed increased AF inducibility despite prolonged atrial refractoriness (305). In an electroanatomic human study, Sanders and colleagues identified that patients with heart failure had right atrial ERP prolongation without changes to rate adaptation or dispersion, conduction slowing, low voltage areas, greater complex electrograms, low voltage areas and AF inducibility compared with age-matched reference patients (297). Electroanatomic data by Prabhu et al demonstrated consistent biatrial changes in patients with heart failure including lower voltage, increased voltage heterogeneity, complex fractionation, slower AF PV cycle length and lower PV antral voltage (306). More recently, this group also demonstrated reversal of substrate in heart failure patients with persistent AF in the randomised CAMERA-MRI study (307) who successfully underwent restoration to sinus rhythm following AF ablation by PVI and posterior wall isolation. Improved LV ejection fraction post-ablation was found to be associated with regression of both ventricular fibrosis on cardiac MRI (308) and clinical right atrial substrate including low voltage zones and scarring on repeat electroanatomic mapping (309).

**Sleep Apnoea**

Obstructive sleep apnoea (OSA) is closely related with AF. Basic data have demonstrated extensive atrial remodelling in dogs exposed to chronic OSA including
shortened atrial refractoriness, alterations in potassium and L-type calcium currents and greater apoptosis and interstitial fibrosis (310). Linz et al performed negative tracheal pressure to simulate OSA in pigs and similarly found shortened ERP and enhanced AF inducibility by vagal activation mechanisms (311). Interestingly, atrial remodelling changes abated after cessation of tracheal pressure.

In humans, to evaluate the impact of OSA on AF, Stevenson and colleagues performed polysomnography in 90 patients with AF and 45 reference patients (19). The investigators identified that patients with AF had a significantly higher apnoea hypopnea index (AHI) compared with controls, and the increased AF burden by phenotype correlated with OSA severity. Dimitri and colleagues performed electroanatomic assessment of patients with and without OSA and found that patients with OSA had no ERP differences, slower coronary sinus and crista terminalis conduction times, prolonged p-wave duration, greater complex fractionated electrograms, increased corrected sinus node recovery times, reduced bipolar voltages and conduction velocities compared with reference patients (312). Consistent with these findings, a recent study by Anter and Josephson comprehensively evaluated atrial electrophysiology in 86 paroxysmal AF patients and 43 controls (313). The authors demonstrated that patients with OSA had lower bi-atrial voltage, slower conduction velocity and higher electrogram fractionation with the left atrial septum identified as a source of abnormality in 74% of patients. Non-PV triggers were markedly more frequent in OSA patients with AF (42% vs 12%, p=0.003) implicating the changes in atrial remodelling related to OSA in the arrhythmia mechanism beyond the pulmonary veins (313).
**Obesity**

Recently, there has been substantial interest in obesity as a novel risk factor of AF (314). Mahajan et al evaluated 10 obese sheep compared with 10 aged-matched lean sheep with comprehensive electrophysiological study, electro-anatomic mapping, histopathology and fibrotic biomarkers (315). Compared with controls, obese sheep demonstrated evidence of bi-atrial remodelling characterised by increased conduction heterogeneity, greater complex electrograms and lower posterior wall voltage and increased AF burden (315). Additionally, obese sheep had evidence of increased atrial interstitial fibrosis and TGF-β1. Human studies have also shown that obese patients demonstrated increased LA pressure and volume and shortened atrial refractoriness (316). Mahajan et al performed a subsequent electro-anatomic study of obese patients and demonstrated consistent findings to the group’s prior ovine model (317). The investigators identified regional changes across the posterior wall of obese patients compared with non-obese control patients including regionally increased conduction heterogeneity, lower voltage and increased fractionation that correlated with epicardial adipose deposits on CMR. These findings suggest that paracrine effects and epicardial adiposity infiltrate may lead to structural remodelling and local fibrosis. Further clinical work from the same group showed that modification of multiple clinical risk factors including weight reduction, blood pressure, lipid and glycaemic control reduced post ablation AF recurrence in the ARREST-AF cohort study (33). The ARREST-AF randomised trial is currently in progress (AZNCTR: ACTRN12613000444785).

**Atrial Septal Defects**

Atrial septal defects (ASD) have been shown to carry a high risk of AF and flutter in up to 20% of patients (318). The impact of chronic volume overload from ASD was
studied by Morton et al and identified that compared with age-matched reference patients, ASD was associated with increased right atrial ERP, conduction slowing and block across the crista terminalis, and sinus node dysfunction (319). Similarly, Roberts-Thomson and co-workers demonstrated consistent findings in the left atrium with uncorrected ASD patients having larger regions of low voltage and increased AF inducibility (320). Taken together, atrial arrhythmias in ASD can arise from both surgical incisions and remodelling-related changes providing the necessary substrate for re-entry.

*Mitral valve disease*

Mitral valve disease if left unchecked, may lead to development of AF (6). Verhuele et al surgically induced mitral regurgitation (MR) in a canine model and showed progressive MR-related changes that included atrial dilatation, prolonged ERPs and histological fibrosis and inflammatory changes (294). Although ERPs were increased, AF inducibility was also significantly increased. This group showed similar results in a follow-up study utilising high-resolution optical mapping with changes of greater conduction heterogeneity in the left atrium which were not seen in the right atrium or control dogs without MR (321). In humans, John et al evaluated 21 patients undergoing mitral valve commissurotomy (MC) for rheumatic mitral stenosis (MS) (298). The investigators found that MS was associated with significantly increased atrial size, prolongation of ERPs, conduction slowing, larger low voltage zones, and increased AF inducibility compared with control patients. Following MC, MS patients had p-wave duration decreased and voltage increased whilst ERPs remained unchanged.
Sinus node disease

AF may lead to sinus node remodelling, and conversely sinus node dysfunction itself may result in predisposition to AF. Sick sinus syndrome is associated with a 10 year risk of AF of 28% (322) and appears to be part of a diffuse process of structural remodelling which also affects the atrial myocardium. Early histopathological studies have found fibrotic changes in relation to sinus node disease (323). Sanders et al utilised electro-anatomic mapping in a group of 16 patients with sinus node dysfunction and 16 age-matched control patients (324). The authors identified that when compared to controls, patients with symptomatic sinus node disease exhibited abnormal right atrial substrate characterised by lower conduction velocities, functional conduction block at the crista terminalis associated with complex fractionated electrograms and lower atrial voltages. The authors concluded that irregular circuitous propagation and conduction block around the crista terminalis may facilitate re-entry and development of AF, similar to the changes observed under atrial stretch conditions.

Hypertension

Hypertension is the most commonly implicated modifiable risk factor in the development of AF (5). Animal studies have demonstrated mechanistic insights to explain this association. Kistler et al evaluated an ovine model of chronic hypertension induced by prenatal corticosteroids and identified generalised conduction slowing and increased AF inducibility without significant changes in atrial refractoriness in hypertensive sheep (148). Additionally, this study showed evidence of structural remodelling comprising of myocyte hypertrophy, myolysis, regional scarring and apoptosis. A subsequent single-clip kidney chronic hypertension ovine model by Lau et al demonstrated consistent findings of conduction slowing, prolonged atrial
refractoriness, increased AF inducibility and interstitial fibrosis with hypertension compared with control animals (325). The time course of these changes was defined in the same model over a 15 week period. This follow-up study identified that hypertensive changes of increased LA dimensions, prolonged atrial refractoriness and inflammatory infiltrates occurred by 5 weeks and atrial fibrosis and conduction slowing occurred at 10 weeks (326). In keeping with animal work, Medi et al demonstrated electro-anatomic substrate changes of conduction slowing, conduction block across the crista terminalis and greater AF inducibility in patients with treated longstanding systemic hypertension (327). Overall, these findings have demonstrated electrical and structural abnormalities associated with hypertension that may predispose to AF.

Alcohol

Alcohol consumption is a known risk factor for atrial fibrillation (21). Although limited data exists studying the clinical substrate associated with alcohol, novel work by Voskoboinik and colleagues utilised cardiac magnetic resonance (CMR) and electro-anatomic mapping in patients with atrial fibrillation stratified according to drinking status (328-330). The investigators showed CMR differences with reductions in left atrial mechanical function (LA volume, emptying fraction and reservoir function) and progressively greater drinking status (329). A subsequent CMR study showed greater ventricular fibrosis assessed by T1 mapping times in drinkers compared with non-drinkers (328). Finally, an electro-anatomic left atrial mapping study by the same group showed that moderate drinkers (8-21 drinks/week) of alcohol exhibited lower global voltage, conduction velocity slowing and greater complex signals compared to non-drinkers (330). Interestingly, these differences were not observed in mild drinkers. The alcohol related changes noted in the aforementioned studies suggest that atrial and
ventricular cardiac remodelling may be key contributors to explaining alcohol related effects in the heart.

**Substrate-based ablation of atrial fibrillation**

Since the seminal study by Haïssaguerre and co-workers, which elegantly identified that ectopic firing from within the pulmonary veins induced AF in a majority of individuals with paroxysmal AF, pulmonary vein isolation remains the foundation of AF ablation. However, achieving durable success rates for persistent AF ablation remains a significant challenge. Beyond PVI, multiple strategies have been employed with variable success including linear ablation, posterior wall isolation, complex fractionated electrograms (CFAE) ablation, rotor ablation of focal impulses and substrate-based ablation. Moreover, adjunctive ablation strategies may predispose to pro-arrhythmic effects due to re-entrant tachycardias such as peri-mitral flutter. In the STAR-AF II trial, Verma and colleagues randomised 549 persistent AF patients to three groups: 1) PVI alone, 2) PVI and CFAE ablation and 3) PVI and linear ablation in a 1:4:4 ratio in an intention-to-treat analysis. The investigators identified that 18 month AF-free survival was not significantly different across each of the groups; PVI, PVI and CFAE and PVI and linear ablation (59% vs 49% vs 46%, p=0.15) (331). These findings suggest that the adjunctive strategies studied did not confer additional benefits, highlighting the requirement for improved patient selection or novel ablation approaches in the context of persistent AF ablation.

**Voltage-guided ablation strategies**

Several observational studies have shown that voltage-guided strategies targeting regions of abnormal substrate may improve outcomes following AF ablation (332-
Rolf and co-workers provided the initial observational evidence that ablation of low voltage areas may result in improved AF recurrence following ablation (332). Kottkamp coined the ‘box isolation of fibrotic areas’ (BIFA) approach by stratifying patients into 4 groups based on prevalence of low voltage scar (<0.5mV) and identified that adjunctive BIFA in patients with low voltage zones reduced AF recurrence (333). Subsequently, a meta-analysis of six observational studies (n=885 patients) showed that adjunctive low-voltage guided ablation in addition to wide antral circumferential PVI may result in a markedly greater freedom from atrial arrhythmia after a mean follow-up period of 17 months (70% vs 43%, OR 3.41; 95% CI 2.22-5.24) (339). Recent randomised evidence on the utility of voltage-guided substrate ablation is conflicting. Kircher et al performed a randomised trial in 124 patients utilising an adjunctive low voltage ablation strategy which demonstrated significantly higher 12 month freedom from arrhythmia compared with a conventional PVI (90% vs 72%, p=0.04) (340). Another randomised trial by Yang and co-workers in 229 persistent AF patients comparing low voltage homogenisation with conventional stepwise strategy failed to show a difference in AF recurrence after 12 months (74% vs 71%, p=0.325). The discrepancy in findings across these observational and randomised studies may be attributed to the significant variability in the mapping strategies, rhythm during mapping (sinus vs pacing vs AF), mapping catheter, inclusion criteria, low voltage cutpoints and prevalence of low voltage zones. This degree of heterogeneity potentially limits the translational and practical applicability of these studies to patients with persistent AF. Additionally, multiple factors such as electrode size and spacing may impact on the degree of mapped substrate. Thus, techniques to improve patient selection, risk stratification and identification of the target ablation zones may help to standardised an approach to this strategy.
Determinants of voltage mapping

Key determinants of the generation of bipolar voltage signals used to create electro-anatomic voltage maps includes: 1) activation direction, 2) electrode spacing, 3) electrode size and 4) tissue contact. The angle and relationship of the recoding bipole to the wave-front dipole influences the time taken for the wave-front to arrive to both electrodes. Single extra-stimulus voltage differences in relation to activation direction has been shown in studies comparing coronary sinus pacing to right atrial pacing (341) and sinus rhythm (342). At a fixed conduction velocity, the electrode spacing determines the relative timing difference in wave-front propagation to each electrode and consequently defines the morphology and amplitude of the bipolar signal (temporal offset between both unipolar signals). A computer simulation of increasing electrode spacing correlated with increasing voltage with a cut-off plateau level in healthy tissue up to 4mm, not seen in abnormal diseased tissue models (343). Far-field signals remote from the recording electrode location have been shown to have a greater influence on bipolar electrograms with increasing electrode spacing leading to near-field electrograms more easily observed with smaller spacing (344). Catheter electrode sizing has been shown to be an important contributor to voltage mapping. Anter and colleagues performed an electro-anatomic study collecting points with a 1mm high-density mapping catheter compared with a 3.5mm ablation catheter (345). They found that the smaller electrode size was associated with smaller area of low voltage areas (<0.5mV) and the bipolar voltage amplitude was significantly higher in these scar regions. These findings suggest that larger electrodes used to map fibrotic regions may be vulnerable to summing both regions of normal tissue and low voltage resulting in overall lower voltages and lower resolution data. Tissue contact has also been shown to modestly correlate with bipolar voltage amplitude up to 5 grams of force (346).
Definitions of abnormal atrial substrate

At present, the term ‘AF substrate’ broadly encompasses both electrical and structural abnormalities including gap junction disruption, atrial dilatation, loss of myofibrils and fibrosis as already discussed. Multiple modalities such as electro-anatomic mapping, cardiac magnetic resonance imaging, pro-fibrotic biomarkers and histopathology have demonstrated direct and indirect evidence of atrial substrate. However, the consensus for the precise definitions of the parameters of fibrosis and scar are lacking. Traditionally, low voltage areas have been defined by voltage less than 0.5mV, which has not necessarily been derived by correlation with underlying atrial substrate. Kapa and colleagues employed a statistical approach for ‘normal’ voltages by adopting the definition of voltages in the lowest 5th percentile as an abnormal cut-off in 26 patients with paroxysmal AF (347). They concluded that cut-offs of 0.2mV in the posterior wall and PV-LA junction and 0.45mV for the other left atrial regions were representative of low voltage zones. Another study by Lin et al in both patients with AF and healthy controls without AF undergoing left sided accessory pathway ablation utilised a similar statistical approach of defining low voltage regions less than 5th percentile of signals (348). This group found that 5% of signals defined as low voltage in their study were below a 0.38mV cut-off in controls and 0.1mV in persistent or long-standing AF characterised as ‘dense scar’. Yagashita and co-workers found a 5% cut-off of 1.17mV in 6 healthy controls with SVT and showed that presence of 0.5-1.1mV zone of relative low voltage was associated with increased AF recurrence, suggesting that clinically important low voltage may exist on a spectrum rather than a fixed cut-off of less than 0.5mV (349). Recent evidence also suggests that single premature extra stimulus testing at decreased coupling intervals may have variable patterns and degrees of attenuation on voltage amplitude (350). Overall, voltage mapping provides a significant
amount of data to potentially personalise ablation strategies however the clinical utility and determinants of this approach requires further evaluation.

**Sex-based differences in atrial fibrillation**

Significant sex-based differences exist in the prevalence and incidence of AF. Framingham Heart data found that men carried a 1.5-fold increase in risk of incident AF even following adjusting for age and other co-morbidities (351). This study reported an AF incidence in women of 1.6 cases per thousand person years compared to 3.8 cases per thousand person years in men. These findings have been consistent across other populations in Europe and Asia.

*Impact of sex on AF pathophysiology*

Currently, the precise pathophysiological mechanisms underlying sex-based differences in AF remains incompletely defined. A number of theories have proposed that lean body mass and anthropomorphic differences may lead to increased left atrial dimensions with a larger atrial mass to facilitate re-entry and account for the increased incidence of AF in men (352,353). A rabbit model found that males had increased PV afterdepolarisations and burst firing, whilst females had a lower PV resting membrane potential (354). Despite less PV firing, females have been found to have an increased prevalence of non-PV triggers of AF in patients undergoing AF ablation (16% in females vs 8.4% in males, p<0.001) (355). However, this finding was unadjusted for between group differences with females being older with larger atrial dimensions. An imaging study of patients with AF demonstrated that females have greater atrial fibrosis on late-gadolinium enhanced CMR sequences compared to men (29.9 ± 6.2 vs 23.0 ± 7.9%, p=0.003) (356). Greater fibrotic change in women may partly explain multiple
clinical associations of increased AF recurrence rates following ablation and cardioversion. However, substrate differences were not found between sexes in an electro-anatomic study by Walters et al. in both atrial and pulmonary venous electrophysiology (357).

**Sex Hormones and AF**

Hormonal differences have been proposed as underpinning sex-related differences in AF. Testosterone deficiency in rats has been implicated in atrial arrhythmogenesis through calcium leak from the sarcoplasmic reticulum with reversal of abnormalities after testosterone replacement (358). Similar results in humans from the Framingham Heart Study showed that lower testosterone was strongly predictive of incidence AF in men aged above 80 years of age (359). Progesterone has been shown to act on slow delayed rectifier currents and L-type calcium currents to result in APD shortening (360). In both animal and human models, oestrogen has been demonstrated to have multiple effects on atrial electrophysiology including increased atrial conduction time, prolonged APD, atrial refractoriness and AV node conduction potentially via potassium channel downregulation or greater calcium influx leading to weaker repolarising currents (361,362). The importance of oestrogen in the incidence of AF in females is uncertain as the majority of females develop AF later in life following menopause. The Women’s Health Initiative found that oestrogen replacement therapy was a univariate predictor of increased incidence of AF compared with placebo or combination oestrogen and progesterone therapy (363). However, after adjustment for heart failure and coronary artery disease, the observed difference was no longer significant. A Taiwanese health insurance registry study analysed data between 1998 and 2008 and demonstrated that conjugated equine oestrogen treatment in post-
menopausal females was an independent predictor of increased incidence of AF compared with oestradiol therapy (adjusted HR 1.96 95%CI 1.03-3.73, p=0.042). Conversely, in Danish women following myocardial infarction, hormone replacement therapy (HRT) was found to be protective from incident AF (HR 0.82, 95%CI 0.68-1.00) with a 37% reduction in risk in females greater than 80 years of age (364). The mechanisms and link of the cardiac effects of sex hormones still requires further investigation. Current evidence suggests that transcriptional and posttranslational effects on protein synthesis impacting potassium and calcium ion channels may partly account for sex-based differences with a multifactorial explanation most likely.

**Sex-based differences in clinical course in AF**

Multiple clinical studies have identified differences in the clinical course of AF in females compared with males. Women with AF reported greater and more marked symptom burden, functional deficit, and morbidity when compared to men (365,366). The Euro Observational Research Program on Atrial Fibrillation (EORP-AF) surveyed over three thousand patients with AF and found that a significantly higher proportion of females reported palpitations compared to males (80.2% vs 68.5%, p<0.001) which was associated with heightened anxiety levels (366). Women have been shown to be hospitalised longer for AF and more likely to experience AF symptoms for greater than 48 hours in duration (367). Whilst there are no significant between sex differences in rates of HFREF in AF patients, women have been found to be more likely to develop heart failure with preserved ejection fraction (HFPEF) in the setting of diastolic impairment (368,369). Studies of sex-based differences in AF-related mortality are mixed. A meta-analysis of outcomes in over four million subjects from 30 studies across 20 years identified that females with a history of AF have a significantly higher
risk of all-cause mortality (1.12, 95% CI 1.07-1.17) and death due to cardiac causes (1.93, 95% CI 1.44-2.60) compared to males (370). In contrast, the contemporary ORBIT-AF registry reported that compared to males, females with AF carry a lower risk of all-cause mortality (HR 0.57, 95%CI 0.49-0.67, p<0.001) and cardiovascular death (365).

There is an expansive body of evidence that has shown that females with AF have a higher risk of stroke above 75 years of age and sex forms part of the widely used CHA2DS2-VASc score (371). Friberg et al studied a Swedish cohort of over 100,000 subjects with AF not treated with anticoagulation and identified that females had a 47% greater stroke risk (372). The sex-based difference in stroke risk was significantly higher in the group of women above 75 years old (HR 1.24, 95% CI 1.18-1.30). Additionally, a registry study of Swedish females aged less than 65 years in the absence of clinical stroke risk factors (CHA2DS2-VASc score = 1) was low, ranging between 0.1-0.2% (373). Thus, recent local Australian clinical guidelines advocate the use of a sexless stroke score below the age of 65 years (374). Women with AF have also been found to experience larger strokes with poorer functional outcomes at discharge (375). Although there is a relative paucity of data underlying the female predisposition to stroke, differences in cardiovascular remodelling, inflammation, greater co-morbidities, and a pro-thrombotic state post-menopause may provide potential explanations (376).

**Sex-based differences in AF management**

From a stroke prevention perspective, evidence investigating the impact of sex on appropriate anticoagulation rates appears conflicting. Lip et al evaluated over 17,000
AF patients with one or more clinical stroke risk factors and found a similar use of anticoagulation between women (60.8%) and men (60.9%) with sub-optimal anticoagulation observed across both groups (377). Consistent findings have been reported in the EORP-AF and ORBIT-AF registry studies (365,366). Conversely, a registry study (378) and a survey of general practitioners in the UK (379) both identified that women were less likely to be prescribed appropriate anticoagulation compared to men. Whilst on anticoagulation treatment, a meta-analysis did not demonstrate any significant between sex differences in bleeding rates (380). Efforts to optimise anticoagulation rates in both men and women, as well as manage modifiable risk factors for AF, have shown to correlate with improved outcomes in specialised integrated care AF clinics (381).

Despite a greater symptom burden, women are less frequently referred for rhythm control strategies compared to men. The ORBIT-AF study found that although anti-arrhythmic use was similar between sexes, women underwent less cardioversions (26.7 vs 32.4%, p<0.001) and AF ablation procedures (4.9 vs 5.9%, p=0.04) and more atrioventricular node ablation procedures (2.9 vs 1.7%, p<0.001) (365). Lower AF ablation rates in women have also been demonstrated in several other registry studies (382-384), with women being older and in AF longer from first diagnosis at time of ablation. The discrepancy in procedural referral patterns may potentially reflect a perception of less optimal outcomes following ablation. Zylla and colleagues recruited 3652 women as part of the Germany Ablation Report undergoing AF ablation who were older with higher paroxysmal AF burden but less cardiovascular disease compared to men (385). The investigators identified that major in-hospital complications were greater in women compared to men (1.9 vs 0.8%, p=0.023) driven
by femoral access site bleeding (6 vs 3%, p<0.001), and 12 month AF recurrence rates were significantly higher in women (50 vs 45%, p=0.017) (385). A large global survey of 34,943 AF ablation procedures also found that women had a near two-fold increased risk of developing the rare complication of cardiac tamponade (OR 1.83, p<0.001) (386). The mechanisms accounting for these differences are incompletely defined but may relate to women having smaller sized atria with thinner walls and more technically difficult femoral access when unaided by vascular ultrasound.
Chapter 2:
Genetic Susceptibility to Atrial Fibrillation is associated with Left Atrial Electrical Remodelling and Adverse Post-Ablation Outcome

INTRODUCTION
Atrial fibrillation (AF) is a complex heterogeneous condition that exists on a clinical spectrum that often begins with the presence of heart failure, valvular heart disease, hypertension, diabetes, sleep disordered breathing and obesity (3). Beyond these traditional risk factors, numerous studies in families and in population cohorts have suggested that AF can have a genetic basis (35). The first genome-wide association study (GWAS) of AF, performed by Gudbjartsson and colleagues, identified a significant association between AF and the single nucleotide variant (SNV), rs2200733, on chromosome 4q25 (42). These findings have been robustly replicated in subsequent studies of up to 500,000 individuals (39). Additionally, this 4q25 locus has been associated with recurrence of AF after catheter ablation (387), ischaemic stroke (49), post-op AF following cardiac surgery (388), response to antiarrhythmic therapy (389), cardioversion (390) and sudden cardiac death (391). Despite the substantial interest in exploring clinical associations of the 4q25 locus, the disease-associated phenotype and pathophysiological mechanisms linking this locus with AF risk remain unknown.

The aim of this study was to undertake a detailed assessment of atrial electrophysiological properties and post-ablation outcomes in patients with AF with and without the rs2200733 SNV. We hypothesized that the presence of this SNV would
be associated with differential extent of electroanatomic remodelling that would contribute to an atrial substrate with adverse impact on AF management.

METHODS

Study Population

185 consecutive patients undergoing AF ablation were prospectively screened. Of these, 102 patients met inclusion criteria and were enrolled (figure 1). Patients were included if they had drug refractory symptomatic paroxysmal (lasting <7 days) or persistent AF (lasting >7 days). Exclusion criteria were: (1) structural heart disease (congestive cardiac failure [LVEF<50%], greater than mild valvular disease, severely elevated pulmonary artery pressure) (2) inability to be electrically cardioverted to sinus rhythm (3) previous AF ablation; (4) amiodarone use; or (5) age < 18 years. All patients underwent routine pre-ablation workup including transthoracic echocardiography. This study was approved by the Royal Melbourne Hospital Research and Ethics Committee.

Genotyping Protocol

After written informed consent was obtained, peripheral blood samples were obtained via venepuncture from all study participants. DNA was extracted using the Illustra Nucleon BACC3 Genomic DNA Extraction kit (GE Healthcare, Chicago, IL, USA). Samples were genotyped for the AF-associated SNV, r2200733 (NC_000004.12:g.110789013C>T)(46), using PCR and Sanger sequencing (Illumina, San Diego, CA, USA). Investigators were fully blinded to the genotype status of each study subject until after completion of all data collection, follow-up and analysis of measured parameters. Patients were categorized as SNV carriers if they were
heterozygous (CT) or homozygous (TT) for the r2200733 variant. Patients with the CC genotype were classified as non-carriers.

**Procedural Study Protocol**

All antiarrhythmic medications including beta-blockers and calcium-channel blockers were withheld at least 5 half-lives prior to procedure. Anticoagulation was managed according to operator preference. All procedures were performed under general anesthesia with peri-procedural transesophageal echocardiography to exclude LA thrombus. Double trans-septal access was performed after heparinization (target ACT 300-350).

Bipolar intracardiac electrograms and 12-lead electrocardiography (ECG) were recorded on a digital amplifier system (EPMed Systems, Chicago, IL, USA). Intracardiac electrograms were filtered from 30 to 500 Hz and measured with digital calipers at 200 mm/s sweep speed.

**Electroanatomical mapping**

Left atrial geometry was constructed with a 20 pole Lasso catheter (Biosense Webster, Irvine, CA, USA, 2-6-2 mm electrode spacing) and merged with a periprocedural CT using the CARTO3 electroanatomical mapping system. Patients who presented to the lab in AF were electrically cardioverted to sinus rhythm. Voltage and activation maps were constructed using the Lasso catheter during constant pacing from the distal coronary sinus (CSD) to standardize the direction of wavefront propagation at a fixed cycle length of 600ms. Complete coverage of the entire LA geometry was performed and correlated to the CT to ensure smooth coverage across all regions with a minimum of 1000 points using the Confidense™ algorithm to ensure even point distribution.
(Biosense Webster). Strict criteria were employed to account for the lack of tissue contact data on the multi-polar mapping catheter with point collection performed only by experienced operators after careful assessment of geometry and fluoroscopic motion. Mapping fill threshold was set at 5mm.

Ablation procedure

Following mapping, experienced high-volume operators (>100 procedures/year case load) created wide antral circumferential ablation lines to achieve pulmonary vein isolation (PVI) utilizing an irrigated-tip radiofrequency ablation catheter. The primary procedural endpoint was demonstration of entrance and exit block of all pulmonary veins on a circular mapping catheter following adenosine challenge for acute reconnection and a mandatory 30 minute waiting period.

Electrogram analysis

Electrogram analysis for each map was performed manually offline. For activation time, each point was analysed at 200 mm/s sweep speed and appropriately annotated at the maximum negative dV/dt for unipolar signals or the peak sharp of the bipolar electrogram. Although data was collected using the Confidense algorithm, an internal point filter to within 5mm of the chamber surface geometry was applied (345) and all residual acquired points were meticulously manually reviewed and annotated. Only points demonstrating characteristics of near-field signals were included. In keeping with prior publications (392), these signals demonstrated at least 2 sharp peaks and were consistent with anatomically adjacent signals in terms of signal quality and electrogram timing. Points that did not fit these criteria, ectopic beats and artefact were excluded. Following extensive manual annotation and exclusion of signals not meeting
the above criteria, signal processing was also performed offline (MATLAB 9.1, Mathworks, MA, USA).

**Global and Regional Atrial Voltage Analysis**

Bipolar voltage was defined as the peak-peak electrogram voltage. Segmental voltage analysis of the left atrium was performed by dividing the chamber into the following 6 segments: anterior, posterior, septal, lateral, roof and inferior (Figure 2A). The mitral annulus and pulmonary veins beyond the antrum were excluded. The mean voltage of each region was calculated.

**Conduction Velocity Analysis**

Conduction velocity (CV) was analysed in MATLAB as previously described using the polynomial algorithm (330,393). In brief, this method assigns a fitting ‘window’ per region with a minimum of 20 points required. Each region is assigned subsets of Cartesian coordinates in space and activation time. These are fitted to a smooth polynomial surface in three-dimensional space, using a standard least squares algorithm, which provides robustness against outliers. The gradient is then calculated from the fit and used to calculate velocity components, with final velocities at each point calculated from the weighted average of velocity components from every fit including that point (394). The mean LA and regional CV was calculated. Atrial conduction slowing (defined as local CV 10 to 20cm/s) and conduction block (defined as <10 cm/s) were expressed as a proportion of total points in each region (395). Conduction heterogeneity was determined by calculating the coefficient of variation (CoV) of conduction velocity.
**Analysis of Complex Signals**

Complex signals were defined as electrograms with \( \geq 3 \) deflections and \( >50\text{ms} \) duration (fractionated potentials [FP]) or 2 separate deflections separate by an isoelectric interval (double potentials [DP]). These signals were manually annotated and tagged. The percentage of complex signals was expressed as a proportion of the number of complex signals divided by the total number of points.

**Assessment of Effective Refractory Period & Sinus Node Function**

Effective refractory period (ERP) at the proximal and distal coronary sinus was performed. Briefly, ERPs were measured using a pacing drive train of 8 beats followed by a single extrastimulus commencing at a coupling interval of 150ms and incrementing by 10ms until local capture was demonstrated. The local ERP was defined as the longest extrastimulus that failed to capture the pacing site. ERPs were measured at 2 drive train cycle lengths (600 and 400ms) and repeated at least twice at each site to ensure consistency. Sinus node recovery time (SNRT), evaluated by 30 s burst pacing trains at every 50ms from 600 to 400ms, was determined as the longest time from the stimulus artefact to the earliest atrial activity. The corrected SNRT was determined by correcting for the underlying sinus cycle length.

**Follow-up and Rhythm Monitoring**

Antiarrhythmic medications and anticoagulation were managed according to physician preference in accordance with guidelines and following a 3-month post-ablation blanking period. Patients underwent systematic comprehensive rhythm monitoring throughout the course of the study. Time to recurrence were determined using either cardiac rhythm management devices (pre-existing pacemaker or insertable cardiac
monitor [ICM – Medtronic Linq™, Minneapolis, MN, USA]) or Holter monitoring at 3, 6, and 12 months post ablation followed by 6-monthly thereafter until study completion. Cardiac arrhythmia detection devices were either interrogated at clinic visits or via remote monitoring.

Statistical Analysis

Data analysis was performed using SPSS software (Version 23, IBM, Armonk, New York). Normality of all quantitative variables was assessed using the Shapiro-Wilk test and normalizing transformations were performed as necessary. Data are expressed as mean ± standard deviation (SD) unless otherwise stated. Power calculation was performed assuming a minor allele frequency of 0.3 and AF recurrence rate of 20%. A sample size of 90 patients is required to detect a hazard ratio of ≤0.5 for arrhythmia free survival based on prior work(387,396) with a power of 80%. A mixed random effects model was used for comparison between multiple regional measures within each patient (ie. CV, voltage). To investigate regional variation, region (6 LA segments) and group (carrier and non-carrier) were modelled as fixed effects with a region x group interaction term. If a signification interaction was present, mixed effects Bonferroni post hoc analysis p-values were reported. Conventional two-group comparisons were made using unpaired t test for continuous variables, or the chi-squared test for categorical variables.

General linear model repeated measures analysis was also performed separately on each parameter to determine univariable predictors of CV heterogeneity and complex signals. Following univariable analysis, all predictors with p<0.1 were included in a multivariable model. Survival curves for freedom from arrhythmia (AF/atrial tachycardia [AT]) and AF were determined using the Kaplan-Meier method and
compared using the log-rank test. Cox proportional hazards regression was used to determine univariate (p<0.1) and multivariate predictors of arrhythmia-free and AF-free survival.

To evaluate the reliability of manual electrogram annotation, the principal investigator and a second observer (C.N.) were asked to classify a blinded sample of 3000 points from the data set using the above study criteria. Cohen’s kappa provided a measure of agreement above that expected by chance. For the principal investigator, kappa statistics were 0.92 (p<0.001) for appropriate near-field signals and 0.91 (p<0.001) for complex signals for intra-observer agreement. For the second observer (C.N.), kappa statistics were 0.86 (p<0.001) for near-field signals and 0.88 (p<0.001) for complex signals for inter-observer agreement.

RESULTS

Baseline Characteristics

A total of 185 patients were screened for the study. After pre-specified exclusions (redo procedures, cardiomyopathy, valvular disease and amiodarone use), 102 patients consented for the study protocol, genotyping and post ablation follow-up (figure 1). Of these, 15 patients who were peri-procedurally cardioverted for AF did not remain in sinus rhythm and 2 patients were not mapped due to patient haemodynamic factors. These individuals were however, retained for follow-up outcomes. The remaining 85 patients underwent detailed three-dimensional electroanatomic mapping. Overall, mean age was 61±9 years, 64% were male and 50% had persistent AF. Of the genotyped cohort, 41/102 patients (40%) were r2200733 carriers (CT, 38 patients [37%]; TT, 3 patients [3%]) with mapping performed in 31/85 (36%) carriers. The baseline patient characteristics including cardiac risk factors and echocardiographic parameters did not
significantly differ between the non-carrier (CC) and carrier (CT/TT) groups (Table 1). Follow-up was completed on a total of 98 (96%) patients (figure 1). Of these, more than half (50/98 [51%]) were continuously monitored by ICM (46/98 [47%]) or dual chamber pacemaker (4/98 [4%]) with the remaining undergoing serial Holter surveillance (48/98 [49%]).

**High Density Electroanatomical mapping**

The mean total number of points collected per patient was 2,239 ± 852. Following internal point filtering (<5mm) and manual point annotation, a mean of 1388 ± 314 points per patient were included with similar numbers of points between carriers and non-carriers (1359 ± 274 vs 1406 ± 336 points, p=0.518). Table 2 summarizes the global and regional electroanatomical parameters measured in both groups.

**Impact of Genotype on Conduction**

The mean global and regional CV were not affected by SNV status (carriers: 38.4 ± 6.8 vs non-carriers: 43.9 ± 5.6 cm/s, p=0.137) (Table 2). However, SNV carriers had significantly greater CV heterogeneity (carriers: 45.7 ± 7.5 vs non-carriers: 35.9 ± 2.3%, p<0.001) (Figure 2B) with marked regional differences across the LA (interactive p[group x region]=0.003) particularly affecting the posterior (p<0.001), lateral (p=0.012) and inferior walls (p=0.001) (Table 2).

Similar regional patterns were also seen for atrial conduction slowing/block (interactive [group x region] p=0.029). SNV carriers had a significantly greater extent of the LA that showed conduction slowing or conduction block (carriers: 31.7 ± 8.2 vs non-carriers: 17.9 ± 1.9%, p=0.013) (Table 2) with changes being most marked in the posterior (carriers: 41.8 ± 30.9 vs non-carriers: 19.1 ± 26.4%, p=0.001) and lateral walls.
(carriers: 44.1 ± 30.9 vs non-carriers: 21.6 ± 30.6%, p=0.003) (Table 2). An example of these conduction differences is shown in Figure 3.

**Impact of Genotype on Electrogram Fractionation**

For the LA overall, SNV carriers showed a significantly higher proportion of complex fractionated electrograms and double potentials (carriers: 9.4 ± 2.9 vs non-carriers: 6.0 ± 1.2, p=0.008) (Table 2). In keeping with the regional differences in conduction, there was significant regional variability in complex signals between the 2 groups (interactive [group x region] p=0.035) again localized to the posterior (11.8 ± 12.4 vs 6.3 ± 5.0%, p=0.007) and lateral walls (14.5 ± 12.8 vs 7.1 ± 7.6%, p=0.002). The distribution of complex signals across the LA in both groups is shown in Figure 4.

**Analysis of LA Voltage**

There were no differences in mean global bipolar voltage (carriers: 1.73 ± 0.20 vs non-carriers: 1.77 ± 0.22, p=0.783) (Table 2). There were no significant regional differences in voltage (interactive [group x region] p=0.822) or proportion of low voltage points (<0.5mV) (carriers: 22.7 ± 4.1 vs non-carrier: 24.0 ± 5.2%, p=0.701).

**Assessment of Effective Refractory Period & Sinus Node Function**

Atrial ERP were similar between carriers and non-carriers from proximal and distal coronary sinus at 2 cycle lengths (Table 3). There were no significant differences in corrected SNRT between groups across each cycle length (Table 3).
Univariable and Multivariable Predictors of Atrial Remodelling

Predictors of atrial remodelling are presented in Table 4. Univariable predictors of CV heterogeneity were persistent AF (p=0.006), SNV carrier status (p=0.001) and AF duration from first diagnosis (p=0.017). However, AF duration (p=0.021) and SNV carrier status (p=0.001) remained as multivariable predictors of CV heterogeneity with only carrier status being a multivariable predictor of regional differences (interactive [group x region] p=0.04). Similarly, univariable predictors of complex signals (fractionated electrograms and double potentials) were female gender (p=0.022), SNV carrier status (p=0.008) and persistent AF (p=0.054). On multivariable analysis, SNV carrier status (p=0.002) and female gender (p=0.022) remained as independent predictors of complex signals.

Clinical Outcomes Following AF Ablation

Pulmonary vein isolation was achieved in 100% of patients (n=102). The average ablation time was 37 ± 10 min and fluoroscopy time was 9.2 ± 5.0 min. Procedural characteristics were not significantly different between carrier and non-carrier groups (Table 5). After a median follow up of 27 months (Q1–3: 19-31) following index AF ablation, overall success was achieved in 67 (68%) patients. SNV carriers had significantly lower single-procedural arrhythmia-free outcome (carriers: 20 [51.3%] vs non-carriers: 47 [79.7%], log-rank p=0.003) and shorter time to arrhythmia recurrence (carriers: 22.0 ± 2.2 vs non-carriers: 29.5 ± 1.5 months, log-rank p=0.003). Similarly, freedom from AF was significantly lower in SNV carriers (carriers: 25 [64%] vs non-carriers: 50 [85%], log-rank p=0.016) with higher antiarrhythmic drug use at last follow up (16 [41%] vs 9 [15%], p=0.008). Kaplan-Meier survival curves are shown in Figure 5. On Cox regression analysis, significant univariable predictors of reduced
arrhythmia-free survival were SNV carrier status (hazard ratio [HR] 0.35; 95% confidence interval [CI] 0.17-0.73, p=0.005), persistent AF (HR 0.36; 95% CI 0.17-0.78, p=0.013) and obstructive sleep apnoea (HR 0.48; 95% CI 0.23-0.96, p=0.037). In a multivariable model, SNV carrier status remained as an independent predictor of lower arrhythmia-free survival (p=0.019).

**DISCUSSION**

Using high-density electroanatomic mapping, we show for the first time, that the rs2200733 SNV is associated with altered atrial electrophysiological characteristics in patients with AF. Specifically, we show that SNV carriers have: (i) significant differences in LA conduction heterogeneity, extent of conduction slowing/block and complex fractionated electrograms, (ii) a distinctive regional distribution of these changes with a predilection to occur in the lateral and posterior walls and (iii) lower single-procedural arrhythmia-free survival and shorter time to recurrence. Our data suggest that genetically-determined AF-induced atrial electrical remodelling provides a substrate for AF maintenance and contributes to poor long-term outcomes following ablation.

*Genetics and AF*

Early studies demonstrated that a family history of AF was associated with a 70% increased risk of AF in offspring (36), suggesting a genetic etiology. Genetics now have an established role in the pathogenesis of AF and numerous rare variants sufficient to cause disease have been identified in familial cases (38). However, familial forms of AF are thought to be relatively uncommon overall. Common variants that affect susceptibility to AF in the general population have been identified by GWAS, with
recent meta-analysis data obtained from >500,000 subjects (39). The chromosome 4q25 locus remains the most significant GWAS locus identified to date, with several distinct haplotypes on chromosome 4q25 independently associated with AF risk. The strongest association has been found for the haplotype tagged by the rs2200733 SNV. Despite more than a decade of research in this field, an explanation for the links between this locus and AF has remained elusive. The only intermediate phenotype reported to date was a subtle PR prolongation but this was only seen in the small numbers of homozygous SNV carriers, with no changes found in heterozygous individuals, which comprise the majority of individuals at AF risk (50).

PITX2 and AF

The 4q25 locus is located in a region of the genome that is devoid of genes, and it has been hypothesized that this region contains a regulatory element that affects gene expression. The closest gene is paired-like homeodomain transcription factor 2 (PITX2), a transcription factor with diverse cardiac and extra-cardiac functions including a role in heart development. There are several isoforms of PITX2, with PITX2c being predominantly present in the heart. Several lines of evidence support PITX2 being a good candidate gene for AF and it has been widely implicated as the target gene of the 4q25 locus. Proposed effects of PITX2c deficiency, based on data from Pitx2c knockout mice, include defective development of the pulmonary vein myocardial sleeve or failure of suppression of sinus node-related genes in the LA with subsequent changes in electrical and structural properties that predispose to AF (51-54). Two studies have identified potential enhancer sequences in the 4q25 locus that interact with the PITX2c promoter (56,57), pointing to PITX2 as the potential target gene of this locus. However, there are currently no data that show convincing evidence
that the rs2200733 haplotype is associated with altered \textit{PITX2c} expression in the adult human LA (59). Since \textit{PITX2c} is mainly active during cardiac development, looking at \textit{PITX2c} expression levels in adult hearts may “miss the boat” and it remains possible that enhancer effects might only be detectable in developmental models.

\textit{Mechanisms of conduction and substrate variability in SNV carriers}

In our study, there were no significant differences in clinical features or structural echocardiographic parameters between carriers and non-carriers. Notably, none of the parameters of LA size were significant multivariate predictors of conduction properties. The most important finding was rs2200733 haplotype-associated LA electrical remodelling which was manifest by regions of conduction slowing and heterogeneity. Increased conduction heterogeneity has been associated with increased AF susceptibility both in animal and human studies (161,175). It is possible that these conduction changes are mediated by alterations in side-to-side cellular electrical coupling, connexin expression and resting membrane changes which may form the substrate for re-entry necessary for maintenance of AF (60). Interestingly, \textit{PITX2} has been implicated in structural changes in intercalated discs and the regulation of multiple downstream targets including gap junction proteins and ion channels (61). Translational work from Syeda and colleagues elegantly demonstrated that \textit{PITX2} plays an integral role in regulating atrial myocyte membrane potential with flecainide-mediated sodium channel blockade being more effective in \textit{PITX2} deficient mice and human models (59).

A key question is why our observed conduction changes display regional differences within the LA. Studies in humans with mitral valve disease have shown relative enrichment of \textit{PITX2} expression at the pulmonary vein-left atrial (PV-LA) junction.
It is intriguing that similar regional posterolateral conduction remodelling has recently been reported in obese patients with LA epicardial fat accumulation (317). It is possible that genetic susceptibility interacts with a regional second factor (wall thickness, hemodynamic stress, paracrine signalling, regional adiposity are potential factors) which increases the local susceptibility to remodelling.

4q25 and AF outcome

Prior studies have suggested that SNV’s at the 4q25 locus are associated with worse outcomes following PVI (387,396-398) while a single large study found no association between common genetic variants and outcome. Recent work by Shoemaker and colleagues in Caucasian populations across 3 centres showed that the rs2200733 SNV is associated with increased risk of AF recurrence (397). Reports in Han Chinese patients have also shown a positive association with AF recurrence (396). In contrast, the largest study to date in 1,068 Korean patients failed to show any association between common genetic variants (across 4q25 including rs2200733, 16q22 and 1q21 loci) and post-ablation outcome (399). The aforementioned studies assessed post-ablation outcomes with mean follow-up times of up to 12 months, and without continuous monitoring. Our study provides evidence in favour of predictive effects of the rs2200733 SNV, and extends previous work by having a significantly longer median follow-up (27 months) and by using a comprehensively monitored contemporary AF ablation cohort with over half receiving devices for arrhythmia detection. As rs2200733 SNV carrier frequencies differ between populations (42), whether phenotypic expression in relation to AF recurrence also varies between ethnicities is unclear. Given the heterogeneity of findings to date however, we cannot exclude the possibility that additional factors as yet undefined might further
modulate SNV effects on patient outcomes. The utility of personalized adjunctive strategies in genetically susceptible patients targeting potential sources of post-ablation AF recurrence such as remodelling-associated substrate and non-PV foci (400) requires further work.

Limitations
Differences in tissue contact can affect bipolar voltage amplitude thereby confounding results. Multipolar catheters do not possess contact force data and may be prone to collecting inaccurate data. However, meticulous attempts were made to ensure that only points in close proximity to the endocardium were collected including manual analysis of all points as described in the Methods section.

CONCLUSION
In patients with AF, carriers of the rs2200733 haplotype demonstrate a relatively greater arrhythmogenic atrial substrate when compared to non-carriers, with increased CV heterogeneity, regions of conduction slowing and block and a greater prevalence of abnormal electrograms. Additionally, carriers were found to have lower arrhythmia-free survival and shorter time to recurrence at long-term follow up. These data provide the first evidence that the rs2200733 SNV haplotype predisposes to AF by effects on LA electrical remodelling and identifies this haplotype as a determinant of long term outcome. The molecular mechanisms underpinning these changes warrant further investigation.
TABLES

Table 1. Baseline demographics of AF study subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-Carrier (CC) (n = 61)</th>
<th>Carrier (CT/TT) (n = 41)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60 ± 9</td>
<td>61 ± 8</td>
<td>0.833</td>
</tr>
<tr>
<td>Female Gender</td>
<td>26 (43)</td>
<td>11 (27)</td>
<td>0.104</td>
</tr>
<tr>
<td>Paroxysmal AF</td>
<td>33 (54)</td>
<td>18 (44)</td>
<td>0.224</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21 (34)</td>
<td>12 (29)</td>
<td>0.704</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>5 (8)</td>
<td>2 (5)</td>
<td>0.529</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>18 (30)</td>
<td>17 (41)</td>
<td>0.186</td>
</tr>
<tr>
<td>Ischaemic Heart Disease</td>
<td>5 (8)</td>
<td>5 (12)</td>
<td>0.505</td>
</tr>
<tr>
<td>TIA/Stroke</td>
<td>3 (5)</td>
<td>1 (2)</td>
<td>0.553</td>
</tr>
<tr>
<td>CHA\textsubscript{2}DS\textsubscript{2}-VASc Score</td>
<td>1.5 ± 1.2</td>
<td>1.2 ± 1.0</td>
<td>0.228</td>
</tr>
<tr>
<td>Obstructive Sleep Apnoea</td>
<td>19 (31)</td>
<td>16 (39)</td>
<td>0.331</td>
</tr>
<tr>
<td>Body Mass Index (kg/m\textsuperscript{2})</td>
<td>28.8 ± 4.6</td>
<td>29.8 ± 4.6</td>
<td>0.249</td>
</tr>
<tr>
<td>Obesity (BMI&gt;30 kg/m\textsuperscript{2})</td>
<td>18 (30)</td>
<td>17 (41)</td>
<td>0.197</td>
</tr>
<tr>
<td>Regular Alcohol Intake</td>
<td>34 (56)</td>
<td>18 (44)</td>
<td>0.239</td>
</tr>
<tr>
<td>Presenting Lab Rhythm (AF)</td>
<td>20 (33)</td>
<td>15 (37)</td>
<td>0.644</td>
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<tr>
<td>Time since AF Diagnosis (months)</td>
<td>58 ± 44</td>
<td>54 ± 56</td>
<td>0.687</td>
</tr>
<tr>
<td>Ethnicity (Anglo-Saxon)</td>
<td>53 (87)</td>
<td>35 (85)</td>
<td>0.773</td>
</tr>
<tr>
<td>Family History of AF (First Degree)</td>
<td>17 (28)</td>
<td>15 (37)</td>
<td>0.160</td>
</tr>
<tr>
<td>- Number of Relatives</td>
<td>1.6 ± 0.7</td>
<td>1.5 ± 0.9</td>
<td>0.841</td>
</tr>
<tr>
<td>- Avg Age at Diagnosis (yrs)</td>
<td>56 ± 11</td>
<td>52 ± 20</td>
<td>0.502</td>
</tr>
<tr>
<td>Antiarrhythmic therapy</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Flecainide</td>
<td>Sotalol</td>
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</tr>
<tr>
<td><strong>Echocardiographic Parameters</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LVEDD (cm)</td>
<td>4.8 ± 0.9</td>
<td>4.9 ± 0.5</td>
<td>0.725</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>57 ± 5</td>
<td>58 ± 6</td>
<td>0.196</td>
</tr>
<tr>
<td>LA Size (cm)</td>
<td>4.1 ± 0.6</td>
<td>4.0 ± 1.0</td>
<td>0.612</td>
</tr>
<tr>
<td>LA Area (cm$^2$)</td>
<td>24.2 ± 4.5</td>
<td>25.1 ± 6.1</td>
<td>0.449</td>
</tr>
<tr>
<td>LA Volume Index (ml/m$^2$)</td>
<td>34.9 ± 10.3</td>
<td>36.3 ± 11.9</td>
<td>0.708</td>
</tr>
<tr>
<td>RA Area (cm$^2$)</td>
<td>20.4 ± 5.5</td>
<td>19.1 ± 5.0</td>
<td>0.357</td>
</tr>
</tbody>
</table>

*Abbreviations:* AF – Atrial Fibrillation, BMI – Body mass index, CHA$^2$DS$_2$-VASc – Congestive Heart Failure, Hypertension, Age, Diabetes, Stroke, Vascular Disease, Sex, LA – Left Atrial, LV – Left Ventricular, LVEF – left ventricular ejection fraction, LVEDD – Left ventricular end diastolic diameter, TIA – Transient Ischaemic Attack, RA – Right atrial
Table 2. Effects of genotype on regional electroanatomic changes.

<table>
<thead>
<tr>
<th></th>
<th>Global</th>
<th>Anterior</th>
<th>Posterior</th>
<th>Inferior</th>
<th>Roof</th>
<th>Septal</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Voltage (mv)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SNV Carrier</td>
<td>1.73 ± 0.20</td>
<td>1.85 ± 0.68</td>
<td>1.65 ± 0.53</td>
<td>1.70 ± 0.68</td>
<td>2.04 ± 0.90</td>
<td>1.43 ± 0.48</td>
<td>1.75 ± 0.78</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>1.77 ± 0.22</td>
<td>2.02 ± 0.78</td>
<td>1.66 ± 0.82</td>
<td>1.66 ± 0.79</td>
<td>2.01 ± 0.78</td>
<td>1.49 ± 0.72</td>
<td>1.77 ± 0.83</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>p(group) = 0.783;</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
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<td>†</td>
</tr>
<tr>
<td></td>
<td>p(interactive*) = 0.822</td>
<td></td>
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<tr>
<td><strong>Conduction Velocity (cm/s)</strong></td>
<td></td>
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</tr>
<tr>
<td>SNV Carrier</td>
<td>38.4 ± 6.8</td>
<td>41.6 ± 21.7</td>
<td>34.2 ± 14.3</td>
<td>47.3 ± 23.9</td>
<td>45.3 ± 22.0</td>
<td>34.6 ± 18.7</td>
<td>27.6 ± 15.4</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>43.9 ± 5.6</td>
<td>44.9 ± 27.2</td>
<td>38.3 ± 20.5</td>
<td>52.1 ± 25.8</td>
<td>46.4 ± 23.8</td>
<td>45.5 ± 25.3</td>
<td>36.4 ± 21.2</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
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<tr>
<td></td>
<td>p(group) = 0.137;</td>
<td>†</td>
<td>†</td>
<td>†</td>
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<tr>
<td></td>
<td>p(interactive*) = 0.537</td>
<td></td>
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<tr>
<td><strong>Conduction Velocity Heterogeneity (%)</strong></td>
<td></td>
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</tr>
<tr>
<td>SNV Carrier</td>
<td>45.7 ± 7.5</td>
<td>36.5 ± 17.2</td>
<td>49.7 ± 19.1</td>
<td>57.6 ± 22.4</td>
<td>42.3 ± 17.5</td>
<td>39.8 ± 21.0</td>
<td>48.2 ± 29.9</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>35.9 ± 2.3</td>
<td>39.0 ± 19.9</td>
<td>33.3 ± 9.3</td>
<td>38.3 ± 24.7</td>
<td>35.7 ± 14.9</td>
<td>34.0 ± 14.4</td>
<td>34.8 ± 16.4</td>
</tr>
<tr>
<td>P-value</td>
<td>p(group) &lt;0.001; p(interactive*) = 0.003</td>
<td>0.576</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.074</td>
<td>0.141</td>
<td>0.012</td>
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<tr>
<td>------------------------------------------------------------------------</td>
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<tr>
<td><strong>Slow &amp; Block Points (%)</strong></td>
<td></td>
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</tr>
<tr>
<td>SNV Carrier</td>
<td>31.7 ± 8.2</td>
<td>24.3 ± 30.4</td>
<td>41.8 ± 30.9</td>
<td>26.7 ± 24.2</td>
<td>24.5 ± 30.6</td>
<td>29.0 ± 29.9</td>
<td>44.1 ± 30.9</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>17.9 ± 1.9</td>
<td>17.0 ± 30.0</td>
<td>19.1 ± 26.4</td>
<td>16.3 ± 26.7</td>
<td>16.2 ± 28.8</td>
<td>17.0 ± 25.2</td>
<td>21.6 ± 30.6</td>
</tr>
<tr>
<td>P-value</td>
<td>p(group) = 0.013</td>
<td>0.313</td>
<td>0.001</td>
<td>0.092</td>
<td>0.238</td>
<td>0.063</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>p(interactive*) = 0.029</td>
<td></td>
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<tr>
<td><strong>Electrogram Fractionation</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(FP/DP [%])</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNV Carrier</td>
<td>9.4 ± 2.9</td>
<td>5.7 ± 7.7</td>
<td>11.8 ± 12.4</td>
<td>9.6 ± 10.5</td>
<td>6.1 ± 7.3</td>
<td>8.7 ± 7.8</td>
<td>14.5 ± 12.8</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>6.0 ± 1.2</td>
<td>3.8 ± 3.8</td>
<td>6.3 ± 5.0</td>
<td>6.7 ± 7.5</td>
<td>5.5 ± 5.2</td>
<td>6.9 ± 10.7</td>
<td>7.1 ± 7.6</td>
</tr>
<tr>
<td>P-value</td>
<td>p(group) = 0.008</td>
<td>0.119</td>
<td>0.007</td>
<td>0.150</td>
<td>0.663</td>
<td>0.413</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>p(interactive*) = 0.035</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD. *interactive p value: group x region. †Post hoc test not performed as interactive p value = NS.

FP/DP – fractionated potentials/double potentials; SNV – single nucleotide variant
Table 3. Effects of genotype on sinus node function and atrial refractory periods.

<table>
<thead>
<tr>
<th></th>
<th>Non-carrier</th>
<th>Carrier</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSNRT 600ms (ms)</td>
<td>304 ± 244</td>
<td>344 ± 151</td>
<td>0.585</td>
</tr>
<tr>
<td>CSNRT 500ms (ms)</td>
<td>411 ± 257</td>
<td>570 ± 371</td>
<td>0.287</td>
</tr>
<tr>
<td>CSNRT 400ms (ms)</td>
<td>416 ± 163</td>
<td>491 ± 384</td>
<td>0.540</td>
</tr>
<tr>
<td>CSd ERP 600ms (ms)</td>
<td>266 ± 62</td>
<td>240 ± 29</td>
<td>0.113</td>
</tr>
<tr>
<td>CSd ERP 400ms (ms)</td>
<td>232 ± 40</td>
<td>220 ± 21</td>
<td>0.361</td>
</tr>
<tr>
<td>CSp ERP 600ms (ms)</td>
<td>234 ± 40</td>
<td>239 ± 29</td>
<td>0.684</td>
</tr>
<tr>
<td>CSp ERP 400ms (ms)</td>
<td>231 ± 30</td>
<td>230 ± 22</td>
<td>0.415</td>
</tr>
</tbody>
</table>

Abbreviations: CSNRT – Corrected Sinus Node Recovery Time; CSd – Distal Coronary Sinus; CSp – Proximal Coronary Sinus, ERP – Effective Refractory Period
Table 4. Univariable and multivariable analysis of conduction heterogeneity and complex signals.

<table>
<thead>
<tr>
<th>Conduction Heterogeneity</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>F Statistic</td>
</tr>
<tr>
<td>SNV Carrier</td>
<td>0.001</td>
<td>13.323</td>
</tr>
<tr>
<td>Age</td>
<td>0.287</td>
<td></td>
</tr>
<tr>
<td>Female Gender</td>
<td>0.721</td>
<td></td>
</tr>
<tr>
<td>Persistent AF</td>
<td>0.006</td>
<td>0.550</td>
</tr>
<tr>
<td>AF Duration (months)</td>
<td>0.017</td>
<td>5.748</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>0.706</td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI&gt;30)</td>
<td>0.382</td>
<td></td>
</tr>
<tr>
<td>Presenting Lab Rhythm AF</td>
<td>0.265</td>
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<tr>
<td>Hypertension</td>
<td>0.844</td>
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<tr>
<td>Dyslipidemia</td>
<td>0.634</td>
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<td>Diabetes Mellitus</td>
<td>0.767</td>
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<td>Ischaemic Heart Disease</td>
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<td>Stroke/TIA</td>
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<td>CHADSVASC</td>
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<td>OSA</td>
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<td>Regular Alcohol Intake</td>
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<td>Familial AF</td>
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<tr>
<td>LV Ejection Fraction</td>
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<tr>
<td>LA Size</td>
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<td>LA Area</td>
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<tr>
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<tr>
<td><strong>LA Volume Index</strong></td>
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<td><strong>Complex Signals</strong></td>
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Table 5. Procedural characteristics

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<td>Fluoroscopy time (mins)</td>
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<td>Radiation dose (mGy x cm$^2$)</td>
<td>15 251 ± 8 942</td>
<td>15 638 ± 9 102</td>
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<tr>
<td>Total RF time (mins)</td>
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<td>39 ± 10</td>
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<td>Peri-procedural DCR</td>
<td>20 (33)</td>
<td>15 (37)</td>
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<tr>
<td>PV isolation</td>
<td>61 (100)</td>
<td>41 (100)</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Figure 1. Patient Selection.

Screening: Consecutive Patients planned for AF ablation (n = 185)

SNV Genotyping (n = 102)

Electroanatomic Mapping (n = 85)

Blinded Offline Analysis (Matlab)

Clinic Follow up & Arrhythmia Detection (n=98)
- Insertable Cardiac Monitor (n=46)
- Dual Chamber Pacemaker (n=4)
- 24hr Holter: 3, 6 & 12 months then 6-monthly (n=48)

Non-carrier (CC) (n = 61)
SNV Carrier (CT/TT) (n = 41)

Redo Procedures – 44
Cardiomyopathy – 18
Valvular Disease – 7
Amiodarone – 12
Refused Consent – 2

Unable to maintain sinus rhythm – 15
Procedural Factors – 2

Lost to follow up – 4*

* 2 non-carriers, 2 SNV carriers lost to follow-up

Figure 1. CONSORT diagram of study protocol and follow-up of study cohorts.
Figure 2. Electroanatomical segmentation. A – Left Atrial Regional Segmentation. B – Mean conduction velocity heterogeneity across the left atrium.
**Figure 3.** Conduction differences between carriers and non-carriers.

**Figure 3.** Representative isochronal activation maps from a posterior view of the left atrium in similarly aged patients with persistent AF in the non-carrier (left) and SNV carrier (right) groups. The red region shows early timing from distal coronary sinus pacing site while the region in blue is late activation. The SNV carrier has a region of isochronal crowding indicative of focal conduction slowing (black arrows) and a greater number of complex electrograms (pink dots) in the posterior and lateral walls. Representative complex electrograms accompanied by signal voltage and duration (white lines). AF – Atrial fibrillation; SNV – single nucleotide variant.
Figure 4. Mean percentage complex fractionated signals in both carrier and non-carrier groups are shown color-coded per segment according to percentage. Carriers have a greater burden of complex signals in the lateral and posterior walls seen in purple.

Figure 5. Post-ablation outcomes.
Figure 5. Kaplan-Meier curve comparing single procedure freedom from arrhythmia (AT/AF) (A) and freedom from AF (B) between single-nucleotide variant (SNV) carriers and non-carriers. AT – Atrial tachycardia; AF – Atrial fibrillation.

Figure 6. Central Illustration.
**Figure 6.** Summary figure of key findings in study: Carriers of the single nucleotide variant rs200733 on chromosome 4q25 is associated with greater electrical remodelling and reduced long-term arrhythmia-free survival which lead to maintenance of AF.
Chapter 3:
Cycle-Length Dependence of Atrial Substrate During High-Density Mapping of Paroxysmal and Persistent Atrial Fibrillation

INTRODUCTION
Pulmonary vein isolation (PVI) is the cornerstone of AF ablation. However, achieving durable long-term arrhythmia free outcomes in patients with persistent AF remains a significant challenge. Thus, numerous ablation strategies beyond PVI have been investigated. Recently, the STAR-AF II randomised controlled trial investigating adjunctive ablation lesion sets targeting complex fractionated abnormal electrograms (CFAE) and linear ablation in patients with persistent AF failed to demonstrate incremental benefits beyond PVI alone (331). Verma et al. has also previously shown that pre-existent left atrial scarring manifest as low voltage zones (LVZ) is an independent marker of procedural failure in patients undergoing AF ablation (117). Various groups have since reported successfully utilising substrate modification strategies to encircle or homogenise LVZs, thought to represent diseased fibrotic atria and play a role in arrhythmia maintenance (401).

The era of ultra high-density mapping technologies has facilitated the identification of substrate at markedly higher resolutions than traditional point-by-point mapping strategies (345). This may facilitate deeper insights into the complexity of AF-related substrate and the arrhythmia mechanism. Recent studies have observed that progressive tight-coupled single premature extra-stimuli can unmask electrophysiologic features that are not otherwise readily apparent at baseline intervals. However, the impact of
cycle-length dependence on high-density substrate maps is yet to be elucidated (341,350).

In this study, we utilise high-density electroanatomic mapping to comprehensively characterise incremental cycle-lengths on the left atrium in patients with AF. We hypothesised that cycle-length variation and rapid pacing would significantly influence the appearance and electro-anatomic characteristics of substrate maps.

METHODS

Study Population

56 consecutive patients undergoing index AF ablation were prospectively enrolled. Patients were included if they had drug refractory symptomatic paroxysmal (lasting <7 days) or persistent AF (lasting >7 days) (402). Patients were excluded if they (1) were unable to be electrically cardioverted to sinus rhythm (2) had previous AF ablation; (3) amiodarone use; or (4) age < 18 years. This study was approved by the Royal Melbourne Hospital Research and Ethics Committee.

Procedural Study Protocol

All antiarrhythmic medications including beta-blockers and calcium-channel blockers were withheld at least 5 half-lives prior to procedure. Anticoagulation was managed according to operator preference. All procedures were performed under general anesthesia with peri-procedural transesophageal echocardiography to exclude LA thrombus. Double trans-septal access was performed after heparinization (target ACT 300-350).

Bipolar intracardiac electrograms and 12-lead surface electrocardiography (ECG) were recorded simultaneously on a digital amplifier system (EPMed Systems, NJ).
Intracardiac electrograms were filtered from 30 to 500 Hz and measured with digital calipers at 200 mm/s sweep speed.

**Electroanatomical mapping**

Left atrial geometry was constructed with a 20 pole Lasso catheter (Biosense Webster, CA, USA, 2-6-2 mm electrode spacing) and merged with a periprocedural CT using the CARTO3 electroanatomical mapping system. Patients who presented to the lab in AF were electrically cardioverted to sinus rhythm. Voltage and activation maps were constructed using the Lasso catheter during constant pacing from the distal coronary sinus (CSD) to standardize the direction of wavefront propagation at 2 distinct cycle lengths (1) 600ms and (2) 300ms. To ensure haemodynamic stability, 2:1 atrial to ventricular conduction was required whilst pacing at the faster 300ms cycle length. If 1:1 conduction was present, the cycle length was decreased by 10ms below 300ms until 2:1 conduction occurred. Complete coverage of the entire LA geometry was performed and correlated to the CT to ensure smooth coverage across all regions with a minimum of 1000 points using the Confidense™ algorithm to ensure even point distribution (Biosense Webster). Strict criteria were employed to account for the lack of tissue contact data on the multi-polar mapping catheter. Point collection was performed only by experienced operators after careful assessment on tactile catheter pressure, fluoroscopic motion and application of an internal point filter to within 5mm of the chamber surface geometry (345). Mapping fill threshold was set at 5mm. New maps and geometry were created for each pacing cycle length. Although data was collected using the Confidense algorithm, all acquired points were also meticulously manually reviewed and annotated. Only points demonstrating characteristics of near-field signals were included. In keeping with prior publications (392), these signals demonstrated at
least 2 sharp peaks and were consistent with anatomically adjacent signals in terms of signal quality and electrogram timing. Points that did not fit these criteria were excluded.

Electrogram analysis

Electrogram analysis was meticulously performed manually offline. For activation time, each point was analysed at 200 mm/s sweep speed and appropriately annotated at the maximum negative dV/dt for unipolar signals or the peak sharp of the bipolar electrogram. Ectopic beats and artefact were excluded. Following extensive manual annotation and exclusion of signals not meeting the above criteria, signal processing was performed offline (MATLAB 9.1, Mathworks, MA, USA).

Global and Regional Atrial Voltage Analysis

Bipolar voltage was defined as the peak-peak electrogram voltage. Segmental voltage analysis of the left atrium was performed by dividing the chamber into the following 6 segments: anterior, posterior, septal, lateral, roof and inferior (Figure 1). The mitral annulus and pulmonary veins beyond the antrum were excluded. The mean, absolute and percentage changes in bipolar voltage were compared for each patient between each of the groups. Global percentage change was classified into three patterns: 1) no or minimal decrease in bipolar voltage (<10%); 2) modest decrease in bipolar voltage (10-25%); or 3) significant decrease in bipolar voltage (>25%). Regional analysis was also performed of the antral region which was defined as the region within a circumferential ring 2cm proximal to the PV ostia, approximating a typical wide circumferential ablation line. Analysis was performed in each of 5 discrete segments around the circumference for both LPVs and RPVs (figure 1). Given varying degrees
of voltage scales in substrate modification studies targeting low voltage regions (LVZ, [<0.5mV]) and transition zones up to 1.3mV, regional assessment of dynamic substrate was also performed in a subset of patients by segmentation of relative lower voltage zones (rLVZ) including electroanatomic surface area measurements on a voltage scale of 0.5-1.3mV (332,338). The heterogeneity of bipolar voltage was determined by calculating the CoV of the different regions in the left atrium.

Conduction Velocity Analysis

Conduction velocity was analysed in MATLAB as previously described using the polynomial algorithm (306,393). In brief, this method assigns a fitting ‘window’ per region with a minimum of 20 points required. Each region is assigned subsets of Cartesian coordinates in space and activation time. These are fitted to a smooth polynomial surface in three-dimensional space, using a standard least squares algorithm, which provides robustness against outliers. The gradient is then calculated from the fit and used to calculate velocity components, with final velocities at each point calculated from the weighted average of velocity components from every fit including that point (394). The mean left atrial conduction velocity was calculated with absolute and percentage changes compared between cycle lengths. Conduction heterogeneity was determined by calculating the CoV of conduction velocity globally and across the different segments in the left atrium.

Analysis of Complex Signals

Complex signals were defined as electrograms with ≥3 deflections >50ms duration (fractionated potentials [FP]) or 2 separate deflections separate by an isoelectric interval (double potentials [DP]). These signals were manually annotated and tagged.
The percentage of complex signals is expressed as a proportion of the number of complex signals divided by the total number of points. Further analysis of complex signals was performed in a subset of patients with significant voltage change by sampling ten representative FP’s or DP’s within or adjacent to rLVZ’s (<1.3mV). Evaluation included manual annotation of complex electrogram duration, number of separate deflections and bipolar voltage.

**Analysis of regions of voltage change and slowed conduction**

Propagation maps at 10ms spacing and isochronal maps at 10ms intervals were reviewed for areas of slowed conduction with isochronal crowding and conduction block. The anatomic locations of these sites of slowed conduction and block, the distribution pattern and changes in response to pacing at the faster cycle length were determined including specific zones such as the antral regions as defined in figure 1. In patients with significant rate-dependant change, further sub-segmental analysis was performed in regions of slowed conduction.

**Statistical Analysis**

Data analysis was performed using SPSS software (Version 23, IBM, Armonk, New York). Normality of all quantitative variables was assessed using the Shapiro-Wilk test and normalising transformations were performed as necessary. Data are expressed as mean ± standard deviation (SD) unless otherwise stated. Two-group comparisons were made using Student’s t test for continuous variables, or the chi-squared test for categorical variables. Three-group comparisons were made using the one-way ANOVA. Correlation was determined by Pearson’s correlation coefficient.
Percent change in bipolar atrial voltage was stratified by AF phenotype (paroxysmal versus persistent). Linear regression analysis was performed separately on each phenotype to determine univariable predictors of voltage change. Following univariable analysis, all predictors with p<0.2 were included in a multivariable model with backward stepwise exclusion to identify independent predictors of voltage change.

RESULTS

Baseline Characteristics
A total of fifty-six patients, underwent 3D electroanatomic mapping (Table 1). The mean age was 60±8 years, mean CHA2DS2-VASc Score was 1.3 ± 1.0 and a mean BMI of 29.8 ± 5.0 kg/m². 30 patients had persistent AF (54%) and the population was predominantly male (67%). The time from first AF diagnosis was 68 ± 60 months and 64% of patients presented for electrophysiology study in sinus rhythm.

High Density Electroanatomical mapping
The mean number of points collected prior to manual annotation and filtering was 2,439 ± 1452 acquired in a mean time of 15 ± 5 minutes per map (Table 2). There were significantly more points collected in the faster 300ms CSD pacing group (2866 ± 589 vs. 2257 ± 469 points, p<0.01).

Assessment of Voltage
Electroanatomic data collected whilst pacing from the distal coronary sinus at a cycle length of 300ms compared to 600ms demonstrated a significant decrease in overall global bipolar voltage (1.56 ± 0.47 vs 1.74 ± 0.48mV, p<0.001; Figure 2). This was seen uniformly in each of the prospectively defined anatomic segments (Figure 2).
There were significantly more low voltage (<0.5mV) points in the 300ms group (26.0±13% vs. 22.5±12%, p=0.009). Three patterns of rate-dependant global bipolar voltage changes were noted during short cycle length pacing; 1) no or minimal decrease in bipolar voltage [<10%] in 34 patients (58%); 2) mild-moderate decrease in bipolar voltage [10-25%] in 15 patients (26%) and 3) significant decrease in bipolar voltage [>25%] in 9 patients (16%). Rate-dependant dynamic substrate with larger local regions of low voltage (<0.5mV) between 300ms and 600ms were most commonly seen in the antral region in 27 patients (48%), followed by the mid posterior wall (20 patients [36%]) and anterior wall (13 patients [23%]; Table 3). The posterior left inferior PVA was the most common antral sub-segment demonstrating rate-dependent voltage change (13 patients [23%]).

Impact of Rate on Conduction

There was evidence of significant global mean conduction slowing in the 300ms pacing group compared with 600ms pacing (30.4 ± 13.2 vs. 38.6 ± 14.1 cm/sec, p<0.001). This was also seen across all LA segments with the exception of the lateral wall (Figure 2). There was a significant positive rate-dependent correlation between absolute change in mean global conduction velocity and bipolar voltage (R = 0.536, p<0.001; Figure 3A). Areas of dynamic conduction slowing were most commonly seen in the posterior wall (20 patients [36%]; Table 3) followed by the posterior left superior PV antrum (18 patients [30%]) and inferior wall (15 patients [27%]).

Rate-Dependant Conduction Slowing in Regional Low Voltage Zones

In line with the regional conduction slowing findings, localised rLVZ’s in patients who experienced greater than 10% change in bipolar voltage (n=58 segments) were most
commonly found in the antral region (n=29 [50%]) followed by the anterior and posterior regions. At 300ms compared with 600ms pacing, these rLVZ’s exhibited significantly lower mean bipolar voltage (0.75 ± 0.19 vs. 1.26 ± 0.55mV, p<0.001), greater proportion of low voltage <0.5mV points (40 ± 14% vs. 27 ± 18, p<0.001), slower conduction velocity (24.5 ± 14 vs. 38.7 ± 14cm/sec, p<0.001) and greater proportion of complex signals (20 ± 10 vs. 7 ± 6%, p<0.001; Table 4).

Assessment of Complex Signals
For the LA overall, there was a significantly higher proportion of complex signals at 300ms pacing compared with 600ms pacing from CSD (8.9 ± 4.4 vs. 5.3 ± 3.2%, p<0.005). This was also demonstrated in all LA segments (Figure 2). There was a significant correlation between the change in proportion of complex signals at 300ms pacing and bipolar voltage change (R = 0.401, p<0.001; Figure 3B) and conduction velocity change (R = 0.432, p<0.001; Figure 3C).

Univariable and Multivariable Predictors of Rate-Dependant Voltage change
Predictors of voltage change when pacing at 300ms versus 600ms are presented in Table 5 according to AF phenotype. In paroxysmal AF, longer duration of AF predicted a larger change in voltage following both univariable and multivariable analysis. In contrast, shorter AF duration and LVEF were univariable and multivariable predictors of voltage change in persistent AF. In persistent AF, the change in atrial voltage was inversely related to AF duration, with longer AF duration predicting more modest voltage differences.
DISCUSSION

This study presents high density electroanatomic mapping data of the AF substrate at variable paced cycle lengths in patients paroxysmal and persistent AF. The main findings of this study are:

1) Pacing at 300ms versus 600ms expands the area of potential atrial substrate characterised by lower LA voltage (global and regional), larger areas of low voltage zones, slower atrial conduction and higher prevalence of complex signals. The reduction of atrial voltage correlates with slowing of conduction.

2) Rate-dependant regional conduction slowing and low voltage zones occurred across all left atrial segments and most frequently within the PV antrum.

3) Changes were most marked in longer duration paroxysmal AF and early persistent AF indicating a critical period in evolution of atrial substrate from dynamic to fixed.

Rate-dependent changes in the atrium

Recent work by Williams et al. utilising a Pentaray catheter positioned at fixed atrial sites and extra-stimulus pacing at incremental short-coupled intervals observed that both conduction velocity and voltage decreased with increasingly closely coupled pacing in a small paroxysmal AF cohort (341,350). In the current study we utilised stable pacing protocols to optimize catheter stability within the beating heart to create repeated high density maps of the atrium to define the nature of cycle-length and rate-dependent substrate. Our study was performed in a large population of both paroxysmal and persistent AF patients allowing investigation of predictors of substrate change. While a degree of atrial substrate is likely fixed and defined by non-conducting tissue, other components may display functional dependency. Thus, under faster paced cycle
lengths, functional block may result in recruitment of less myocardium and enhancement of low voltage zones. We posit that regions of ‘latent substrate’, vulnerable electrically and structurally remodelled atrial tissue, may be revealed only under conditions of electrophysiologic stress induced by rapid conduction. The significance of this ‘latent substrate’ is uncertain and may represent an interim stage of rate-dependent remodelling in the progression from paroxysmal into persistent AF.

**Mechanisms of rate-dependent conduction**

Animal and human studies across multiple clinical contexts have observed rate-dependency, CV restitution and increased atrial fibrosis in association with progressive atrial remodelling. Extracellular matrix protein deposition by cardiac fibroblasts leads to atrial fibrosis and decreased local tissue excitability potentially forming conduction barriers (403). Altered gap-junctional coupling and reduced connexin expression may affect both conduction and voltage (350). Restitution alternans of action potential duration (APD) has been shown to be a key determinant of electrical and rotor stability; a potential driver of persistent AF. Short diastolic intervals from faster rates may lead to large fluctuations in APD alternans and functional gradients of repolarization which may promote destabilization of re-entrant spiral waves (404). Simulations in cardiac tissue have demonstrated a transition from concordant alternans to discordant alternans in response to rapid ventricular pacing. Discordant alternans markedly increases dispersion of refractoriness and vulnerability to re-entry important in the fibrillatory mechanism (405). Koller et al. demonstrated that APD alternans occurred earlier and over a wider range in patients with structural heart disease in response to a dynamic pacing protocol (406). In an animal myocyte model, vulnerability to re-entry was more pronounced due to rapid pacing in a zig-zag pattern in regions of asymmetry suggesting
the importance of structural heterogeneity in arrhythmia induction (407). Re-entry around a fixed line of conduction block with features of both microanatomical and functional block was demonstrated at steeper CV restitution slopes that only occurred at high pacing rates.

Acceleration-dependent slowing of atrial conduction has been shown to precede AF initiation. Lalani et al. postulated that steep CV restitution may be related to electrical remodelling whilst slower and broader restitution may be dependent on structural remodelling seen more frequently in persistent AF (408). Our data supports the notion of rate-dependency in fibrotic tissue exhibiting abnormally slow transverse conduction and high-degree of anisotropy. Regions of rate dependent ‘latent substrate’ may represent ‘at risk’ areas of anatomical and functional block with interstitial fibrosis unmasked by faster pacing rates. In the present study, long-standing PAF and early PeAF independently predicted voltage change, suggesting that these AF phenotypes are characterised by a dynamic vulnerable substrate. Left unchecked, the substrate may progress toward a fixed pattern that associates with an increasingly advanced clinical AF phenotype.

Antral remodelling in AF

Since the landmark study by Haissaguerre (83), PVI has evolved from a predominantly ostial lesion set to a wide area circumferential approach (WACA) encompassing the pulmonary venous antral region (409). McLellan and colleagues demonstrated no significant difference in post-ablation AF recurrence in paroxysmal AF patients between a minimal wide circumferential minimal ablation strategy and more extensive segmental PVI ablation (410). The PV-LA junction within the PV antral region appears to play a critical role in the arrhythmogenesis in AF (91). The
electrophysiological characteristics of this region in response to acute stretch may provide substrate conducive to re-entry (291). Kapa et al. identified lower voltage cut-offs at the PV-LA junction and posterior wall compared to the remainder of the atria (0.2mV vs 0.45mV) (347). The present study extends these observations implicating the PV-LA junction, and specifically the posterior PV antrum as the most common site of progressive rate-dependent changes in conduction slowing and low voltage zones. These findings are in keeping with a potentially greater degree of fibrosis in the antral region which may represent an important arrhythmogenic target covered by a wide circumferential PVI approach.

Substrate modification in AF ablation

A novel approach described in several recent observational studies involves substrate modification of atrial scarring. Areas of low electroanatomic bipolar atrial voltage have been used as a surrogate for fibrosis with studies showing positive correlation to both cardiac MRI and histology (411,412). These low voltage zones are associated with increased risk of post ablation AF recurrence (117) and have emerged as potential targets beyond PVI to improve outcomes following AF ablation (333,413). Rate-dependency was demonstrated in relatively lower voltage zones (regions 0.5-1.3mV) with a decrease in voltage at faster 300ms pacing rate from 1.26 ± 0.55mV to 0.75 ± 0.19mV. Although this value is beyond the traditional 0.5mV cut-off used by electroanatomic studies to define LVZs, it may identify important substrate and underscores the limitations of static atrial maps. These regions of ‘latent substrate’ may only be exposed at rapid rates and altering the voltage mask in a personalised approach for each patient. Rapid atrial pacing may thus represent a feasible and practical standardised novel adjunctive manoeuvre to identify further regions of target substrate
in a voltage-guided strategy. This requires validation and further research in clinical trials.

**Limitations**

Differences in tissue contact can affect bipolar voltage amplitude thereby confounding results. Multipolar catheters do not possess contact force data and may collect internal far-field signals. However, meticulous attempts were made ensure adequate tissue contact including use of fluoroscopy, verification with contact force ablation catheter and maintaining consistency in geometry between maps in collected in the same patient. In addition, points were only accepted if they were within 5mm from the surface geometry to reduce as performed in previous studies to reduce intra-cavitary point collection (345). We did not utilise a control cohort for comparison of rate-dependent changes therefore it is unknown whether these differences are more likely to occur or be more exaggerated in patients with AF compared with reference patients without AF. Further research into differential pacing maps of patients with normal left atria would be of significant interest.

**CONCLUSION**

Patients with paroxysmal and persistent AF exhibit evidence of rate-dependent electrical and electroanatomic remodelling in the left atrium manifest by uniformly lower global and segmental voltage, slower conduction velocity and increased proportion of complex signals at a higher pacing rate. These changes occurred more commonly in the pulmonary venous antral region and may represent areas of ‘latent substrate’. These abnormalities may represent areas of ‘latent substrate’ and provide
further insight to unmasking important areas and potential mechanisms for AF maintenance beyond the pulmonary veins.
### TABLES

**Table 1. Baseline characteristics of study patients.**

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<thead>
<tr>
<th>Baseline Patient Characteristics (n = 56)</th>
<th></th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>Male</td>
<td>39 (67)</td>
</tr>
<tr>
<td>AF Type</td>
<td></td>
</tr>
<tr>
<td>- Paroxysmal</td>
<td>26 (46)</td>
</tr>
<tr>
<td>- Persistent</td>
<td>30 (54)</td>
</tr>
<tr>
<td>Duration of AF (months)</td>
<td>68 ± 60</td>
</tr>
<tr>
<td>Hypertension</td>
<td>22 (39)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
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</tr>
<tr>
<td>Diabetes</td>
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<tr>
<td>Ischaemic Heart Disease</td>
<td>5 (9)</td>
</tr>
<tr>
<td>TIA/Stroke</td>
<td>2 (4)</td>
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<td>CHA₂DS₂-VASc score</td>
<td>1.3 ± 1.0</td>
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</table>

**Echocardiographic Parameters**

| LV Ejection Fraction (%)               | 57 ± 5   |
| LA Size (cm)                           | 4.3 ± 0.5|
| LA Area (cm²)                          | 24.4 ± 5 |

**Abbreviations:** AF – Atrial Fibrillation, CHA₂DS₂-VASc – Congestive Heart Failure, Hypertension, Age, Diabetes, Stroke, Vascular Disease, Sex, LA – Left Atrial, LV – Left Ventricular, TIA – Transient Ischaemic Attack
Table 2. Mean Number of Electroanatomic Points Acquired per Patient following annotation.

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<th>Segment</th>
<th>Number of Points</th>
<th>p-value</th>
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<tr>
<td></td>
<td>600ms Pacing</td>
<td>300ms Pacing</td>
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<tr>
<td>Global</td>
<td>2257 ± 469</td>
<td>2866 ± 589</td>
</tr>
<tr>
<td>Posterior</td>
<td>487 ± 134</td>
<td>676 ± 186</td>
</tr>
<tr>
<td>Anterior</td>
<td>425 ± 110</td>
<td>580 ± 123</td>
</tr>
<tr>
<td>Roof</td>
<td>372 ± 96</td>
<td>450 ± 179</td>
</tr>
<tr>
<td>Inferior</td>
<td>350 ± 77</td>
<td>367 ± 107</td>
</tr>
<tr>
<td>Lateral</td>
<td>222 ± 68</td>
<td>245 ± 79</td>
</tr>
<tr>
<td>Septal</td>
<td>226 ± 70</td>
<td>279 ± 121</td>
</tr>
</tbody>
</table>
Table 3. Regional areas of rate dependent change in voltage and conduction slowing between 600ms and 300ms distal coronary sinus pacing.

<table>
<thead>
<tr>
<th>Location of Dynamic Substrate</th>
<th>Lower Voltage Zones (No. of patients [%])</th>
<th>Conduction Slowing (No. of patients [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PV Antral Sub-Segments</strong></td>
<td></td>
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</tr>
<tr>
<td>Posterior Left Superior PVA</td>
<td>9 (15)</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Posterior Left Inferior PVA</td>
<td>13 (23)</td>
<td>12 (21)</td>
</tr>
<tr>
<td>Anterior Left Superior PVA</td>
<td>7 (13)</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Anterior Left Inferior PVA</td>
<td>6 (11)</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Posterior Right Superior PVA</td>
<td>8 (14)</td>
<td>10 (18)</td>
</tr>
<tr>
<td>Posterior Right Inferior PVA</td>
<td>6 (11)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Anterior Right Superior PVA</td>
<td>7 (13)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Anterior Right Inferior PVA</td>
<td>6 (11)</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Roof Left PVA</td>
<td>10 (18)</td>
<td>10 (18)</td>
</tr>
<tr>
<td>Roof Right PVA</td>
<td>7 (13)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Any Antral Segment</td>
<td>25 (45)</td>
<td>32 (57)</td>
</tr>
<tr>
<td>Posterior</td>
<td>21 (38)</td>
<td>20 (36)</td>
</tr>
<tr>
<td>Anterior</td>
<td>16 (29)</td>
<td>14 (25)</td>
</tr>
<tr>
<td>Roof</td>
<td>12 (21)</td>
<td>13 (23)</td>
</tr>
<tr>
<td>Inferior</td>
<td>12 (21)</td>
<td>15 (27)</td>
</tr>
<tr>
<td>Septal</td>
<td>7 (13)</td>
<td>11 (20)</td>
</tr>
<tr>
<td>Lateral</td>
<td>6 (11)</td>
<td>6 (11)</td>
</tr>
</tbody>
</table>

*Abbreviations: PVA – Pulmonary Vein Antrum*
Table 4. Regional changes in relative low voltage zones in patients with significant rate-dependent changes in global bipolar voltage.

<table>
<thead>
<tr>
<th>rLVZ Characteristics (n=58 regions)</th>
<th>600ms Pacing</th>
<th>300ms Pacing</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Points</td>
<td>163 ± 39</td>
<td>252 ± 62</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean Bipolar Voltage (mV)</td>
<td>1.26 ± 0.55</td>
<td>0.75 ± 0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Proportion of Points &lt;0.5mV (%)</td>
<td>27.1 ± 18.4</td>
<td>40.4 ± 14.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Conduction Velocity (cm/sec)</td>
<td>38.7 ± 13.4</td>
<td>24.5 ± 14.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Proportion of Complex Signals (%)</td>
<td>7.1 ± 5.7</td>
<td>20.4 ± 10.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: rLVZ – relative Low Voltage Zones
Table 5. Univariate and multivariable analysis of predictors of percent bipolar voltage change between 600ms and 300ms pacing by AF phenotype.

<table>
<thead>
<tr>
<th>Co-variates</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Paroxysmal AF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.002 (-0.006-0.009)</td>
<td>0.620</td>
</tr>
<tr>
<td>Female</td>
<td>0.090 (-0.211-0.031)</td>
<td>0.140</td>
</tr>
<tr>
<td>AF Duration (months)</td>
<td>-0.003 (-0.004--0.001)</td>
<td>0.009</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.005 (-0.008-0.018)</td>
<td>0.452</td>
</tr>
<tr>
<td>OSA</td>
<td>0.093 (-0.027-0.214)</td>
<td>0.124</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.091 (-0.230-0.048)</td>
<td>0.190</td>
</tr>
<tr>
<td>CHA₂DS₂-VASc</td>
<td>-0.043 (-0.115-0.290)</td>
<td>0.230</td>
</tr>
<tr>
<td>LA size (mm)</td>
<td>0.005 (-0.002-0.011)</td>
<td>0.173</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>-0.003 (-0.150-0.008)</td>
<td>0.543</td>
</tr>
<tr>
<td>Persistent AF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.001 (-0.008-0.010)</td>
<td>0.778</td>
</tr>
<tr>
<td>Female</td>
<td>0.0003 (-0.145-0.144)</td>
<td>0.996</td>
</tr>
<tr>
<td>AF Duration (months)</td>
<td>0.001 (0.0002-0.002)</td>
<td>0.017</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.004 (-0.010-0.018)</td>
<td>0.522</td>
</tr>
<tr>
<td>OSA</td>
<td>-0.068 (-0.184-0.047)</td>
<td>0.236</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.011 (-0.130-0.107)</td>
<td>0.848</td>
</tr>
<tr>
<td>CHA₂DS₂-VASc</td>
<td>-0.014 (-0.068-0.039)</td>
<td>0.582</td>
</tr>
<tr>
<td>LA size (mm)</td>
<td>0.003 (-0.005-0.010)</td>
<td>0.437</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>-0.013 (-0.028-0.001)</td>
<td>0.063</td>
</tr>
</tbody>
</table>
FIGURES

Figure 1. Segmental comparison between pacing at 600ms and 300ms.

Figure 1. Anteroposterior (AP) and posteroanterior (PA) views of the LA divided into six segments: anterior, posterior, inferior, lateral, septal, roof and inferior walls for regional analysis. The pulmonary venous antral region, defined as the circumferential region 2cm from the pulmonary vein-LA junction was divided into 5 segments on each side for sub-segmental analysis of voltage and conduction change in response to different pacing strategies. 1 – anterior left superior PVA; 2 – anterior left inferior PVA; 3 – posterior left inferior PVA; 4 – posterior left superior PVA; 5 – left roof PVA; 6 – anterior right superior PVA; 7 – anterior right inferior PVA; 8 – posterior right inferior PVA; 9 – posterior right superior PVA; 10 – right roof PVA.
Figure 2. Left atrial electrophysiological parameters.

**Rate Dependant Change**

**Left Atrial Voltage**

* p<0.0001

**Left Atrial Conduction Velocity**

* p<0.0001

**Left Atrial Complex Signals**

* p<0.005

Figure 2. Segmental comparison between rate and direction dependant change.
Figure 3. Correlations of change in electrophysiological parameters.

A. Voltage Vs Conduction Velocity

B. Voltage Vs Complex Points
Figure 3. Correlation between absolute change in bipolar voltage (A-B), conduction velocity (B-C) and complex points (B-C) between 600ms and 300ms pacing from the distal coronary sinus.
Chapter 4:

Dynamic Direction-Dependent Atrial Substrate

During High Density Mapping of Paroxysmal and Persistent AF: Implications for Substrate Ablation

INTRODUCTION

It is well-recognised that pulmonary vein isolation is alone insufficient to eradicate AF for a large percentage of patients with persistent AF and for some patients with paroxysmal forms of the arrhythmia. An emerging strategy which involves identification of abnormal atrial substrate during sinus rhythm has been proposed and preliminary observational evidence supports this approach (333,401,413). However, the sensitivity of sinus rhythm mapping for identification of abnormal substrate is uncertain.

Low voltage zones, regions of fractionation and slowed conduction have classically been considered markers of diseased tissue that enable arrhythmia perpetuation, and that may sustain arrhythmia even after successful trigger elimination (414). These regions have been associated with interstitial fibrosis, gap junction remodelling and tissue anisotropy (131). Although this remodelling is by nature structural, whether sinus rhythm mapping is sufficiently sensitive to detect early but potentially mechanistically important structural change is unknown. Many early studies have also used relatively low density mapping techniques with a large ablation bipole insensitive for detection of localized abnormalities.

We hypothesised that direction-dependent pacing would produce significant changes in the substrate map beyond changes in cycle-length with important implications for
substrate ablation approaches. We also investigated effects on electrophysiologic parameters and regionality of substrate change.

METHODS

Study Population

17 consecutive patients undergoing index AF ablation were prospectively enrolled. Patients were included if they had drug refractory symptomatic paroxysmal (lasting <7 days) or persistent AF (lasting >7 days) (402). Patients were excluded if they (1) were unable to be electrically cardioverted to sinus rhythm (2) had previous AF ablation; (3) amiodarone use; or (4) age < 18 years. This study was approved by the Royal Melbourne Hospital Research and Ethics Committee.

Procedural Study Protocol

All antiarrhythmic medications including beta-blockers and calcium-channel blockers were withheld at least 5 half-lives prior to procedure. Anticoagulation was managed according to operator preference. All procedures were performed under general anesthesia with peri-procedural transesophageal echocardiography to exclude LA thrombus. Double trans-septal access was performed after heparinization (target ACT 300-350).

Bipolar intracardiac electrograms and 12-lead surface electrocardiography (ECG) were recorded simultaneously on a digital amplifier system (EPMed Systems, NJ). Intracardiac electrograms were filtered from 30 to 500 Hz and measured with digital calipers at 200 mm/s sweep speed.
**Electroanatomical mapping**

Left atrial geometry was constructed with a 20 pole Lasso catheter (Biosense Webster, CA, USA, 2-5-2 mm electrode spacing) and merged with a periprocedural CT using the CARTO3 electroanatomical mapping system. Patients who presented to the lab in AF were electrically cardioverted to sinus rhythm. Voltage and activation maps were constructed using the Lasso catheter during constant pacing from the distal coronary sinus (CSD) to standardize the direction of wavefront propagation at 2 distinct cycle lengths (1) 600ms and (2) 300ms. Additional maps were also created to assess site-dependent directionality while pacing from the ablation catheter positioned at (3) the left superior pulmonary vein (LSPV) at a fixed cycle length of 300ms. To ensure haemodynamic stability, 2:1 atrial to ventricular conduction was required whilst pacing at the faster 300ms cycle length. If 1:1 conduction was present, the cycle length was decreased by 10ms below 300ms until 2:1 conduction occurred. Complete coverage of the entire LA geometry was performed and correlated to the CT to ensure smooth coverage across all regions with a minimum of 1000 points using the Confidense™ algorithm to ensure even point distribution (Biosense Webster). Strict criteria were employed to account for the lack of tissue contact data on the multi-polar mapping catheter. Point collection was performed only by experienced operators after careful assessment on tactile catheter pressure, fluoroscopic motion and application of an internal point filter to within 5mm of the chamber surface geometry (345). Mapping fill threshold was set at 5mm. New maps and geometry were created for each pacing cycle length. Although data was collected using the Confidense algorithm, all acquired points were also meticulously manually reviewed and annotated. Only points demonstrating characteristics of near-field signals were included. In keeping with prior publications (392), these signals demonstrated at least 2 sharp peaks and were
consistent with anatomically adjacent signals in terms of signal quality and electrogram timing. Points that did not fit these criteria were excluded.

Electrogram analysis

Electrogram analysis was meticulously performed manually offline. For activation time, each point was analysed at 200 mm/s sweep speed and appropriately annotated at the maximum negative dV/dt for unipolar signals or the peak sharp of the bipolar electrogram. Ectopic beats and artefact were excluded. Following extensive manual annotation and exclusion of signals not meeting the above criteria, signal processing was performed offline (MATLAB 9.1, Mathworks, MA, USA).

Global and Regional Atrial Voltage Analysis

Bipolar voltage was defined as the peak-peak electrogram voltage. Segmental voltage analysis of the left atrium was performed by dividing the chamber into the following 6 segments: anterior, posterior, septal, lateral, roof and inferior (Figure 1). The mitral annulus and pulmonary veins beyond the antrum were excluded. The mean, absolute and percentage changes in bipolar voltage were compared for each patient between each of the groups. Given varying degrees of voltage scales in substrate modification studies targeting low voltage regions (LVZ, [<0.5mV]) and transition zones up to 1.3mV, regional assessment of dynamic substrate was also performed by segmentation of relative lower voltage zones (rLVZ) including electroanatomic surface area measurements on a voltage scale of 0.5-1.3mV (332,338). The heterogeneity of bipolar voltage was determined by calculating the CoV of the different regions in the left atrium.
Conduction Velocity Analysis

Conduction velocity was analyzed in MATLAB as previously described using the polynomial algorithm (306,393). The mean left atrial conduction velocity was calculated with absolute and percentage changes compared between cycle lengths. Atrial conduction slowing was defined as a local conduction velocity of 10 to 20cm/s and conduction block as <10 cm/s (415). Conduction heterogeneity was determined by calculating the CoV of conduction velocity globally and across the different segments in the left atrium.

Analysis of Complex Signals

Complex signals were defined as electrograms with ≥3 deflections >50ms duration (fractionated potentials [FP]) or 2 separate deflections separate by an isoelectric interval (double potentials [DP]). These signals were manually annotated and tagged. The percentage of complex signals is expressed as a proportion of the number of complex signals divided by the total number of points. Further analysis of complex signals was performed by sampling ten representative FP’s or DP’s within or adjacent to rLVZ’s (<1.3mV). Evaluation included manual annotation of complex electrogram duration, number of separate deflections and bipolar voltage. In addition, semi-automated CARTO fractionation map indices were collected for shortest complex interval (SCI), average complex interval (ACI) and interval confidence level (ICL). Surface area measurements were performed at arbitrarily defined consistent scales of SCI < 65ms, ACI < 70ms and ICL > 5 deflections for adequate identification of margins on electroanatomic maps.
Analysis of regions of voltage change and slowed conduction

Propagation maps at 10ms spacing and isochronal maps at 10ms intervals were reviewed for areas of slowed conduction with isochronal crowding and a vertical line of conduction block. The anatomic locations of these sites of slowed conduction and block, the distribution pattern and changes in response to pacing direction were determined.

Statistical Analysis

Data analysis was performed using SPSS software (Version 23, IBM, Armonk, New York). Normality of all quantitative variables was assessed using the Shapiro-Wilk test and normalising transformations were performed as necessary. Data are expressed as mean ± standard deviation (SD) unless otherwise stated. Two-group comparisons were made using Student’s t test for continuous variables, or the chi-squared test for categorical variables. Three-group comparisons were made using the one-way ANOVA. Correlation was determined by Pearson’s correlation coefficient.

RESULTS

Baseline Characteristics

Seventeen patients underwent 3D electroanatomic mapping with 3 complete maps created whilst pacing at 600ms and 300ms from the distal coronary sinus and pacing from the left superior pulmonary vein at 300ms. The mean age was 61±9 years, mean CHA2DS2-VASc Score was 1.2 ± 0.9 and a mean BMI of 29.7 ± 4.5 kg/m². Nine patients had PeAF (53%) and they were predominantly male (59%). Time from first AF diagnosis was 66 ± 57 months and 53% of patients presented in sinus rhythm.
High Density Electroanatomical mapping

The mean number of points collected prior to manual annotation and filtering was 2,079 ± 816 acquired in a mean time of 16 ± 5 minutes per map. There were significantly more points collected in the 300ms CSD pacing group compared to 300ms LSPV pacing and 600ms CSD pacing (2366 ± 589 vs. 1975 ± 469 vs 1867 ± points, p<0.01).

Direction-Dependant Voltage Differences: LSPV Pacing

Pacing from the left superior PV at 300ms resulted in a significant reduction in mean global voltage compared to distal CS pacing at 300ms (1.49 ± 0.44 vs 1.21 ± 0.42mV, p<0.01; Figure 2). Voltage heterogeneity was markedly greater in response to LSPV pacing (85 ± 20% vs 31 ± 10%, p<0.0001). Regionally, there were dramatic differences when pacing from the LSPV compared to distal CS at 300ms with significantly lower mean voltages in the posterior (1.04 ± 0.43 vs 1.47 ± 0.53mV, p=0.01), inferior (1.02 ± 0.34 vs 1.40 ± 0.52mV, p=0.002) and roof (1.33 ± 0.55 vs 1.79 ± 0.89mV, p=0.01) segments (figure 2). LSPV pacing resulted in significantly greater low voltage points (32 ± 12% vs 25 ± 9%, p<0.001), particularly in the posterior wall (38 ± 15% vs 25 ± 10%, p=0.015). In contrast, the remaining anterior, septal and lateral walls remained relatively unchanged.

A representative example of the progressive differences in voltages between pacing strategies is shown in Figure 3.

Variance in Low Voltage Areas with Rate and Direction: Effect on Target Area Size of Potential Substrate Ablation

On electroanatomic bipolar voltage maps with a scale of 0.5-1.3mV, low voltage zones (LVZ [<0.5mV]) and relative lower voltage zones (rLVZ [<1.3mV]) typically targeted
in adjunctive ablation approaches were identified. There was a significant progressive increase in LVZ surface area between 600ms, 300ms and LSPV pacing (1.17 ± 1.82 vs. 2.52 ± 2.20 vs 4.28 ± 1.59 cm² respectively, p<0.001 [Figure 4]). Similarly, there was a significant increase in rLVZ surface area between groups (3.59 ± 2.88 vs. 7.39 ± 4.83 vs. 9.80 ± 3.21 cm², p<0.001). Of clinical significance, these changes represent a relative 115 ± 88% and 266 ± 36% respective increase in the LVZ region that would be the target of potential substrate modification.

**Regional Conduction Slowing or Conduction Block: LSPV Pacing**

Conduction velocity significantly decreased exclusively in the posterior wall when pacing from the LSPV compared with CSD pacing at 300ms (39.9 ± 16.6 vs 24.4 ± 13.0 cm/sec, p=0.008). This was associated with significantly greater conduction heterogeneity (82 ± 22% vs 39 ± 16%, p<0.02). Notably, when pacing the pulmonary veins, a vertical line of conduction slowing or conduction block was observed in 69% of patients compared with 15% with CSD pacing. This posterior line of block was associated with a corresponding low voltage zone running between the pulmonary veins in the posterior wall (figure 5F).

A representative example of the progressive differences in conduction between pacing strategies in the same patient is shown in Figure 5D-F.

**Impact of Wavefront Direction on Complex Signals**

Regional variation was again present when pacing from the LSPV with greater complex signals compared to CSD pacing in the posterior wall (18.2 ± 12 vs 5.9 ± 3%, p=0.01) and roof (15.9 ± 9 vs 6.5 ± 7%, p=0.006) with the remaining segments unchanged (figure 2).
In LVZ regions, there was a significant progressive increase from CSD pacing at 600ms to 300ms to LSPV pacing in multiple parameters including: fractionated signal duration (50.4 ± 1.9 vs 55.5 ± 6.5 vs 58.0 ± 6.9ms, p=0.002); number of deflections (5.9 ± 0.6 vs 8.2 ± 2.4 vs 9.3 ± 3.2, p<0.001) and area of automated fractionation map indices (table 2). Additionally, fractionated signal voltage progressively decreased with each pacing strategy (1.12 ± 0.22 vs 0.74 ± 0.27 vs 0.59 ± 0.37, p<0.001). Representative fractionation maps according to each pacing strategy in the same patient for each of the three automated indices (SCI, ACI, ICL) is shown on figure 6.

**DISCUSSION**

This current study using repeated high density electroanatomic mapping at different cycle lengths and with change in wavefront direction demonstrated the dynamic nature of a left atrial substrate map with potentially important implications for approaches to substrate ablation. The detailed findings of this study are:

1) Pacing from left superior pulmonary vein compared with distal coronary sinus pacing significantly expands the area of potential atrial substrate characterised by lower LA voltage (global and regional), larger areas of low voltage zones, reduced atrial conduction velocity and greater and more fractionated complex signals.

2) Marked direction-dependant regional variation in the appearance of left atrial substrate occurs when pacing from the left superior pulmonary vein compared with the distal coronary sinus. These changes are consistent in the posterior wall and characterised by a vertical line of block in the majority of patients, increased conduction heterogeneity, lower bipolar voltages and increased complex signals.
Mechanisms of direction-dependant conduction in the left atrium

Both basic and clinical studies have clearly demonstrated the dynamic nature of atrial conduction and its relevance to AF onset and maintenance (350,416). Furthermore, studies of atrial structural remodelling have shown that fibrotic change amplifies CV rate-dependency, conduction anisotropy and conduction heterogeneity (408). Structural separation with fibrosis and reduced side-to-side cellular coupling increase anisotropic conduction (183,417); and excess extra-cellular matrix proteins produced by fibroblasts can interrupt cardiomyocyte-bundle continuity, leading to local conduction disturbances and reentrant arrhythmias (418). Both endocardial and epicardial mapping studies have demonstrated the key role functional anisotropic conduction delay and block plays in the initiation of atrial arrhythmias (109,395). A study in atria extensively remodelled due to severe mitral stenosis also demonstrated the impact of wavefront directionality on atrial voltage (342). Markides and colleagues elegantly described an anatomically determined line of functional conduction block in the posterior left atrium associated with interatrial macroreentry and PV ectopic initiation of AF (109). Histologic evaluation correlated this line of block in the posterior left atrium with change of myocardial fiber orientation demarked by the septopulmonary bundle (SPB). Detailed mapping of the epicardial surface during pacing from multiple sites, confirmed a stereotyped line of posterior left atrial block (395). The present study extends these results to high-density mapping of the endocardium, by demonstrating highly regional direction-dependant changes in the appearance of atrial substrate predominantly localised to the posterior wall in response to PV pacing.
Dynamic atrial substrate and remodelling

The appearance of fractionated electrograms during “electrophysiologic stress” may identify regions important in the initiation and maintenance of AF (341,419). The current study describes the impact of these well-established phenomena on the extent and anatomic distribution of the abnormal left atrial substrate in a large population of patients with paroxysmal and persistent AF, emphasising the dynamic nature of the ablation substrate target area. The posterior left atrium and antral regions were particularly sensitive to changes in cycle length and wavefront direction; both areas have been identified as important to atrial arrhythmia mechanism (91,291). This marked variation in regional substrate highlights the inherent limitations in a static approach to substrate identification and ablation. The profound substrate differences in the posterior wall due to wavefront activation from regions of known ectopic triggers may contribute to circuitous activation around lines of block promoting re-entry, anisotropy, fractionation and vulnerability to a primary arrhythmia mechanism. With more advanced remodelling during longer lasting persistent AF the substrate may be relatively fixed and therefore less sensitive to changes in cycle length or directionality.

Voltage-guided ablation strategy

Static bipolar atrial voltage maps have been developed as the electrophysiologic surrogate for fibrosis and have emerged as potential targets beyond PVI to improve outcomes following AF ablation (333,413). Kottkamp et al. pioneered a patient-tailored box isolation of fibrotic areas (BIFA) strategy in patients with low voltage areas achieving multi-procedure success rates up to 83% at a mean of 1.17 procedures per patient (333). Other studies that have targeted LVZs or the posterior wall have reported similar results (335,338,420). However, the extent and distribution of LVZ’s have
differed significantly between studies due to differences in the mapping rhythm, the mapping bipole and the stimulation protocol used. Importantly, voltage cut-points for low voltage regions have also not been standardised and may demonstrate both anatomic regional and patient to patient variation (347). Indeed, it may be necessary to adjust the voltage scale to identify voltage gradients in an individualised manner. In the current study we employed a voltage scale for display used in prior substrate ablation studies (332,338). However, rate and direction dependent changes in voltage are demonstrable independent of chosen cut-points. Low voltage regions may not be synonymous with substrate in some patients with AF and this study challenges the assumptions inherent in the use of voltage maps to guide ablation. The study highlights that the potential ablation target zone may change dramatically under varying pacing conditions. The extent to which regions of latent or dynamic substrate (exposed at short cycle length and with variation in direction of wavefront propagation) are critical to a substrate ablation approach requires further study. However, the above-described work indicating that these regions seem important to atrial arrhythmia mechanism provide support for this hypothesis.

Studies which have attempted to correlate electroanatomic low voltage with fibrosis identified on MRI have yielded divergent results (411). The reasons for this are likely multi-factorial relating in large part to the MRI protocol used. However, we have also demonstrated the variable and dynamic extent of the electroanatomic substrate by pacing protocol and it is unclear which protocol will correlate best with MRI data. Finally, a recent study mapping the epicardial left atrial surface in dogs demonstrated that the recording of omnipolar electrograms from a grid catheter can extract maximal voltages from AF signals not influenced by directional factors, collision or fractionation (421). Whether this approach will provide a clearer picture of dynamic
atrial substrate compared to sinus rhythm and pacing approaches is worthy of further study.

Limitations

The right atrium plays an important mechanistic role in the perpetuation of AF and data from high density right atrial maps would provide further insight into the presence of dynamic substrate in patients with AF. However, to date substrate-based approaches have predominantly focussed on left atrial ablation and the current study required completion of three detailed left atrial maps.

CONCLUSION

Substrate maps in patients with paroxysmal and persistent AF exhibit dynamic properties which can significantly impact the area of defined abnormal substrate, highlighting the potential limitations of utilising static low voltage maps to characterize AF substrate in substrate-based ablation strategies. Direction-dependent changes were highly regional and confined to posterior and inferior walls. Whether this latent substrate is important in the design of substrate ablation approaches to improve AF ablation outcomes requires further study.
TABLES

Table 1. Baseline Characteristics.

<table>
<thead>
<tr>
<th>Baseline Patient Characteristics (n = 17)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 8</td>
</tr>
<tr>
<td>Male</td>
<td>10 (59)</td>
</tr>
<tr>
<td>AF Type</td>
<td></td>
</tr>
<tr>
<td>- Paroxysmal</td>
<td>8 (47)</td>
</tr>
<tr>
<td>- Persistent</td>
<td>9 (53)</td>
</tr>
<tr>
<td>Duration of AF (months)</td>
<td>66 ± 57</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.7 ± 4.5</td>
</tr>
<tr>
<td>Obstructive Sleep Apnoea</td>
<td>6 (35)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Ischaemic Heart Disease</td>
<td>0 (0)</td>
</tr>
<tr>
<td>TIA/Stroke</td>
<td>1 (6)</td>
</tr>
<tr>
<td>CHA₂DS₂-VASc score</td>
<td>1.2 ± 0.9</td>
</tr>
<tr>
<td>Lab Rhythm</td>
<td></td>
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<tr>
<td>- Sinus Rhythm</td>
<td>9 (53)</td>
</tr>
<tr>
<td>- Atrial Fibrillation</td>
<td>8 (47)</td>
</tr>
<tr>
<td>Echocardiographic Parameters</td>
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<tr>
<td>LV Ejection Fraction (%)</td>
<td>54 ± 6</td>
</tr>
<tr>
<td>LA Size (cm)</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td>LA Area (cm²)</td>
<td>25.7 ± 5</td>
</tr>
</tbody>
</table>
Table 2. Complex Signal Characteristics in patients with progressive change in pacing rate and direction.

<table>
<thead>
<tr>
<th>Complex Electrogram Characteristics</th>
<th>600ms Pacing</th>
<th>300ms Pacing</th>
<th>LSPV Pacing</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean FP/DP Duration (ms)</td>
<td>50.4 ± 1.9</td>
<td>55.5 ± 6.5</td>
<td>58.0 ± 6.9</td>
<td>p=0.002</td>
</tr>
<tr>
<td>Mean Number of FP/DP Deflections</td>
<td>5.9 ± 0.6</td>
<td>8.2 ± 2.4</td>
<td>9.3 ± 3.2</td>
<td>p&lt;0.001</td>
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<td>Mean FP/DP Voltage (mV)</td>
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<td>0.59 ± 0.37</td>
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<td>Mean SCI Area (cm²)</td>
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<td>Mean SCI (ms)</td>
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<td>66 ± 22</td>
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<td>Mean ACI (ms)</td>
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Abbreviations: FP – Fractionated Potential; DP – Double Potential; SCI – Shortest Complex Interval; ACI – Average Complex Interval; ICL – Interval Confidence Level; LSPV – Left Superior Pulmonary Vein
FIGURES

Figure 1. Segmentation of the left atrium into regions.

**Figure 1.** Anteroposterior (AP) and posteroanterior (PA) views of the LA divided into six segments: anterior, posterior, inferior, lateral, septal, roof and inferior walls for regional analysis.
Figure 2. Global and segmental comparison of direction-dependent conduction differences between left superior pulmonary venous (LSPV) pacing and distal coronary sinus (CSd) pacing at 600ms and 300ms.
Figure 3. Electroanatomic maps demonstrating significant progressive increase in low voltage zones <0.5mV typically targeted in adjunctive scar homogenization ablation strategies according to different pacing methods in 3 different patients (area in red). Complex fractionated electrograms from corresponding regions in patient 3 (right side), a 72 year old male with early persistent AF, are longer with lower voltages in response to each subsequent pacing strategy. This Figure highlights the impact of pacing CL and wavefront direction on the potential atrial area included in substrate ablation. (Central Illustration).
Figure 4. Area of low voltage zone.

Figure 4. Variance in low voltage zone (LVZ) area (<0.5mV) and relative low voltage zone (rLVZ) area (<1.3mV) according to pacing strategy. CSD – Distal coronary sinus pacing at 600ms & 300ms; LSPV – left superior pulmonary vein pacing.
Figure 5. Electroanatomic maps of conduction.

Figure 5. Electroanatomic maps of different pacing strategies from a PA View in same patient. Bipolar voltage maps demonstrate marked progressive increase in low voltage zones between pacing from the distal coronary sinus at 600ms (A), 300ms (B) and the left superior pulmonary vein at 300ms (C). Isochronal maps (10ms intervals) show associated progressive conduction slowing between 600ms (D), 300ms (E – black arrows) and significant vertical posterior line of block with PV pacing at 300ms (F – black arrows). Complex fractionated signals are represented by white dots.
Figure 6. Electroanatomic fractionation maps using three semi-automatic mapping system fractionation indices (Shortest Complex Interval [SCI], Average Complex Interval [ACI], Interval Confidence Level [ICL]) between 600ms distal coronary sinus pacing (A, D, G), 300ms distal coronary sinus pacing (B, E, H) and directional left superior pulmonary vein pacing (C, F, I) strategies in the same patient. There is a progressive increase in surface area representing severity of fractionation on each of the indexes showed by the regions in red, yellow and green.
Chapter 5:

Gender related differences in atrial remodelling in patients with atrial fibrillation: Relationship to ablation outcomes

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disorder worldwide. Similar to other cardiovascular diseases, significant sex differences exist in patients with AF. Population studies have demonstrated that incident AF is higher in males compared with females (351). However, females with AF have been observed to have a greater symptom burden and potentially higher morbidity and mortality(370,422,423). Studies of AF ablation have also shown significant sex-based differences including higher risk of arrhythmia recurrence (424,425), increased periprocedural complications and hospitalisation (426) and lower ablation referral rates (427) in females compared to males with AF. Although the reported impact of female sex on AF ablation outcomes has not been uniform, a large recent systematic review and meta-analysis of post-ablation outcomes found lower AF-free survival in women⁵.

Currently, there are limited mechanistic data of sex-related differences in AF pathophysiology to explain potential differences in outcome. Whilst a greater burden of atrial fibrosis in females compared with males has been shown on MRI sequencing (356), a prior low density electroanatomic mapping study did not show a gender-based difference in atrial substrate (357).
In the current study we used high-density, high resolution mapping to characterise sex-based differences in the atrial substrate and correlated these with AF ablation outcomes.

METHODS

Study Population
This study prospectively recruited 45 patients undergoing catheter ablation of supraventricular tachycardia (SVT) without a history of AF and 97 patients with symptomatic refractory AF undergoing index pulmonary vein isolation. Patients were included if they had drug refractory symptomatic paroxysmal (lasting <7 days) or persistent AF (lasting >7 days) (402). Patients were excluded if they (1) had previous AF ablation; (2) advanced structural heart disease (congestive heart failure, greater than moderate valvular disease); (3) were unable to be electrically cardioverted to sinus rhythm; (4) amiodarone use; or (5) age < 18 years. This study was approved by the Royal Melbourne Hospital Research and Ethics Committee.

Procedural Study Protocol
All antiarrhythmic medications including beta-blockers and calcium-channel blockers were withheld at least 5 half-lives prior to procedure. Anticoagulation was managed according to operator preference. AF procedures were performed under general anesthesia with peri-procedural transesophageal echocardiography to exclude LA thrombus. Double trans-septal access was performed after heparinization (target ACT 300-350). SVT procedures were performed under routine sedation or general anesthesia according to operator preference.

Bipolar intracardiac electrograms and 12-lead surface electrocardiography (ECG) were recorded simultaneously on a digital amplifier system (EPMed Systems, NJ).
Intracardiac electrograms were filtered from 30 to 500 Hz and measured with digital calipers at 200 mm/s sweep speed.

**Electroanatomical mapping**

In AF patients, left atrial geometry was constructed with a 20 pole Lasso catheter (Biosense Webster, Irvine, CA, USA, 2-6-2 mm electrode spacing) and merged with a periprocedural CT using the CARTO3 electroanatomical mapping system. In the SVT cohort, right atrial (RA) geometry was constructed with a 20 pole Pentaray catheter (Biosense Webster, 2-6-2mm electrode spacing). Patients who presented to the lab in AF were electrically cardioverted to sinus rhythm. Voltage and activation maps were constructed using the multipolar catheter during constant pacing from the distal coronary sinus (CSD) for LA mapping or from the proximal coronary sinus (CSP) for RA mapping at 2 distinct cycle lengths (1) 600ms and (2) 300ms. Complete coverage of the entire LA and RA geometry was performed and correlated to the CT if available to ensure smooth coverage across all regions with a minimum of 1000 points using the Confidense™ algorithm to ensure even point distribution (Biosense Webster). Strict criteria were employed to account for the lack of tissue contact data on the multi-polar mapping catheter. Point collection was performed only by experienced operators after careful assessment of geometry and fluoroscopic motion. Mapping fill threshold was set at 5mm.

**Ablation procedure**

In the AF group, following mapping, experienced high-volume operators (>100 procedures/year case load) created wide antral circumferential ablation lines to achieve pulmonary vein isolation (PVI) utilizing an irrigated-tip radiofrequency ablation
catheter. The primary procedural endpoint was demonstration of entrance and exit block of all pulmonary veins on a circular mapping catheter following adenosine challenge for acute reconnection and a mandatory 30 minute waiting period. In the SVT group, ablation strategy was performed according to the electrophysiological study guided diagnosis (ie. atrioventricular nodal re-entrant tachycardia or atrioventricular re-entrant tachycardia) with a non-irrigated or irrigated tip ablation catheter as appropriate at operator discretion.

Electrogram analysis

Electrogram analysis for each map was performed manually offline. For activation time, each point was analysed at 200 mm/s sweep speed and appropriately annotated at the maximum negative dV/dt for unipolar signals or the peak sharp of the bipolar electrogram. Although data was collected using the Confidense algorithm, an internal point filter to within 5mm of the chamber surface geometry was applied (345) and all residual acquired points were meticulously manually reviewed and annotated. Only points demonstrating characteristics of near-field signals were included. In keeping with prior publications (392), these signals demonstrated at least 2 sharp peaks and were consistent with anatomically adjacent signals in terms of signal quality and electrogram timing. Points that did not fit these criteria, ectopic beats and artefact were excluded. Following extensive manual annotation and exclusion of signals not meeting the above criteria, signal processing was also performed offline (MATLAB 9.1, Mathworks, MA, USA).
**Global and Regional Atrial Voltage Analysis**

Bipolar voltage was defined as the peak-peak electrogram voltage. Segmental voltage analysis was performed by dividing the chamber into the following 6 left atrial segments: anterior, posterior, septal, lateral, roof and inferior (Figure 1A) and 5 right atrial segments: anterior, posterior, lateral, septal and inferior (Figure 1B). In the LA, the mitral annulus and pulmonary veins beyond the antrum were excluded and in the RA, the tricuspid annulus and the region beyond the vena cava were excluded. The mean voltage of each region was calculated.

**Conduction Velocity Analysis**

Conduction velocity (CV) was analysed in MATLAB as previously described using the polynomial algorithm (330,393). In brief, this method assigns a fitting ‘window’ per region with a minimum of 20 points required. Each region is assigned subsets of Cartesian coordinates in space and activation time. These are fitted to a smooth polynomial surface in three-dimensional space, using a standard least squares algorithm, which provides robustness against outliers. The gradient is then calculated from the fit and used to calculate velocity components, with final velocities at each point calculated from the weighted average of velocity components from every fit including that point (394). The mean left and right atrial CV was calculated and compared between cycle lengths. Conduction heterogeneity was determined by calculating the coefficient of variation (CoV) of conduction velocity.

**Analysis of Complex Signals**

Complex signals were defined as electrograms with ≥3 deflections >50ms duration (fractionated potentials [FP]) or 2 separate deflections separate by an isoelectric
interval (double potentials [DP]). These signals were manually annotated and tagged. The percentage of complex signals is expressed as a proportion of the number of complex signals divided by the total number of points.

Assessment of Effective Refractory Period & Sinus Node Function

Effective refractory period (ERP) at the proximal and distal coronary sinus was performed. Briefly, ERPs were measured using a pacing drive train of 8 beats followed by a single extrastimulus commencing at a coupling interval of 150ms and incrementing by 10ms until local capture was demonstrated. The local ERP was defined as the longest extrastimulus that failed to capture the pacing site. ERPs were measured at 2 drive train cycle lengths (600 and 400ms) and repeated at least twice at each site to ensure consistency. Sinus node recovery time (SNRT), evaluated by 30 s burst pacing trains at every 50ms from 600 to 400ms, was determined as the longest time from the stimulus artefact to the earliest atrial activity. The corrected SNRT was determined by correcting for the underlying sinus cycle length.

Follow-up and Arrhythmia Detection

Antiarrhythmic medications and anticoagulation were managed according to physician preference in accordance with guidelines and following a 3-month post-ablation blanking period. Patients underwent systematic comprehensive rhythm monitoring throughout the course of the study. Time to recurrence were determined using either cardiac rhythm management devices (pre-existing pacemaker or insertable cardiac monitor [ICM – Medtronic Linq™, Minneapolis, MN, USA]) or Holter monitoring at 3, 6, and 12 months post ablation followed by 6-monthly thereafter until study completion. Cardiac arrhythmia detection devices were either interrogated at clinic
visits or via remote monitoring. Repeat ablation procedures were performed in patients with refractory symptomatic recurrent atrial arrhythmia with adjunctive lesion sets (cavotricuspid isthmus and posterior wall isolation) performed according to operator preference.

**Statistical Analysis**

Data analysis was performed using SPSS software (Version 23, IBM, Armonk, New York). Normality of all quantitative variables was assessed using the Shapiro-Wilk test and normalising transformations were performed as necessary. Data are expressed as mean ± standard deviation (SD) unless otherwise stated. Power calculation was performed assuming an absolute difference in AF recurrence rate of 20%. A sample size of 90 patients is required to detect a hazard ratio of ≤0.5 for arrhythmia free survival based on prior work with a power of 80%. A mixed random effects model was used for comparison between multiple regional measures within each patient (ie. CV, voltage). Generalised estimating equations were used for nested categorical variables (ie. Percentage fractionated points). To investigate regional variation in measured parameters, region (6 LA or 5 RA segments) and group (male and female) were modelled as fixed effects with a region x group interaction term. If a significance interaction was present, mixed effects Bonferroni post hoc analysis p-values were reported.

Conventional two-group comparisons were made using unpaired t test for continuous variables, or the chi-squared test for categorical variables for data without multiple measures or levels (ie. Gender, LA size).

General linear model repeated measures analysis was also performed separately on each parameter to determine univariable predictors of voltage, CV and complex
signals. Following univariable analysis, all predictors with \( p<0.1 \) were included in a multivariable model. Survival curves for freedom from arrhythmia (AF/atrial tachycardia [AT]) were determined using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards regression was used to determine univariate (\( p<0.1 \)) and multivariate predictors of arrhythmia-free survival following single and multiple procedures.

**RESULTS**

*Baseline Characteristics*

In total, 27 out of 45 patients (60%) were female in the SVT group and 35 out of 97 patients (36%) were female in the AF group. Overall, the mean age was 61±8 years in the AF group and 47±16 in the SVT group. AF was persistent in 50% of the AF group. The baseline patient characteristics did not significantly differ between males and females in each group and in particular, there were no gender differences in hypertension, diabetes or vascular disease (Table 1). Follow-up was completed in all patients. Of these, more than half (54/97 [56%]) were continuously monitored by ICM (50/97 [52%]) or dual chamber pacemaker (4/98 [4%]) with the remaining undergoing serial Holter surveillance (43/97 [44%]).

*High Density Electroanatomical mapping*

The mean total number of points collected was 2,123 ± 891 per map. Following internal point filtering (5mm) and manual annotation, a mean of 1311 ± 557 points per LA map and 1337 ± 495 per RA map were included, with no significant differences between male and female groups. Table 2 summarises the global and regional electroanatomical parameters measured in both groups.
Analysis of Voltage

In the AF group, the mean global bipolar LA voltage was significantly lower in females compared with males pacing at 600ms (1.46 ± 0.15 vs 1.87 ± 0.16, p=0.003) and 300ms (1.27 ± 0.18 vs 1.57 ± 0.18mV, p=0.013) (Figure 2A). These differences were observed uniformly across all LA regions without any significant inter-regional gender differences in voltage (p=0.955) (Figure 3).

In the SVT group, mean RA voltage was also significantly lower in females compared with males at 600ms pacing (1.84 ± 0.20 vs 2.50 ± 0.40mV, p<0.001) and similarly uniformly seen across all RA segments. However, there was no significant difference between genders when pacing at 300ms (1.91 ± 0.35 vs 2.16 ± 0.28mV, p=0.271). Representative electroanatomic substrate maps demonstrating these differences are shown in figure 4 and 5.

Impact of Gender on Conduction

In AF patients, the mean global conduction velocity was lower in females at both 600ms pacing (35.5 ± 6.0 vs 45.0 ± 6.1cm/s, p=0.024) and 300ms pacing (19.3 ± 2.3 vs 35.0 ± 2.1cm/s, p<0.001) (Figure 2B). This difference was seen uniformly across the left atrium (interactive p[group x region]=0.407) (Table 2). Mean CV heterogeneity was similar between groups (39.7 ± 4.7 vs 39.4 ± 1.4%, p=0.911).

In SVT patients, mean global conduction velocity was significantly lower in females at 600ms pacing (37.9 ± 5.0 vs 48.4 ± 2.5 cm/s, p=0.002) (Figure 2) and similarly observed uniformly across the right atrium (interactive p-value=0.496) (Figure 3) (Table 2). Mean CV was not significantly different between genders at 300ms pacing (29.5 ± 5.5 vs 32.3 ± 4.7 cm/s, p=0.379) (Figure 2). CV heterogeneity was significantly
higher in females at 600ms pacing (38.5 ± 5.0 vs 31.0 ± 3.5%, p=0.035) with no significant difference at 300ms pacing (37.2 ± 6.0 vs 36.4 ± 4.4%, p=0.858).

Impact of Gender on Electrogram Fractionation
There was a significantly higher proportion of complex fractionated electrograms and double potentials in females in the AF group compared with males at 600ms pacing (9.0 ± 1.7 vs 6.2 ± 1.6%, p=0.026) and 300ms pacing (12.0 ± 1.8 vs 8.5 ± 1.5%, p=0.013) (Figure 2). In keeping with differences in voltage and CV, these findings were observed uniformly at both cycle lengths (interactive [group x region] p=0.023 [600ms], p=0.122 [300ms]).

A significantly higher proportion of right atrial complex signals was also seen in female SVT patients at 600ms pacing (6.8 ± 3.4 vs 2.8 ± 1.7%, p=0.014) but no difference was seen at 300ms pacing (6.4 ± 5.3 vs 6.0 ± 3.9%, p=0.729).

Assessment of Effective Refractory Period & Sinus Node Function
There were no significant gender-based differences in atrial refractoriness at the proximal and distal coronary sinus at 2 cycle lengths (Table 3). In addition, there were no gender differences in corrected sinus node recovery times across multiple cycle lengths (Table 3).

Univariable and Multivariable Predictors of Voltage, CV & Complex Signals
Predictors of atrial remodelling in both AF and SVT cohorts during 600ms pacing are presented in Table 4. For the AF cohort, significant univariate predictors of low voltage were age (p=0.001), female gender (p=0.003), persistent AF (p=0.048), AF duration (p=0.002) and hypertension (p=0.034). Of these parameters and including CHA2DS2-
VASc score and smoking (univariate p-value<0.1), only age (p=0.002), female gender (p=0.024), persistent AF (0.042) and AF duration (p=0.009) remained as multivariable predictors of low voltage. In SVT patients, only female gender was a univariate and multivariate predictor of low voltage (p=0.005).

Univariate predictors of conduction slowing in the AF cohort were female gender (p=0.011) and hypertension (p=0.026). On multivariable analysis, only female gender (p=0.046) remained as a predictor of conduction slowing. Whereas in the SVT group, both age (p=0.039) and female gender (p=0.016) were multivariate predictors of conduction slowing.

Similarly, female gender was a univariate and multivariate predictor of complex fractionated signals in AF patients (p=0.013) and SVT patients (p=0.013).

*Clinical outcomes post AF ablation*

In the AF group, pulmonary vein isolation was achieved in 100% of patients (n=97). The average fluoroscopy time was 9.8 ± 6.1 minutes; and ablation time was 39 ± 9 minutes. Procedural characteristics were not significantly different between males and females. After a median follow up of 27 months (Q1–3: 18-32) following index AF ablation, overall success was achieved in 68 (70%) patients. Female patients had significantly lower single-procedural arrhythmia-free survival (20 [57%] vs 48 [77%], log-rank p=0.041) and shorter time to arrhythmia recurrence (22.8 ± 2.2 vs 26.4 ± 1.6 months, log-rank p=0.041). Kaplan-Meier survival curves are shown in Figure 6. On Cox regression analysis, univariate predictors (p<0.1) of reduced arrhythmia-free survival were female sex (hazard ratio [HR] 0.48; 95% confidence interval [CI] 0.23-0.96, p=0.046), persistent AF (HR 0.25; 95% CI 0.10-0.61, p=0.002), LVEF (HR 0.94; 95% CI 0.87-0.99, p=0.048) and obstructive sleep apnoea (p=0.062). In a multivariate
model, female sex (p=0.035) and persistent AF (p=0.029) remained as independent predictors of single-procedure arrhythmia recurrence.

A total of 16 patients (16%) underwent repeat ablation for symptomatic arrhythmia recurrence (female: 8 (23%) vs male: 8 (13%), p=0.258). Multi-procedure success was achieved in 76 (78%) patients. Following a mean of 1.1 ± 0.4 procedures per patient, female patients had significantly lower multi-procedural arrhythmia-free outcome (females: 23 [66%] vs males: 53 [86%], log-rank p=0.019) (Figure 6B). Univariate predictors of multi-procedure arrhythmia recurrence included female sex (HR 0.37; 95% CI 0.16-0.88, p=0.024), persistent AF (HR 0.32; 95% CI 0.12-0.88, p=0.028) and obstructive sleep apnoea (p=0.053). On multivariable analysis, female sex (p=0.014) and persistent AF (p=0.032) were independent predictors of multi-procedure arrhythmia recurrence.

**DISCUSSION**

Using high-density electroanatomic mapping, we demonstrated significant sex-related differences in atrial electrophysiology both in AF and in SVT patients. These were characterised by a more advanced atrial substrate including: (i) lower atrial voltage (ii) reduced conduction velocity and (iii) greater proportion of complex fractionated signals. Atrial substrate differences were consistently observed at both cycle lengths in AF patients and in one pacing cycle length in the SVT group. Additionally, female patients with AF had significantly higher single and multi-procedure arrhythmia recurrence following pulmonary vein isolation. Taken together, these findings indicate a significant sex-based influence on atrial remodelling may account for adverse post-ablation outcomes in females.
Sex and AF Epidemiology

It is now well established that the epidemiology of AF differs between men and women. The age-adjusted incidence of AF has been shown to be higher in men compared to women in multiple cohorts (351). This difference has been attributed to taller stature and a greater prevalence of AF risk factors such as coronary artery disease in men (428). Larger left atria and increased left ventricular wall thickness in men have been associated with increased risk of AF and may also explain the lower incidence of AF among women. However, certain AF risk factors such as HT, valvular disease and heart failure with preserved ejection fraction may be more common in women (429). In most epidemiologic studies, women with AF are older than men with AF. In the Framingham Heart Study for example, 74% of women with AF were aged ≥70 years, compared with 58% of men (351).

Clinical Outcomes of AF

Numerous sex-related differences in AF presentation and response to treatment have been described. Females may have a more complicated clinical course involving faster ventricular rates, longer AF episodes (430), increased risk of stroke (376), increased post cardioversion recurrence (431) and reduced response to pulmonary vein isolation (355,432,433). A recent meta-analysis of 19 studies of over 150,000 patients demonstrated that females had lower arrhythmia-free survival following ablation compared with males (odds ratio [OR]: 0.75, 95% CI 0.69-0.81, p<0.0001) (424). Similarly, sub-analysis of the FIRE AND ICE randomised trial in paroxysmal AF ablation showed a significant impact of female sex on increased arrhythmia recurrence (425). A large audit of greater than 50,000 patients in the US found that females undergoing AF ablation were older with a higher prevalence of co-morbidities, and
experienced significantly higher readmission rates for complications and recurrent arrhythmia (OR 1.48, p<0.0001) (426). Consistent with the aforementioned studies, our study showed significantly lower single as well as multi-procedural arrhythmia-free survival in females compared to males.

These observations suggest that AF mechanisms may differ according to sex. However, studies investigating sex-related differences in AF pathophysiology are limited and such differences, if present, are poorly understood.

**Sex and Atrial Remodelling**

Prior studies investigating the impact of AF risk factors on atrial remodelling have shown consistent results (134). Voltage reduction, conduction slowing and heterogeneity have been observed in the context of ageing (302), hypertension (327), sleep apnoea (312), obesity (317) and heart failure (306). However, prior studies investigating sex differences in atrial substrate are limited with available data showing inconsistent results. Women have been shown to possess greater degrees of atrial fibrosis when compared to men in histologic studies including fibrotic markers (434) and on delayed gadolinium enhancement imaging with cardiac magnetic resonance (356) in patients with and without AF. A small prior electroanatomic study of patients with and without AF did not observe any gender related differences in atrial electrophysiology (357). However, this study employed lower density point-by-point mapping method potentially of insufficient resolution and/or power to detect subtle differences between groups. The current study using both higher resolution (small electrode spacing) and higher density observed consistent gender related differences in the AF population at 2 cycle lengths. In addition, the presence of some gender related differences present in the SVT population point to a primary impact of sex on atrial
electrophysiology. It is noteworthy that in the current study, differences in atrial remodelling could not be explained by differences in atrial or ventricular size or by sex differences in the prevalence of AF risk factors. However, unmeasured factors such as lower atrial wall thickness may potentially account for lower atrial voltages in women (435).

Mechanisms of Sex-related Differences in the Atrium

While gender differences in ventricular repolarization and QT interval are relatively well-established, the exact mechanisms for sex-related differences and the impact of sex hormones in AF remain incompletely understood. Progesterone has been implicated in the modulation and enhancement of slow delayed rectifier potassium current and inhibition of L-type calcium currents which serve to shorten action potential duration (360). Oestrogen opposes the effect of progesterone and has been shown to play a role in cardiac ion channel regulation. Oestrogen receptors are highly expressed in cardiomyocytes and oestrogen administration has been linked with prolonged action potential duration, atrial and atrioventricular nodal conduction time and atrial refractoriness primarily through effects on increased calcium influx leading to weaker repolarising currents or potassium channel downregulation (361,362). Menstrual variations are associated with increased episodes of SVT particularly during the luteal phase of higher progesterone and lower oestrogen levels (436). This relationship has not been observed with AF and there is disparate evidence suggesting the net effect of sex hormones may be somewhat protective. A small study has shown less atrial ERP shortening in pre-menopausal women (437) which may in part account for an increased incidence of AF in postmenopausal women beyond increasing co-morbidities. However, hormone replacement therapy when compared with placebo, did
not have an impact on incident AF in the randomised Women’s Health Initiative trial (363). Lastly, a study in paroxysmal AF patients identified greater non-pulmonary vein triggers for AF in women when compared to men (16% vs 8.4%, p<0.001), although this difference was unadjusted for potential confounders including co-morbidities and atrial size (355).

Limitations
Data in SVT patients was obtained from right atrial maps as the majority of patients did not have a clinical indication to perform transeptal puncture. In contrast, data in AF patients was collected in the left atrium. Therefore, comparisons cannot be made between these groups. Further examination of left atrial maps, histology and imaging in a SVT group would help to determine whether gender differences in patients with atria unaffected by AF (including stratification of pre- and post-menopausal females) may be potentially attributed to baseline hormonal differences or local factors such as pericardial adipose deposits.

CONCLUSION
The current study demonstrated significant sex-related differences in atrial substrate in AF and SVT patients characterised by lower voltage, reduced conduction velocity and increased complex fractionated signals in females compared with males. These changes may contribute to sex-based differences in the clinical course of females with AF and may in part explain the higher reported risk of recurrence. The mechanisms underlying these differences and their clinical impact warrant further investigation.
# TABLES

**Table 1.** Baseline Demographics.

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### Echocardiographic Parameters

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<tr>
<td><strong>LVEDD (cm)</strong></td>
<td>4.6 ± 5.0</td>
<td>4.5 ± 4.7</td>
<td>0.774</td>
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<tr>
<td><strong>LVEF (%)</strong></td>
<td>58 ± 9</td>
<td>63 ± 7</td>
<td>0.183</td>
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<tr>
<td><strong>LA Size (cm)</strong></td>
<td>3.7 ± 3.0</td>
<td>3.5 ± 2.9</td>
<td>0.188</td>
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<tr>
<td><strong>LA Area (cm²)</strong></td>
<td>17.3 ± 3.0</td>
<td>18.0 ± 2.2</td>
<td>0.446</td>
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<tr>
<td><strong>LA Volume Index (ml/m²)</strong></td>
<td>33.6 ± 8.2</td>
<td>30.5 ± 7.0</td>
<td>0.429</td>
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<tr>
<td><strong>RA Area (cm²)</strong></td>
<td>15.6 ± 2.8</td>
<td>14.9 ± 1.6</td>
<td>0.453</td>
</tr>
</tbody>
</table>

**Abbreviations:** AF – Atrial Fibrillation, CHA²DS²-VASc – Congestive Heart Failure, Hypertension, Age, Diabetes, Stroke, Vascular Disease, Sex, LA – Left Atrial, LV – Left Ventricular, TIA – Transient Ischaemic Attack
Table 2. Regional Electroanatomic differences according to gender in the AF population.

<table>
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<tr>
<th>Voltage (mv)</th>
<th>Global</th>
<th>Anterior</th>
<th>Posterior</th>
<th>Inferior</th>
<th>Roof</th>
<th>Septal</th>
<th>Lateral</th>
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<tr>
<td>Female</td>
<td>1.46 ± 0.15</td>
<td>1.66 ± 0.60</td>
<td>1.39 ± 0.53</td>
<td>1.38 ± 0.56</td>
<td>1.68 ± 0.68</td>
<td>1.21 ± 0.45</td>
<td>1.45 ± 0.58</td>
</tr>
<tr>
<td>Male</td>
<td>1.87 ± 0.16</td>
<td>2.05 ± 0.79</td>
<td>1.77 ± 0.76</td>
<td>1.77 ± 0.80</td>
<td>2.16 ± 0.88</td>
<td>1.58 ± 0.69</td>
<td>1.92 ± 0.88</td>
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<tr>
<td>P-value</td>
<td>p(group) = 0.003; p(interactive*) = 0.955</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
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</tr>
<tr>
<td>Female</td>
<td>1.84 ± 0.21</td>
<td>2.16 ± 0.70</td>
<td>1.61 ± 0.37</td>
<td>1.82 ± 0.79</td>
<td>-</td>
<td>1.75 ± 0.57</td>
<td>1.89 ± 0.71</td>
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<tr>
<td>Male</td>
<td>2.50 ± 0.38</td>
<td>3.04 ± 1.00</td>
<td>1.97 ± 0.47</td>
<td>2.52 ± 0.76</td>
<td>-</td>
<td>2.51 ± 0.76</td>
<td>2.44 ± 0.53</td>
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<tr>
<td>Female</td>
<td>35.5 ± 6.0</td>
<td>40.7 ± 30.3</td>
<td>33.4 ± 24.5</td>
<td>41.5 ± 18.8</td>
<td>39.5 ± 22.8</td>
<td>32.9 ± 21.3</td>
<td>24.9 ± 15.1</td>
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<td>Male</td>
<td>45.0 ± 6.1</td>
<td>45.1 ± 22.0</td>
<td>38.3 ± 14.5</td>
<td>54.5 ± 26.1</td>
<td>48.7 ± 22.8</td>
<td>45.7 ± 24.9</td>
<td>37.6 ± 21.7</td>
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<tr>
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<td></td>
<td>p(interactive*) = 0.407</td>
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<td>Female</td>
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<td>42.3 ± 13.0</td>
<td>32.2 ± 14.5</td>
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<td>Male</td>
<td>48.4 ± 2.5</td>
<td>50.9 ± 13.0</td>
<td>46.5 ± 13.3</td>
<td>46.8 ± 11.8</td>
<td>-</td>
<td>51.5 ± 13.0</td>
<td>46.4 ± 12.5</td>
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<tr>
<td>P-value</td>
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<td>†</td>
<td>†</td>
<td>-</td>
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<tr>
<td>p(group)</td>
<td>p(interactive*) = 0.002;</td>
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<td>p(interactive*) = 0.149</td>
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<td><strong>Electrogram Fractionation</strong></td>
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</tr>
<tr>
<td>Female</td>
<td>9.0 ± 1.7</td>
<td>5.7 ± 5.4</td>
<td>11.8 ± 12.4</td>
<td>9.8 ± 10.4</td>
<td>8.7 ± 6.6</td>
<td>9.5 ± 13.8</td>
<td>10.8 ± 9.8</td>
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<td>7.3 ± 7.9</td>
<td>6.5 ± 7.6</td>
<td>4.1 ± 4.9</td>
<td>6.3 ± 6.4</td>
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<tr>
<td>P-value</td>
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<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
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<td>p(interactive*) = 0.758</td>
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<tr>
<td>Female</td>
<td>6.8 ± 3.4</td>
<td>2.2 ± 1.7</td>
<td>10.5 ± 3.4</td>
<td>5.6 ± 3.1</td>
<td>-</td>
<td>6.0 ± 3.2</td>
<td>9.9 ± 4.9</td>
</tr>
<tr>
<td>Male</td>
<td>2.8 ± 1.7</td>
<td>1.0 ± 1.2</td>
<td>5.1 ± 2.6</td>
<td>1.4 ± 1.4</td>
<td>-</td>
<td>3.0 ± 2.0</td>
<td>3.7 ± 2.1</td>
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<tr>
<td>P-value</td>
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<td>†</td>
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<td></td>
<td>p(interactive*) = 0.294</td>
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Values are mean±SD. *interactive: group x region. †Post hoc test not performed interactive p value=NS. FP/DP – fractionated/double potentials
Table 3. Sinus Node Function and Atrial Refractory Periods.

<table>
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<tr>
<th></th>
<th>SVT Patients</th>
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<th>AF Patients</th>
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<th></th>
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<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>p-value</td>
<td>Male</td>
<td>Female</td>
<td>p-value</td>
</tr>
<tr>
<td>CSNRT 600ms (ms)</td>
<td>233 ± 157</td>
<td>309 ± 115</td>
<td>0.191</td>
<td>326 ± 250</td>
<td>283 ± 169</td>
<td>0.583</td>
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<tr>
<td>CSNRT 500ms (ms)</td>
<td>221 ± 125</td>
<td>357 ± 151</td>
<td>0.084</td>
<td>403 ± 163</td>
<td>391 ± 343</td>
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<tr>
<td>CSNRT 400ms (ms)</td>
<td>201 ± 134</td>
<td>250 ± 97</td>
<td>0.304</td>
<td>365 ± 118</td>
<td>453 ± 212</td>
<td>0.382</td>
</tr>
<tr>
<td>CSd ERP 600ms (ms)</td>
<td>228 ± 23</td>
<td>224 ± 24</td>
<td>0.760</td>
<td>257 ± 45</td>
<td>265 ± 69</td>
<td>0.699</td>
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<tr>
<td>CSd ERP 400ms (ms)</td>
<td>216 ± 20</td>
<td>206 ± 15</td>
<td>0.290</td>
<td>229 ± 29</td>
<td>232 ± 43</td>
<td>0.809</td>
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<tr>
<td>CSp ERP 600ms (ms)</td>
<td>241 ± 32</td>
<td>220 ± 27</td>
<td>0.133</td>
<td>236 ± 38</td>
<td>232 ± 43</td>
<td>0.892</td>
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<tr>
<td>CSp ERP 400ms (ms)</td>
<td>229 ± 34</td>
<td>208 ± 22</td>
<td>0.118</td>
<td>224 ± 28</td>
<td>234 ± 24</td>
<td>0.431</td>
</tr>
</tbody>
</table>

CSNRT = Corrected Sinus Node Recovery Time; CSd = Distal Coronary Sinus; CSp = Proximal Coronary Sinus, ERP = Effective Refractory Period
Table 4. Univariable and multivariable analysis of voltage and conduction velocity.

<table>
<thead>
<tr>
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<th>AF Group</th>
<th></th>
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<tbody>
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<td>Multivariate</td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
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<td>F Statistic</td>
<td>p-value</td>
<td>F Statistic</td>
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<td>3.732</td>
<td>0.001</td>
<td>10.647</td>
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<tr>
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<td>0.003</td>
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<td>Saxon)</td>
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<td>AF Duration (months)</td>
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<td>0.397</td>
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<td>Obesity (BMI&gt;30)</td>
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<td>0.134</td>
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<td>Value</td>
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FIGURES

Figure 1. Electroanatomic segmentation.

Figure 1. Regional analysis of the left atrium in 6 segments (top) and the right atrium in 5 segments (bottom).
Figure 2. Comparison of atrial electrophysiology and substrate between genders at multiple cycle lengths in both AF and SVT cohorts. Females showed lower voltage (A), slower conduction velocity (B) and greater complex signals (C) compared with male patients in both groups. AF – atrial fibrillation; SVT – supraventricular tachycardia.
Figure 3. Segmental comparison of electrophysiologic parameters.

**AF Group**
LA 600ms Pacing

**SVT Group**
RA 600ms Pacing

Figure 3. Segmental voltage, conduction velocity and complex signals in AF group (A-C) and SVT group (D-F) at 600ms pacing cycle length between male and female patients. AF – atrial fibrillation; LA – left atrium; RA – right atrium; SVT – supraventricular tachycardia.
Figure 4. Electroanatomic substrate differences (AF Group).

**Figure 4.** Representative left atrial electroanatomic maps in male (A-B) and female (C-D) patients with AF from posteroanterior (PA) views. Voltage maps demonstrate greater degree of remodelling in females with AF (C) as shown by the larger lower voltage region shown in red across the posterior right antral region. Activation maps also show corresponding isochronal crowding consistent with slowed conduction velocity in females (D) compared with males (B).
Figure 5. Representative right atrial electroanatomic maps in male (A-B) and female (C-D) patients in the SVT group without AF from right lateral (RL) views. Voltage maps demonstrate greater degree of remodelling in females in the SVT group as shown by a larger vertical area of red, yellow and green across the crista terminalis (C). Activation maps also show significantly greater isochronal crowding and conduction block across this line in females (D) compared with males (B).
Figure 6. Kaplan Meier curves of single-procedure (A) and multi-procedure (B) arrhythmia-free survival differences between male and females with AF.
Chapter 6:

Sinus Node Remodelling in Atrial Fibrillation:

Insights from High Density Mapping

INTRODUCTION

The sinus node is a small crescent-shaped structure located at the junction of the superior vena cava and the right atrium (RA) along the sulcus terminalis containing clusters of specialized pacemaker myocytes (438). Prior work has shown that sinus node conduction and the site of earliest activation occurs over a widespread area anatomically disparate to the location of the sinus node pacemaker cells (439,440). Variable preferential pathways of sinus node activity beyond the pacemaker complex have been shown in both preclinical animal and human models (141,441). A dynamic interplay has been observed between sinus node activity and atrial arrhythmias, which frequently occur in up to 70% of patients presenting with sinus node dysfunction (SND) (442). Sanders et al demonstrated atrial remodelling in patients with significant SND prior to the onset of atrial fibrillation (AF) and reported reduced atrial voltage, conduction slowing and a caudal shift in the sinus pacemaker complex (324). Subsequent work using a similar low-resolution mapping approach has demonstrated sinus node remodelling in different patient populations. However, the characterisation of sinus node and regional remodelling in humans including persistent forms of AF at high-density remains unknown. The advent of high density, high resolution mapping has provided new insights into arrhythmia mechanism across a broad spectrum of atrial and ventricular arrhythmias. In the current study we used high density, high-resolution
mapping to characterise the nature of sinus node and atrial remodelling in patients with paroxysmal and with persistent atrial fibrillation.

We hypothesised that high-density 3-dimensional electroanatomic would demonstrate more extensive sinus node and regional atrial remodelling in patients with persistent AF (PsAF) compared with those with paroxysmal AF (PAF) or control patients.

**METHODS**

*Study Population*

The study population comprised 25 consecutive prospectively enrolled patients undergoing index AF ablation (11 patients in paroxysmal AF group and 14 patients in persistent AF group). As a reference, 25 age-matched control patients with structurally normal hearts undergoing ablation for supraventricular tachycardia (SVT) were studied. Patients were included if they had drug refractory symptomatic paroxysmal (lasting <7 days) or persistent AF (lasting >7 days) (402). Patients were excluded if they (1) were unable to be electrically cardioverted to sinus rhythm (2) had previous AF ablation; (3) amiodarone use; or (4) age < 18 years. This study was approved by the Royal Melbourne Hospital Research and Ethics Committee.

*Procedural Study Protocol*

All antiarrhythmic medications including beta-blockers and calcium-channel blockers were withheld at least 5 half-lives prior to procedure. Anticoagulation was managed according to operator preference. All procedures were performed under general anesthesia with peri-procedural transesophageal echocardiography to exclude LA thrombus. Double trans-septal access was performed after heparinization (target ACT
SVT procedures were performed under routine sedation or general anesthesia according to operator preference.

Bipolar intracardiac electrograms and 12-lead surface electrocardiography (ECG) were recorded simultaneously on a digital amplifier system (EPMed Systems, NJ). Intracardiac electrograms were filtered from 30 to 500 Hz and measured with digital calipers at 200 mm/s sweep speed.

**Electroanatomical mapping**

In both AF and control groups, right atrial (RA) geometry was constructed with a 20 pole Pentaray catheter (Biosense Webster, CA, USA, 2-6-2 mm electrode spacing) using the CARTO3 electroanatomical mapping system. Patients who presented to the lab in AF were electrically cardioverted to sinus rhythm. Voltage and activation maps were constructed using the multipolar catheter during sinus rhythm, and constant pacing from the proximal coronary sinus (CS) at 2 distinct cycle lengths (1) 600ms and (2) 300ms. Complete coverage of the entire RA geometry was performed with a minimum of 1000 points using the Confidense™ algorithm to ensure even point distribution (Biosense Webster). Strict criteria were employed to account for the lack of tissue contact data on the multi-polar mapping catheter. Point collection was performed only by experienced operators after careful assessment, fluoroscopic motion and application of an internal point filter to within 5mm of the chamber surface geometry (345). Mapping fill threshold was set at 5mm. Although data was collected using the Confidense algorithm, all acquired points were also manually reviewed and annotated. Only points demonstrating characteristics of near-field signals were included. In keeping with prior publications (392), these signals demonstrated at least
2 sharp peaks and were consistent with anatomically adjacent signals in terms of signal quality and electrogram timing. Points that did not fit these criteria were excluded.

Electrogram analysis

Electrogram analysis was performed manually offline. For activation time, each point was analysed at 200 mm/s sweep speed and appropriately annotated at the maximum negative dV/dt for unipolar signals or the peak sharp of the bipolar electrogram. Ectopic beats and artefact were excluded. Following extensive manual annotation and exclusion of signals not meeting the above criteria, signal processing was performed offline (MATLAB 9.1, Mathworks, MA, USA).

Sinus node mapping and definitions

Mapping of the sinus node complex was performed during stable baseline sinus rhythm. Sinus node activation was characterised by determining the positions of the earliest activation site (EAS) and sinus break-out (SBO). EAS was defined as the earliest unipolar electrogram with a ‘QS’ pattern and SBO as the earliest unipolar electrogram with an ‘RS’ pattern and an abrupt increase in dV/dt indicating rapid depolarization of surrounding atrial myocardium as previously described (141,441). EAS and SBO locations were described on the 3D geometry relative to the SVC–RA junction in the superior-inferior direction and the crista terminalis anterior-posteriorly during baseline sinus rhythm. The conduction time between the EAS and SBO and direction of activation was measured. The sinus node activation area was arbitrarily defined as the region demarcated by the initial 20ms from the earliest activation time on a local activation timing map to standardize measurements. Voltage analysis of points in the EAS-SBO region was also recorded. The crista terminalis was defined
anatomically from the 3D map extending from the SVC-RA junction to the inferior vena cava.

Sinus node activation was defined as unicentric as a single EAS which spreads to activate the atria and multicentric for two or more EAS separated by ≥10 mm with an activation time difference of ≤5 ms as previously described (443). Number of SBO sites was defined using the same criteria.

**Global and Regional Atrial Voltage Analysis**

Bipolar voltage was defined as the peak-peak electrogram voltage. Global right atrial and regional analysis including the crista terminalis were performed. The tricuspid annulus and superior and inferior vena cava were excluded. The area of regional relative low voltage zone (rLVZ) using a scale of 0.5-1.3mV was measured across the crista terminalis (332).

**Conduction Velocity Analysis**

Conduction velocity was analysed in MATLAB as previously described using the polynomial algorithm (306, 393). In brief, this method assigns a fitting ‘window’ per region with a minimum of 20 points required. Each region is assigned subsets of Cartesian coordinates in space and activation time. These are fitted to a smooth polynomial surface in three-dimensional space, using a standard least squares algorithm, which provides robustness against outliers. The gradient is then calculated from the fit and used to calculate velocity components, with final velocities at each point calculated from the weighted average of velocity components from every fit including that point (394). Both the mean RA and regional conduction velocity across
the crista terminalis were calculated. Conduction heterogeneity was determined by calculating the CoV of conduction velocity. Propagation maps were analysed during sinus rhythm for the presence and location of functional lines of conduction slowing and block.

**Analysis of Complex Signals**

Complex signals were defined as electrograms with ≥3 deflections >50ms duration (fractionated potentials [FP]) or 2 separate deflections separate by an isoelectric interval (double potentials [DP]). These signals were manually annotated and tagged both globally and regionally across the crista terminalis. The percentage of complex signals is expressed as a proportion of the number of complex signals divided by the total number of points.

**Assessment of Effective Refractory Period & Sinus Node Function**

Effective refractory period (ERP) at the proximal and distal coronary sinus was performed. Briefly, ERPs were measured using a pacing drive train of 8 beats followed by a single extrastimulus commencing at a coupling interval of 150ms and incrementing by 10ms until local capture was demonstrated. The local ERP was defined as the longest extrastimulus that failed to capture the pacing site. ERPs were measured at 2 drive train cycle lengths (600 and 400ms) and repeated at least twice at each site to ensure consistency. Sinus node recovery time (SNRT), evaluated by 30s burst pacing trains at every 50ms from 600 to 400ms, was determined as the longest time from the stimulus artefact to the earliest atrial activity. The corrected SNRT was determined by correcting for the underlying sinus cycle length.
Statistical Analysis

Data analysis was performed using SPSS software (Version 23, IBM, Armonk, New York). Normality of all quantitative variables was assessed using the Shapiro-Wilk test and normalising transformations were performed as necessary. Data are expressed as mean ± standard deviation (SD) unless otherwise stated. Two-group comparisons were made using Student’s t test for continuous variables, or the chi-squared test for categorical variables. Three-group comparisons were made using the one-way ANOVA. Correlation was determined by Pearson’s correlation coefficient.

General linear model repeated measures analysis was used for comparison between multiple regional measures within each patient (ie. CV, voltage). Generalised estimating equations were used for nested categorical variables (ie. Percentage fractionated points). To investigate the variation of AF type in measured parameters, rhythm (sinus rhythm, 600ms pacing and 300ms pacing) and group (control vs AF group) were modelled as fixed effects with a rhythm x group interaction term.

RESULTS

Baseline Characteristics

Fifty patients underwent 3D electroanatomic mapping during sinus rhythm and 600ms and 300ms pacing from the proximal coronary sinus. Baseline demographic data was evenly matched for age and gender with no significant differences between groups (Table 1). However, patients with persistent AF had significantly larger left atria (p=0.004) and right atria (p=0.015). The mean age was 59±9 years, 24 patients (48%) were male and mean BMI of 30.6 ± 5.1 kg/m².
Sinus node function and atrial refractoriness

There was a significant stepwise prolongation in CSNRT from control to paroxysmal to persistent AF groups for each cycle length (table 2): at 600ms (281 ± 86 vs 405 ± 117 vs 496 ± 147ms, p<0.001); at 500ms (299 ± 62 vs 414 ± 138 vs 517 ± 165ms, p<0.001); at 400ms (284 ± 82 vs 425 ± 156 vs 540 ± 151ms, p<0.001). Baseline sinus rhythm cycle length was also progressively longer in each group (729 ± 115 vs 767 ± 92 vs 946 ± 185ms, p<0.001). However, ERPs at both proximal and distal coronary sinus was not significantly different across the groups.

High Density Electroanatomical mapping

The mean number of points collected prior to manual annotation and filtering was 2,329 ± 1312. There were no significant differences in global and regional points collected between groups.

Sinus node pacemaker location

Sinus node complex characteristics are summarised in table 3. The earliest sinus node activation site (EAS) was located progressively more caudal relative to the SVC-RA junction in control to paroxysmal to persistent AF (5.9 ± 7.2 vs 9.9 ± 2.6 vs 15.1 ± 5.5mm, p<0.001). These caudal shifts were also seen in the location of earliest sinus break-out (SBO) (10.1 ± 8.8 vs 16.5 ± 4.9 vs 24.1 ± 6.3, p<0.001).

Sinus node complex activation

Across the three groups, persistent AF patients displayed a more focal sinus node origin and extent of remodelling. Comparing the control group with PAF and PeAF respectively, there was a progressive increase in the proportion of patients with
unicentric sinus node activity (7 [28%] vs 4 [36%] vs 12 [86%], p=0.002) and progressive decrease in the number of pacemaker sites (4.1 ± 2.2 vs 3.3 ± 1.8 vs 1.6 ± 1.5, p=0.002). This was associated with a larger area of sinus node activation in the control group compared with both AF groups (6.2 ± 2.6 vs 4.6 ± 1.7 vs 2.1 ± 1.0 cm², p<0.001) (Figure 1A). Coupled with this, sinus node voltage was progressively lower across groups (2.19 ± 0.51 vs 1.75 ± 0.34 vs 1.31 ± 0.56mV, p<0.001) (Figure 1B) and EAS-SBO conduction times were slower (9.6 ± 3.6 vs 13.8 ± 5.3 vs 18.9 ± 5.9 ms, p<0.001). The number of SBO sites also decreased from control to PAF and PeAF patients (3.3 ± 1.1 vs 3.3 ± 1.8 vs 1.6 ± 1.5, p=0.002) (Table 3). The earliest SBO site relative to the EAS was most frequently inferior (56%) and anterior away from the crista terminalis (32%). However, there were no significant differences in the location of the earliest SBO site across the 3 groups (p=0.639).

A representative example of the progressive differences in sinus node remodelling between groups is shown in Figure 2.

**Global atrial substrate**

Details on global right atrial parameters are shown in table 4. Overall, persistent AF patients demonstrated significantly greater substrate compared with paroxysmal AF and control patients. On global analysis of the 3 groups, this included progressively reduced voltage (p<0.004), conduction velocity (p=0.004) and greater complex signals (p=0.038). There were significant positive correlations in pooled control and AF patients between sinus node voltage and global RA voltage (R=0.597, p<0.001) (figure 3A), global RA conduction velocity (R=0.513, p<0.001) (figure 3B) and proportion of complex signals (R=-0.417, p=0.002) (figure 3C).
Similarly, the persistent AF group had significantly increased regions of atrial substrate at the crista terminalis (table 4). In keeping with global substrate changes, there were stepwise decreases in voltage (1.85 ± 0.58 vs 1.68 ± 4.0 vs 1.21 ± 0.59 mV, p=0.005), conduction velocity (34.5 ± 15.2 vs 31.1 ± 7.2 vs 23.0 ± 7.1 cm/s, p=0.021) and an increase in complex signals (5.3 ± 2.9 vs 8.7 ± 5.1 vs 12.4 ± 4.5%, p<0.001). There were also significant incremental increases in conduction heterogeneity index at the crista terminalis across the 3 groups (0.30 ± 0.10 vs 0.37 ± 0.12 vs 0.48 ± 0.14, p<0.001).

A vertical functional line of conduction slowing or block was observed in 9 (36%) control patients with 3 (12%) located posteriorly in the sinus venosus and 6 (24%) in the lateral RA (table 4). Compared to other two groups, there were progressively more patients with functional line of block in the persistent AF group (paroxysmal AF: 6 [55%] vs persistent AF: 11 [79%], p=0.038 overall). This line of block was also located more posteriorly in patients with persistent AF (paroxysmal AF: 2 [18%] vs persistent AF: 9 [64%], p=0.038 overall).

**Dynamic atrial substrate change at the crista terminalis**

Alteration in pacing rate and direction (sinus rhythm, coronary sinus pacing at 600ms and 300ms) was associated with significant substrate change at the crista terminalis in the control group and combined paroxysmal and persistent AF patients. The extent of dynamic rate and direction-dependent change was significantly greater in AF patients compared with control patients for voltage decrease (p<0.001), conduction velocity slowing (p<0.001) and increased complex signals (p<0.001) (Figure 4). This finding is
consistent with a greater degree of latent substrate in patients with AF compared with controls.

**DISCUSSION**

The current study performed high density, high resolution electroanatomic mapping of the sinus pacemaker complex and the right atrium in patients with paroxysmal and persistent AF patients and compared these to maps obtained from a control population. The key findings of this study were:

1) Significant sinus node remodelling is present in AF patients when compared to age-matched controls. This is characterised by a reduction in sinus node region voltage, slower preferential pathway conduction times, caudal shifts in the earliest activation site and reduced atrial pacemaker activation and sinus break-out multicentricity. These differences were significantly more marked in patients with persistent compared with paroxysmal AF.

2) Greater progressive global and regional atrial remodelling in patients with AF compared to controls. High density, high resolution mapping demonstrated regions of functional conduction slowing and block which were most prevalent in persistent AF patients. Anatomically, these regions were most frequently observed in the posterior RA along an SVC-IVC line or adjacent to this region at the crista terminalis.

**Sinus node physiology**

Histologically, the sinus node consists of specialised tissue spanning a mean length of 13.5mm at the upper pole of the sulcus terminalis at the junction of the right atrium and superior vena cava (135). However, Boineau and colleagues demonstrated that the
functional sinus pacemaker complex is distributed over a three-four fold larger area than seen histologically (440). This observation of multiple sinus impulse breakthrough sites is thought to relate to the fragmented caudal portion of the node and radiating sinoatrial interdigitating connections which merge with the surrounding atrial myocardium. Pacemaker cells are surrounded by a dense fibrous matrix of interstitial collagen and connective tissue which increases in content with age (444). Cardiac ion-channels including calcium ($I_{\text{CAL}}$), sodium, potassium rectifier ($I_{Kf}$ and $I_{Ks}$) and hyperpolarization-activated cyclic-nucleotidode (HCN) channel mediated ‘funny’ ($I_{f}$) currents are involved in sinus node automaticity. This activity is subject to modulation by numerous intrinsic and extrinsic factors including autonomic regulation (445), anti-arrhythmic drugs (446) and sinus node remodelling (443) with resultant shifts in the site of leading pacemaker activation.

Recent studies utilising integrated intramural optical mapping combined with 3D histologic reconstruction in isolated preparations have demonstrated the 3-dimensional nature of the SAN; multiple pacemaker sites and multiple preferential sino-atrial conduction pathways exist (447,448). With suppression of one pacemaker or conduction pathway, activation of a subsidiary pacemaker with conduction over an alternate pathway maintains SAN function. This redundancy of pacemakers and conduction pathways results in a robust system resistant to failure. In the current study we demonstrated that in patients with atrial fibrillation and more markedly in persistent AF, this sinus node reserve is significantly attenuated. Not only was the multicentric activation pattern reduced; but sino-atrial break-out and conduction were also impaired.
Atrial fibrillation and sinus node remodelling

AF and SND often co-exist and manifest clinically as tachycardia-bradycardia syndrome (449). Evidence suggests that both impaired sinus node function may lead to AF and that AF may result in sinus node dysfunction. Elvan et al demonstrated impaired sinus node function in a rapid atrial pacing canine model of chronic AF (137). Similar findings were reported in humans following rapid pacing (138) and post cardioversion of chronic AF (139). Electroanatomic mapping studies have demonstrated sinus node remodelling under ‘atrial stretch’ conditions including ventricular pacing (296), atrial septal defect (319), atrial flutter (141) and heart failure (443). These studies showed consistent findings of a caudal shift in the earliest site of activation, decreased sinus node multicentricity and slower conduction. To date, there is limited data evaluating the nature of sinus node remodelling in different AF populations. In the current study, we created high-density (>2000 points/map) multipolar maps, using closely spaced 1mm ring electrodes to provide high resolution. The study demonstrated the presence of AF phenotype-dependent sinus node remodelling characterised by fewer sinus activation sites, caudal shift of earliest activity, smaller sinus node activation area, lower sinus node voltage and longer conduction pathway times. All findings were more marked in persistent AF patients compared to paroxysmal AF. The capacity of the sinus node for reverse remodelling has been observed following restoration of sinus rhythm following termination of atrial flutter (140) and AF ablation (450). Thus, strategies to reduce the progression to persistent AF at an early stage may potentially help to minimise the impact of remodelling on sinus node function.
Role of electroanatomic atrial substrate

An important contributor common to the pathogenesis of SND and atrial substrate is the development of fibrosis (159). Stimulated by aforementioned ‘atrial stretch’ conditions, arrhythmias and structural heart disease, fibroblasts deposit collagen in response to signalling proteins such as angiotensin II and tumour growth factor β. This fibrotic process disrupts normal atrial myocardial architecture and side-to-side cell coupling resulting in heterogeneous anisotropic conduction facilitating re-entry and arrhythmogenesis. Increased fibrofatty replacement surrounding the sinus node complex may lead to sinoatrial block or impaired impulse generation and age-related fibrosis has been found to correlate with lower heart rates (451). Beyond fibrosis, AF-related interruption in connexin expression, structural cellular changes, and atrial myocyte apoptosis are potential mechanisms implicated in the remodelling process (154). Sanders and colleagues evaluated patients with symptomatic SND and found evidence of a diffuse atrial myopathy including functional conduction delay across the crista terminalis in the absence of incident AF (324). A more recent study by Chang et al in patients with AF and SND (defined by CSNRT greater than 550ms) demonstrated greater regional atrial remodelling in the location of the sinus node with lower voltage and longer RA and crista terminalis activation times compared with those without SND (452). Distinct from this point-by-point mapping study which only investigated patients with paroxysmal AF, our study also included patients with persistent AF as well as age-matched controls at high-density. We found that sinus node remodelling occurred together with remodelling at the crista terminalis and the global RA suggestive of a more diffuse process extending beyond the sinus node in both patients with and without AF. Interestingly, a third of age-matched controls exhibited a line of functional block which may serve as a susceptible anatomically-determined site to remodelling and
predisposition to arrhythmia as this was observed significantly more frequently in paroxysmal and persistent AF patients. Additionally, we have previously shown dynamic left atrial substrate changes in response to different pacing rate and direction of activation in AF patients (453). Sinus node remodelling correlated with more widespread atrial remodelling and the presence of anatomically determined regions of functional conduction block. This vulnerable substrate may form part of a primary underlying myopathic process, which contributes both to development of sinus dysfunction and to persistence of atrial fibrillation.

Limitations
Cardioversion performed in persistent AF patients to restore sinus rhythm may confound interpretation of results. Thus, it is difficult to quantify whether observed sinus node and atrial differences are due to underlying atrial substrate in AF or potentially transient electrical remodelling in the context of the recent AF episodes. However, less than half of the persistent AF group presented to the lab in AF and prior studies have demonstrated that atrial substrate exists even in the absence of recent AF (133).

CONCLUSION
This study demonstrates that patients with AF have significant sinus node remodelling which is more pronounce in persistent than in paroxysmal AF. This is characterised by a reduction in sinus node region voltage, slower preferential pathway conduction times, caudal and posterior shifts in the earliest activation site and reduced atrial pacemaker activation multicentricity. These changes occur in the context of more wide-spread atrial remodelling both of which may contribute to atrial arrhythmogenesis.
### Table 1. Baseline Characteristics.

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<td>LA Size (cm)</td>
<td>3.7 ± 0.3</td>
<td>3.9 ± 0.4</td>
<td>4.2 ± 0.5</td>
<td>0.782</td>
</tr>
<tr>
<td>LA Area (cm²)</td>
<td>18.2 ± 2.4</td>
<td>20.4 ± 4.3</td>
<td>23.8 ± 3.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Abbreviations: AF – Atrial Fibrillation, CHA₂DS₂-VASc – Congestive Heart Failure, Hypertension, Age, Diabetes, Stroke, Vascular Disease, Sex, LA – Left Atrial, LV – Left Ventricular, TIA – Transient Ischaemic Attack</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Differences in sinus node function and atrial refractoriness between control and AF groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=25)</th>
<th>Paroxysmal AF Group (n=11)</th>
<th>Persistent AF Group (n=14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSNRT 600ms drive (ms)</td>
<td>281 ± 86</td>
<td>405 ± 117</td>
<td>496 ± 147</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSNRT 500ms drive (ms)</td>
<td>299 ± 62</td>
<td>414 ± 138</td>
<td>517 ± 165</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSNRT 400ms drive (ms)</td>
<td>284 ± 82</td>
<td>425 ± 156</td>
<td>540 ± 151</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline cycle length (ms)</td>
<td>729 ± 115</td>
<td>767 ± 92</td>
<td>946 ± 185</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Effective refractory periods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSp 600ms drive (ms)</td>
<td>253 ± 46</td>
<td>244 ± 39</td>
<td>222 ± 37</td>
<td>0.282</td>
</tr>
<tr>
<td>CSp 400ms drive (ms)</td>
<td>243 ± 49</td>
<td>238 ± 27</td>
<td>217 ± 35</td>
<td>0.472</td>
</tr>
<tr>
<td>CSd 600ms drive (ms)</td>
<td>255 ± 44</td>
<td>232 ± 25</td>
<td>217 ± 32</td>
<td>0.099</td>
</tr>
<tr>
<td>CSd 400ms drive (ms)</td>
<td>246 ± 49</td>
<td>234 ± 29</td>
<td>213 ± 31</td>
<td>0.246</td>
</tr>
</tbody>
</table>

Abbreviations: AF – atrial fibrillation, CSNRT – corrected sinus node recovery time, CSp – proximal coronary sinus, CSd – distal coronary sinus
Table 3. Sinus node function across control and AF groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=25)</th>
<th>Paroxysmal AF Group (n=11)</th>
<th>Persistent AF Group (n=14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus node area points</td>
<td>79 ± 50</td>
<td>73 ± 54</td>
<td>84 ± 59</td>
<td>0.863</td>
</tr>
<tr>
<td>Type of sinus node activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multicentric</td>
<td>18 (72)</td>
<td>7 (64)</td>
<td>2 (14)</td>
<td>0.002</td>
</tr>
<tr>
<td>Unicentric</td>
<td>7 (28)</td>
<td>4 (36)</td>
<td>12 (86)</td>
<td>0.002</td>
</tr>
<tr>
<td>Number of pacemaker sites</td>
<td>4.1 ± 2.2</td>
<td>3.3 ± 1.8</td>
<td>1.6 ± 1.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Sinus node voltage (mV)</td>
<td>2.19 ± 0.51</td>
<td>1.75 ± 0.34</td>
<td>1.31 ± 0.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Area of sinus node activation</td>
<td>6.2 ± 2.6</td>
<td>4.6 ± 1.7</td>
<td>2.1 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earliest activation site level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>18 (72)</td>
<td>3 (27)</td>
<td>3 (21)</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>4 (16)</td>
<td>6 (55)</td>
<td>8 (57)</td>
<td>0.015</td>
</tr>
<tr>
<td>Low</td>
<td>3 (12)</td>
<td>2 (18)</td>
<td>3 (21)</td>
<td></td>
</tr>
<tr>
<td>Earliest activation site-SVC</td>
<td>5.9 ± 7.2</td>
<td>9.9 ± 2.6</td>
<td>15.1 ± 5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>distance (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of SBO sites</td>
<td>3.3 ± 1.1</td>
<td>2.3 ± 1.0</td>
<td>1.7 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Earliest SBO site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior</td>
<td>14 (56)</td>
<td>6 (55)</td>
<td>8 (57)</td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>9 (36)</td>
<td>4 (36)</td>
<td>3 (21)</td>
<td>0.639</td>
</tr>
<tr>
<td>Superior</td>
<td>2 (8)</td>
<td>1 (9)</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (14)</td>
<td></td>
</tr>
<tr>
<td>SBO-SVC distance (mm)</td>
<td>10.1 ± 8.8</td>
<td>16.5 ± 4.9</td>
<td>24.1 ± 6.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Earliest activation to SBO time (ms)</td>
<td>9.6 ± 3.6</td>
<td>13.8 ± 5.3</td>
<td>18.9 ± 5.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Abbreviations:* AF – atrial fibrillation, CSNRT – corrected sinus node recovery time, CSp – proximal coronary sinus, CSd – distal coronary sinus, SBO – sinus break-out
Table 4. Conduction and electroanatomic parameters of the crista terminalis and global RA between each group during sinus rhythm.

<table>
<thead>
<tr>
<th>Crista Terminalis</th>
<th>Control Group (n=25)</th>
<th>Paroxysmal AF Group (n=11)</th>
<th>Persistent AF Group (n=14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional line of block</td>
<td>9 (36)</td>
<td>6 (55)</td>
<td>11 (79)</td>
<td>0.038</td>
</tr>
<tr>
<td>Site of line of block</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>3 (12)</td>
<td>2 (18)</td>
<td>9 (64)</td>
<td>0.008</td>
</tr>
<tr>
<td>Lateral</td>
<td>6 (24)</td>
<td>4 (36)</td>
<td>2 (14)</td>
<td>0.008</td>
</tr>
<tr>
<td>Number of points</td>
<td>372 ± 92</td>
<td>364 ± 114</td>
<td>405 ± 273</td>
<td>0.244</td>
</tr>
<tr>
<td>CT voltage (mv)</td>
<td>1.85 ± 0.58</td>
<td>1.68 ± 4.0</td>
<td>1.21 ± 0.59</td>
<td>0.005</td>
</tr>
<tr>
<td>CT conduction velocity (cm/s)</td>
<td>34.5 ± 15.2</td>
<td>31.1 ± 7.2</td>
<td>23.0 ± 7.1</td>
<td>0.021</td>
</tr>
<tr>
<td>CT conduction velocity heterogeneity index</td>
<td>0.30 ± 0.10</td>
<td>0.37 ± 0.12</td>
<td>0.48 ± 0.14</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>CT complex signals (FP/DP)</td>
<td>5.3 ± 2.9</td>
<td>8.7 ± 5.1</td>
<td>12.4 ± 4.5</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Global number of points</td>
<td>2432 ± 843</td>
<td>2106 ± 1252</td>
<td>2358 ± 1184</td>
<td>0.671</td>
</tr>
<tr>
<td>Global RA voltage (mV)</td>
<td>2.73 ± 0.68</td>
<td>2.09 ± 0.32</td>
<td>1.54 ± 0.65</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Global RA conduction velocity (cm/s)</td>
<td>40.2 ± 14.7</td>
<td>31.4 ± 12.8</td>
<td>23.7 ± 12.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Global RA complex signals (FP/DP) (%)</td>
<td>5.7 ± 4.9</td>
<td>8.0 ± 3.3</td>
<td>10.7 ± 7.0</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Abbreviations: CT – crista terminalis, DP – double potentials, FP – fractionated potentials, RA – right atrium
FIGURES

Figure 1. Differences in sinus node parameters.

**Figure 1.** Stepwise decreases in sinus node activation area (A) and sinus node voltage (B) across control to paroxysmal AF to persistent AF groups. AF – atrial fibrillation.
Figure 2. Right lateral (RL) views of right atrial maps demonstrate progressive sinus node remodelling. Red areas indicate earliest activation regions (<10ms) on activation maps (top) and low voltage zones (LVZ, <0.5mV) on voltage maps (bottom). Control maps are characterised by diffuse sinus node activation with multiple early sites (A) with healthy tissue in pink (B). Paroxysmal AF (PAF) maps show a caudal shift and fewer early activation sites (C) with a larger region of low voltage at the sinus node and crista terminalis (D) and a larger number of complex signals (light pink and green dots) compared to controls. Persistent AF maps show advanced remodelling at the sinus node and crista terminalis with a further caudal shift and focal site of early activation (E) corresponding with a significantly larger LVZ (F) and greater complex signals compared to PAF and control examples.
Figure 3. Correlations between sinus node voltage and global right atrial (RA) voltage (A), RA conduction velocity (B) and RA complex signals (C).
Figure 4. Electrophysiologic parameters at the crista terminalis.

**Figure 4.** Impact of dynamic rate-dependent conduction on the crista terminalis between sinus rhythm and proximal coronary sinus (CS) pacing at 600ms and 300ms cycle lengths.
Despite a recent surge in management options for atrial fibrillation (AF), there continues to be a steady but appreciable attrition in the success rates of the maintenance of sinus rhythm. This is particularly apparent in the treatment of persistent AF, which remains elusive. Thus, there have been considerable efforts in the search for further therapeutic strategies. Contemporary data suggest that lifestyle measures such as risk factor control, weight loss, alcohol abstinence and control of sleep apnoea should form the ‘fourth pillar’ in integrated AF care. Additionally, adjunctive ablation strategies may afford incremental benefits in patients with persistent AF. However, patient specific-factors such as genetics and the nature of substrate within an individual are incompletely understood. Recently, the advent of ultra high-density electroanatomic multipolar mapping technologies that collect thousands of points per map facilitate a far greater resolution and understanding of the arrhythmia mechanism and electrophysiologic substrate than ever before. This thesis explores novel personalised mechanisms which influence atrial substrate including genetics, pacing strategies, gender and sinus node remodelling.

Chapter 2 evaluates the first electroanatomic association between the common single nucleotide variant (SNV) rs2200733 at the chromosome 4q25 and AF. We observe that SNV carriers possess greater conduction heterogeneity, proportion of the left atrium (LA) exhibiting conduction slowing or block and complex fractionated signals. These changes are global and regional across the posterior and lateral walls. Accompanying these differences in atrial substrate are poorer outcomes in carriers at long-term follow-
up after AF ablation. These findings present a mechanism by which the 4q25 locus may predispose to the maintenance of AF and higher recurrence rates. The genetic susceptibility to regional substrate may also identify a subset of patients who may benefit from adjunctive ablation strategies targeting this abnormal substrate. However randomised clinical studies of the utility of genetic variant-guided posterior wall isolation and targeted ablation strategies are required. Further work to identify the molecular mechanisms underpinning the electrical changes mediated by the 4q25 pathway including downstream transcription factors and enhancers will significantly improve our understanding of this important and common variant in AF pathogenesis.

Chapters 3-4 present the impact of rate and direction on atrial substrate in patients with AF. In chapter 3, we evaluate changes in high-density electroanatomic maps at two paced cycle-lengths in 73 patients with paroxysmal and persistent AF. Rapid pacing resulted in a uniform reduction in voltage, conduction slowing and increased complex fractionated signals across all LA segments. This dynamic or ‘latent’ substrate was significantly associated with long duration paroxysmal AF and short duration persistent AF. This suggests that there may be a critical period where substrate may transition to a more ‘burnt-out’ fixed phenotype seen in patients with long-standing persistent AF. Chapter 4 extends these findings to assess direction-dependent conduction pacing from the pulmonary veins (PV), the predominant site of ectopic triggers initiating AF. In contrast to rate-dependency, altering the direction of wavefront activation produced a highly regional and dramatic increase in the area of relative low voltage zones across the posterior and inferior LA walls. A posterior line of block demarcating a functional region of conduction slowing or abrupt change in fibre orientation in response to PV pacing suggests the potential importance of directionality in the arrhythmia mechanism.
and evaluation of substrate. These findings identify inherent limitations in the use of static electroanatomic maps as a clinical tool for substrate-based ablation approaches. Moreover, the extent to which regions of latent rate or direction-dependent substrate are critical to a substrate-based ablation approach remains unknown. Further studies are required to characterise the clinical significance of the dynamic substrate particularly in relation to fibrosis, MRI correlation and ablation outcomes.

The importance of sex-based differences in cardiovascular disease is well-established, however significant gaps in knowledge remain to explain these differences, particularly in relation to AF. Chapter 5 focusses on electrophysiologic sex-based differences in a large group of both patients with and without AF and long-term outcomes. We find that women have several significant differences compared with men including lower voltage, conduction slowing and a higher proportion of complex signals and lower single and multi-procedure arrhythmia-free survival following ablation. The electrophysiologic differences are consistent across both the AF and control groups and may in part explain the increased susceptibility of women to AF-related complications and the observed higher recurrence rates following ablation. Further study is necessary to elucidate the mechanisms of greater atrial substrate in women potentially contributing to or interacting with non-pulmonary vein triggers which have also previously been observed to occur at a higher burden in women.

Sinus node dysfunction (SND) is an important entity that often occurs in parallel to AF-related remodelling and co-exists in patients with clinical AF. Remodelling across the crista terminalis (CT) has also been implicated in SND even prior to the onset of AF. Chapter 6 utilises high-density mapping to define the sinus node and regional atrial
substrate in patients with AF compared to age-matched controls without AF. A key finding of this study is that there is an AF phenotype-dependent relationship with sinus node and atrial remodelling. More pronounced differences in persistent AF patients include a caudal shift in sinus node earliest activation site (EAS) and sinus break-out (SBO), lower number of EAS, longer EAS-SBO conduction times, lower sinus voltage and longer sinus node recovery times. We observe evidence of a diffuse atrial myopathy with greater ‘latent’ substrate at the CT in AF patients compared to controls. This study provides new insights into old observations in sinus node remodelling varying according to AF phenotype. The impact of reverse remodelling in specific AF patients with greater evidence of sinus node dysfunction or presence of ‘latent’ CT substrate requires further study.

The management of AF has greatly evolved over the past few decades owing to rapidly progressive technologies and procedural strategies to help restore sinus rhythm. However, there remains no durable ‘cure’ for AF. This thesis presents novel personalised mechanisms which influence atrial substrate and contribute to the development and perpetuation of AF. Beyond the paradigm of lifestyle-directed management, the rising utility of genetics to help guide treatment or form the basis of novel pharmacological agents to block downstream proteins is an exciting prospect. Genetic sequencing techniques are progressively faster and more affordable, particularly in relation to whole genome sequencing. The use of ‘big data’ in genetic analysis and steadily improving computational power to facilitate algorithms to explore the genetic landscape and identify important mechanisms holds significant promise.
Randomised trials of adjunctive ablation techniques such as posterior wall isolation in CAPLA and STAR-AF III are under way and are expected to provide important insights into the optimal treatment of persistent AF. Such targeted strategies may be further bolstered by novel personalised determinants of atrial substrate including dynamic substrate, 4q25 variant genotyping or gender. Based on the findings presented in this thesis, further research into patient-tailored ablation strategies in persistent AF guided by these novel determinants to improve long-term outcomes and minimise extensive ablation are warranted. This may help to evaluate whether female, genotype positive patients that exhibit marked dynamic substrate in response to differential pacing may potentially benefit from PVI with adjunctive substrate modification; and conversely whether male, genotype negative patients without inducible latent substrate may achieve comparable post-ablation success rates with PVI alone. A prospective clinical trial to assess the efficacy of a ‘substrate-score’ is required to test these hypotheses. Although AF is a complex and challenging condition, this thesis highlights the importance of detailed understanding of the mechanisms underlying atrial architecture, conduction and substrate in AF.
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