# Study Protocol and Statistical Analysis Plan

# Elucidation of the Influence of Sleep Apnea on Risk of Atrial Fibrillation

# NCT02576587

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**STUDY PROTOCOL**
**Elucidation of the Influence of Sleep Apnea on Risk of Atrial Fibrillation**

## 1. ABSTRACT

The NHLBI strategic plan has highlighted a need to identify strategies to halt the atrial fibrillation (AF) epidemic and reduce its related morbidity and financial burden. The growing rate of AF, with projections of afflicting up to 16 million individuals by the year 2050, is not fully explained by known risk factors, underscoring the need to identify novel triggers. Sleep-disordered breathing (SDB) is common in patients with cardiovascular disease and its attendant hypoxemia and autonomic dysfunction create a milieu that is likely to enhance AF propensity. Thus, SDB may represent a novel target for AF prevention and treatment strategies. Although our prior cross-sectional work has shown a 2-4 fold higher odds of AF related to SDB, these and other reports have *not included* cardiac structural data or autonomic/biochemical measures, and have addressed only arrhythmic events occurring during an overnight sleep study. In this proposal, we will examine paroxysmal AF (PAF), an early stage risk factor for persistent AF, and relevant to this proposal because it occurs prior to extensive cardiac electrical remodeling/fibrosis. PAF provides an ideal setting to investigate the immediate influences of SDB and examine temporal patterns given its intermittent nature. We will perform a case control study with each group matched on important confounders such as age (±5 years), gender, race (white versus non-white), and obesity (+/-2 kg/$m^2$) and characterized using detailed collection of overnight sleep study data, echocardiographic measures, biomarkers and 7-21 day continuous ECG monitoring. Cases (PAF, n=150) will be recruited from our local AF clinics, and controls (n=150) from medicine and cardiology clinics. The following existing gaps will be addressed: clarify the extent that respiratory event frequency, apnea subtype (obstructive versus central), and hypoxia are associated with PAF independent of cardiac structural abnormalities (i.e. systolic dysfunction and left atrial enlargement); explore the extent to which cardiac morphology, pathways of inflammation/oxidative stress and autonomic dysfunction mediate the SDB-PAF relationship and identify whether temporal patterns of AF paroxysms differ in patients with SDB.
Data generated from this research is key for developing new strategies to reduce AF-related morbidity including stroke, heart failure and also death. Expected results will be of high impact given ability to identify and inform future screening and treatment approaches for management and/or prevention of AF and identify key outcomes for clinical trials.

The primary aims of the study are to:

SA 1. Quantify the extent that SDB and its subtypes are associated with PAF. SDB will be assessed by the standard metric: the Apnea Hypopnea Index (AHI) and also Central Apnea Index, Periodic Breathing, Obstructive Apnea Hypopnea Index and % sleep time <90%SaO2. For each, exposure response relationships and thresholds that confer increased risk for PAF will be defined.

SA 2. Examine PAF burden (defined as frequency of paroxysms over a 24 hour period and average duration of paroxysms) via 7-21 day continuous ECG monitoring relative to SDB in those with PAF.

SA 3. Examine the effects of SDB (AHI≥15) treatment for 3 months on PAF burden and also measures of autonomic function, cardiac morphology and biochemical measures in those with SDB and PAF.

## 2. OVERVIEW

The *Elucidation of the Influence of Sleep Apnea on Risk of Atrial Fibrillation* study Is employs a case control study design to investigate the extent to which there is an independent relationship of SDB and PAF. Cases will be defined as clinically identified patients with PAF and controls as those without AF. In order to rigorously address important biologic confounding influences, the cases and controls will be individually matched based upon age (±5 years), gender, race (white versus non-white), and obesity (+/-2 $kg/m^2$). Those participants with both PAF and SDB (Apnea Hypopnea Index, AHI≥15) will be asked to return for a follow up exam after 3 months of SDB treatment in the Case Dahms Clinical Research Unit (DCRU) and the Cleveland Clinic CRU for collection of the same measures collected at the baseline exam to observe for any significant changes with the purpose of collecting effect size data to inform future clinical trials.

In addition, ten participants with SDB who are CPAP treatment naive with paroxysmal AF and 10 control participants will be recruited for a sub- study. The 10 participants of the CPAP treatment group undergo MRI scanning before and after 12 weeks of CPAP treatment in a 7T imaging protocol designed to study resting state networks at high spatial resolution. Cognitive testing and questionnaires will be performed before and after treatment. The strength of connectivity between elements of the DMN will be compared before and after treatment. Controls will only be given the cognitive battery and not undergo the MRI brain scan.

The total duration of the study is 4 years. The duration for any individual participant is up to from one to 13 weeks months, including a 3-month treatment period for those with moderate to severe SDB, i.e. AHI≥15.

**Study Time Line**

| **Study Year** | **1** | | | | **2** | | | | **3** | | | | **4** | | | | **5** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calendar Year** | **2011** | | **2012** | | | | **2013** | | | | **2014** | | | | **2015** | | | | **2016** | |
| **Quarter** | **3** | **4** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** | **1** | **2** |
| Hiring, Training, Refining Protocols | | | | | | | | | | | | | | | | | | | | |
| Study Recruitment | | | | | | | | | | | | | | | | | | | | |
| Collection of ECHO Data | | | | | | | | | | | | | | | | | | | | |
| Collection of Continuous ECG Data | | | | | | | | | | | | | | | | | | | | |
| Collection of Biochemical Data | | | | | | | | | | | | | | | | | | | | |
| Data Analysis | | | | | | | | | | | | | | | | | | | | |

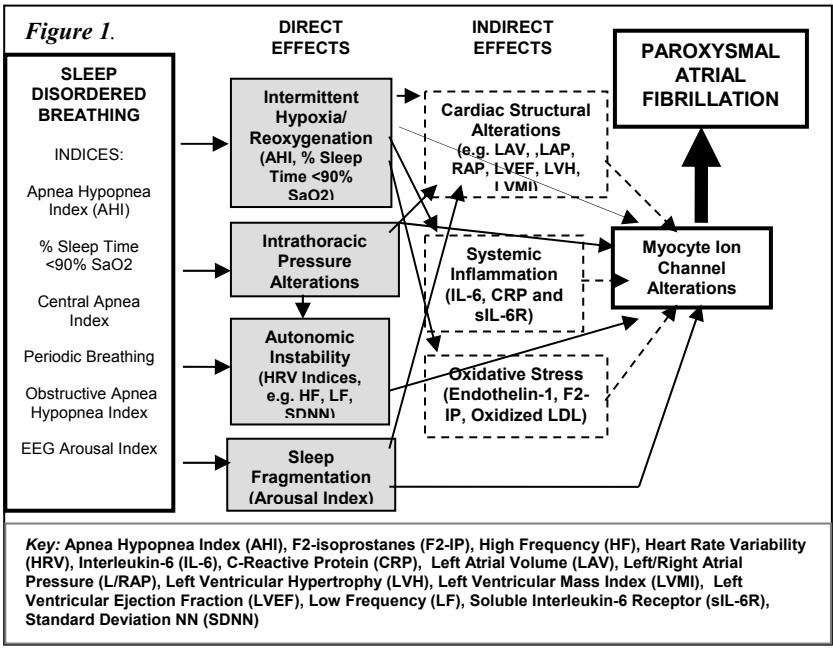
## 3. BACKGROUND AND RATIONALE

**3. A. 1. Atrial Fibrillation: Burgeoning Epidemic and Need for New Prevention and Treatment Approaches.** AF is the most common sustained arrhythmia [3], affecting ~2.2 million individuals in the US [4], and potentially up to 16 million Americans by the year 2050 [5]. AF is an independent predictor of mortality [6] and increased stroke, implicated in ~75,000 strokes per year [7]. Annual costs for AF treatment have been estimated at $6.7 billion, including $2.9 billion for hospitalizations [8]. Therefore, improved AF disease management strategies are needed to reduce hospitalizations and costs [9]. Furthermore, AF treatments such as ablation and anti-arrhythmic medications are fraught with inconsistent results and pro-arrhythmic side effects [10]. There is a critical need to identify alternative AF treatment and prevention strategies such as those targeting SDB.

**3. A. 2. Paroxysmal Atrial Fibrillation: An Ideal Condition to Examine Electrophysiologic Triggers and Diurnal Patterns.** PAF is often asymptomatic and occurs early in the continuum of persistent AF development [11]. Half of those with PAF have no obvious cause [12] which may be due to unrecognized triggers such as SDB. The longer PAF persists, the more difficult it is to restore sinus rhythm and prevent recurrence which emphasizes the need to expeditiously identify and address potential contributing factors such as SDB at this stage of disease [13]. Patients initially presenting with PAF often progress to longer, non–self-terminating bouts [13]. Importantly, PAF often begins before electrical and extensive structural cardiac remodeling has occurred and provides an ideal substrate for examining acute physiologic triggers such as those that occur in SDB. In individuals with symptomatic PAF, there is a 12:1 ratio of asymptomatic versus symptomatic episodes [14], therefore continuous ECG monitoring is helpful to provide more detailed, objective data on paroxysms as described in the current proposal. Furthermore, unlike continuous AF, which does not allow reliable detection of autonomic dysregulation via heart rate variability (HRV) indices from ECG data; in PAF, HRV analyses for autonomic function assessment can be derived from the normal sinus rhythm (NSR) period data.

**3. A. 3. Atrial Fibrillation: Unexplained Risk.** Given the excess burden of AF and also high prevalence of SDB (~10-15%) [15], there is a need to better understand AF risk as it pertains to SDB. Hypertension is a risk factor for AF possibly via mechanisms of atrial fibrosis [16] and left ventricular hypertrophy resulting in atrial dilatation [17]. Obesity is a risk for incident AF based upon Framingham data [18]. A graded relationship between body mass index and progression from PAF to permanent AF in a longitudinal cohort followed over a 21-year period has also been described [19]. However, the role of SDB was not assessed in either study. As increasing SDB has coincided with the obesity epidemic, it is reasonable to suspect that unrecognized SDB would be a contributor to the AF epidemic. Heart failure is also a significant AF predictor [20] and other bidirectional relationships are likely at play. Despite identification of these AF risk factors [21], Framingham data as a whole have shown an increased AF prevalence even after adjusting for these known risks [22]. Hence, the underlying explanations of increasing AF prevalence have not been completely realized, and investigation of other biologically

plausible risks is critical. The incomplete explanation of AF etiology is "disquieting because, in the absence of identifiable predisposing factors, targeting preventive therapy is difficult" [13]. SDB can be such a factor, and be recognized and managed in clinical practice and in AF pathways for prevention.




**3. A. 4. Sleep Disordered Breathing Potentially Accounts for Unexplained Atrial Fibrillation Risk:**

**Pathophysiology (Figure 1).** SDB is a highly prevalent and disabling disorder, afflicting ~15% [23] of adults and as many as 30-50% [24] of patients with cardiac disease. SDB exposes the individual to chronic intermittent hypoxemia and wide intrathoracic pressure swings altering autonomic balance, preload and afterload and also enhances inflammatory and oxidative stresses, which operate to bolster a pro-arrhythmogenic milieu. Animal and human experimental studies have identified potential mechanisms by which SDB either through direct or indirect pathways alters the functional and cardiac structural substrate for AF arrhythmogenesis.

***1) Direct SDB Effects Potentially Contributing to PAF.*** *(gray boxes and solid arrows in Figure 1)*

***a. Intermittent Hypoxia/Reoxygenation.*** Apneas and hypopneas in SDB are associated with oxygen desaturation and ventilatory overshoot hyperoxia. AF is associated with activation of hypoxic factors including hypoxia inducible factor-1α (HIF-1α) [25, 26], a transcription factor which is also up-regulated in SDB [27]. The role of hypoxia in AF is further exemplified by reduction in post-operative AF with the use of supplemental oxygen in those with hypoxia [28]. Retrospective clinical data also implicate nocturnal hypoxia as a risk of incident AF [29].

***b. Intrathoracic Pressure Alterations.*** Obstructive apneas and hypopneas cause repetitive forced inspiration against a closed airway, which generates substantial negative pressures in the chest cavity (approaching -65mmHg) [30]. These cyclical increased intrathoracic pressures in SDB result in acute left atrial and pulmonary vein stretch [30-33] thereby potentially resulting in stretch-mediated ion channel activation triggering AF.

***c. Autonomic Instability.*** This is a known AF risk [34, 35] and occurs in SDB via increased vagal activity during obstructive apneas and hypopneas as well as post-apnea and hypopnea related hypercarbia or hypoxia-induced sympathoexcitation [36, 37]. Vagally mediated influences favor atrial macroreentry and reduce the effective refractory period, whereas sympathetic influences favor abnormal automaticity and triggered atrial activity

[38]. Compelling animal data support attenuation of apnea-mediated AF after autonomic blockade (via ganglionated plexi neural ablation) highlighting the importance of the role of the autonomic system in SDB and AF [39]. HRV analyses have shown increased Standard Deviation of NN intervals (SDNN), increased high frequency (HF) and reduced low frequency (LF) preceding PAF episodes suggesting that sympathovagal imbalances contributing to PAF [40]. These data support the value of performing HRV in the proposed research to investigate SDB-related autonomic instability triggers in PAF.
***d. Sleep Fragmentation.*** SDB-related EEG arousals assessed by the arousal index based upon polysomnography (PSG) lead to sleep fragmentation and are associated with sympathetic activation [41].

***2) Indirect SDB Effects Potentially Contributing to PAF.*** *(dashed outlined boxes and arrows in Figure 1)*
***a. Cardiac Structural Alterations.*** SDB leads to increased wall stress, increased afterload, increased atrial size [32, 33] and impaired diastolic function [32, 42]. Our published data demonstrate increased left ventricular mass index in SDB compared to those without SDB, mechanistically explained by the nocturnal degree of hypoxia [43]. Known risks for AF include increased left atrial volume and strain, left ventricular hypertrophy and left atrial wall motion velocity [44], all which should be measured in any research on AF in SDB.
***b. Systemic Inflammation.*** SDB markers include increased *interleukin-6 (IL-6) [45], soluble IL-6 receptor (sIL-6R)*[46] *and high sensitivity C-Reactive Protein (hs-CRP)* [47]. Importantly, increased hs-CRP levels are implicated in atrial structural and electrical remodeling [48, 49] and increased IL-6 levels have been associated with AF [50].
***c. Oxidative Stress*** SDB-related intermittent hypoxia-reoxygenation results in oxidative stress [51-54] which may alter myocyte ion channel function. Animal data show increased oxidized glutathione [55] and NADPH oxidase levels in the left atrial appendage [56] in persistent AF. Initiation and perpetuation of AF involves inflammation and oxidative stress, e.g. *endothelin-1* has been increasingly implicated as a pathogenic factor in AF resulting in upregulation of oxidative stress pathways [57-59]. There are methods to quantify *F2-isoprostanes*, a recognized marker in SDB [60-62] which are prostaglandin-like compounds derived from free radical-catalyzed peroxidation of arachidonic acid. This has allowed facile and accurate assessment of oxidative stress in vivo [63] and will be measured in the current proposal.

**3. A. 5. Sleep Disordered Breathing Potentially Accounts for Unexplained Atrial Fibrillation Risk: Clinical and Epidemiologic Data.** The existing clinical and epidemiologic data in SDB are currently limited by a lack of objective assessments of cardiac and autonomic function [2, 64-67], lack of consideration of central apnea [64, 67], inconsistent arrhythmia adjudication (Sleep Heart Health Study), insufficient sample size [66], retrospective data in a skewed population [29] and the SDB data in AF studies depend largely on subjective measures (lack of objective sleep study data) [64, 68].
***1) Clinical Data.*** The published relationships between SDB and AF, while in general compelling, have flaws. One well referenced study found a significantly higher prevalence of SDB (by questionnaire) in AF patients, many with established structural

heart disease, than in age-matched controls [64]. In contrast, another study found a similar prevalence of SDB in AF patients and controls [66]; close reading suggests that this study was likely under-powered with a control sample potentially biased towards having SDB [66]. A small case control study matched for age and sex and excluding those with LVEF<50% demonstrated a 3-fold increased adjusted odds of AF in those with moderate to severe SDB, but was limited by not taking into account the contribution of central sleep apnea and also did not include detailed assessment of cardiac structural data or autonomic function measures [67]. Although data support that effectively treated SDB in compliant patients reduces AF recurrence after cardioversion, the comparison group were those non-compliant with SDB treatment, a group perhaps more likely to engage in other unhealthy behaviors increasing AF recurrence [69]. There are also conflicting data as to whether SDB predicts procedural failure after AF ablation [68, 70].

***2) Epidemiologic Data.*** We have performed epidemiologic observational studies which have demonstrated statistically significant associations of SDB and AF (odds ratio point estimates of 2-4) even after taking into account a host of potential confounding factors such as age, sex, race, body mass index, and self-reported co-morbidities such as hypertension, diabetes mellitus, cardiovascular disease and heart failure [1, 2]. A major limitation of these large-scale studies is dependence on self-reported diagnoses and a lack of objective cardiac function data to account for the confounding influence of structural cardiac disease. Even when examining the association of Central Sleep Apnea-Cheyne Stokes Respirations (CSA-CSR) with AF, the directionality is unclear, as CSR-CSA could either predispose to AF development or merely represent a marker of cardiac dysfunction leading to AF. Other limitations include lack of measurement or consideration of markers of autonomic function, systemic inflammation, oxidative stress and consideration of only nocturnal ECG data from the sleep study to ascertain AF.

The goal of the current application is to address the shortcomings of existing data by the detailed collection of cardiac structural data, autonomic function and biomarkers of inflammation oxidative stress and careful consideration of these influences in the SDB-PAF relationship. Relationships of obstructive and central apnea with PAF will be explored. We will also investigate interactions of PAF and age in SDB given our prior work showing increased odds of arrhythmia with younger age [1]. Moreover, diurnal variation of PAF with SDB will also be examined given the immediate and chronic SDB-related physiologic stresses (i.e. intermittent hypoxia, intrathoracic pressure alterations and autonomic influences). Furthermore, effects of SDB treatment on PAF will also be examined to provide data for future studies.

## 4. ORGANIZATIONAL STRUCTURE

Experts in large-scale epidemiologic studies, engineering, biostatistics and electrophysiology will meet weekly during the first several study months and then monthly.

**Case Western Reserve University Administrative Center, (Director: R Mehra, PI):** Dr. Mehra is an Associate Professor of Medicine at the Cleveland Clinic Lerner College of Medicine and Director of Sleep Disorders Research in the Sleep Center of the Neurologic Institute. She has acquired experience in clinical trials/epidemiologic research with a focus on cardiovascular outcomes. She will oversee the Administrative Center which will be charged with providing overall scientific direction and strategic planning for the study, ensuring adherence to study objectives and timelines, maintaining high levels of data integrity and providing fiscal oversight. Members of the center will include a study coordinator, research assistant, sleep technologists, data manager and statisticians. The Center will be responsible for development of informed consent forms, obtaining IRB approval and enrollment of participants. It will develop data collection instruments, provide data management and quality control, closely monitor study recruitment, set up meetings and calls and provide minutes. Dr. Kingman P. Strohl will serve as the Responsible Principal Investigator at the University Hospitals Case Medical Center site to oversee recruitment and data integrity. The Center will coordinate the processing and merging of files from the different cores. The statistical core will be led by Dr. Jeffrey Albert, Associate Professor of Biostatistics, an expert in mediation analysis and causal inferences who will oversee programming of data and performing interim and final analyses for manuscript writing. ***Data Sharing:*** The Center will integrate the collected PSG, ECHO and ECG raw signal data in a customized instance of Physio-MIMI system developed by Dr. Zhang (PI) at Case as an NCRR-funded Multi-CTSA-site project. Data sharing will be facilitated by Physio-MIMI's extensible Role-Based Access Control mechanism, which allows for fine-controlled access to data. Curated data and application tools will be made available for long-term data sharing and dissemination.

**Electrophysiology (EP) /Autonomic Nervous System Physiology Core (Co-Directors: Drs. Mackall and Loparo).** Dr. Judith Mackall is the Director of the UHCMC EP Center with longstanding clinical experience in the care of patients with AF and as an investigator in several AF trials. She will work with the PI and administrative center staff to facilitate recruitment for the study and provide input in the interpretation and analysis of data. Dr. Kenneth Loparo, Nord Professor of Engineering, will apply his expertise in signal processing, system dynamics and nonlinear and stochastic control to the proposal. He has an interest in signal processing of sleep study and ECG data and also has worked with Dr. Mehra on other projects. Dr. Al Waldo, Director of EP Research at UHCMC, is internationally renowned for his extensive contributions and work in the realm of AF mechanisms and pathogenesis, and will apply his expertise in the conduct of this study, interpreting outcomes and providing input for analyses of data. The Core will establish and ensure quality standards, establish standardized procedures for acquisition and processing of digitized ECG data and oversee the transmission of incoming raw ECG data to the Administrative Center for ECG extraction/spectral analyses applying robust algorithms.

**Echocardiography Core (Director: Dr. Brian Hoit).** Dr. Hoit is the Director of the Noninvasive Echocardiography Laboratory at UHCMC with extensive experience in

noninvasive cardiac testing and will oversee the performance and interpretation of ECHOs for the proposed research. The Administrative Center will work with members of his team to transfer ECHO data files to the center for analyses.

## 5. SAMPLE SELECTION

**Cases.** Patients with PAF who present to Electrophysiology (EP) Clinic at UHCMC and the Cleveland Clinic Foundation (CCF) will be approached for recruitment in the study.

*Inclusion Criteria for Cases:* 1) *PAF defined by recurrent episodes of AF, which self-terminate within a 7-day period (based upon AHA consensus statement [77])*, 2) Age 18-80 years, 3) Individuals able to participate in ≥ 2 overnight/daytime sleep and physiologic assessments over a 3 month period.

*Exclusion Criteria for Cases:* 1) PAF with rapid or uncontrolled rate (≥120bpm), 2) Post-operative PAF, 3) History of cardiac ablation or successful electro-cardioversion for PAF (ablation for other arrhythmias such as AVNRT and if PAF persists after cardioversion is acceptable ), 4) Valvular stenosis, prosthesis or significant valvular insufficiency [i.e. those with moderate or greater severity of aortic stenosis (aortic valve area <1.5 $cm^2$), mitral regurgitation which is moderate or more severe in degree (>20% regurgitant fraction) or moderate or greater severity mitral stenosis (mitral valve area <1.5 $cm^2$)], 5) Atrial septal defect, 6) Infiltrative/restrictive cardiomyopathy, 7) Sick sinus syndrome, 8) Previously diagnosed SDB on specific SDB treatments (CPAP, oral appliances), 9) Severe chronic insomnia, 10) Circadian rhythm disorder (e.g. shift work sleep disorder, delayed or advanced sleep phase syndrome), 11) Insufficient sleep syndrome defined by reported sleep duration < 4 hrs, 12) Supplemental oxygen use, 13) Unstable medical conditions (e.g., new onset or changing angina, a myocardial infarction or congestive heart failure exacerbation documented within the previous 3 months, systolic heart failure (Left Ventricular Ejection Fraction < 35%), high grade cardiac dysrhythmia/heart block, stroke with functional limitations, uncontrolled hypertension (BP>170/110), abdominal aneurysm >5.5 cm or >1 cm growth/year, uncontrolled diabetes mellitus (HbA1c>9.0), pulmonary hypertension, non-skin cancer diagnosis or treatment within the previous year, end stage renal and hepatic failure, immunodeficiencies (HIV, HCV), uncontrolled hypo- or hyperthyroidism), 14) Psychiatric disorders which are inadequately treated, 15) Compromised competence, 16) Alcohol abuse (currently drinks >5 alcoholic drinks/day), 17) Pregnancy, 18) Inability to provide informed consent, 19) Illicit drug use over last 6 months, 20) Rate controlling anti-arrhythmic medication (Classes I-III and V) with no further clinical occurrence of PAF, 21) has an Implantable cardioverter-defibrillator. *Rationale for criteria:* The goal of this study is to include those patients with PAF that is not secondary to the post-operative period or valvular disease and without ablation as these processes would result in alteration of atrial physiology and preclude assessment of independent SDB effects on AF which is independent of these conditions. Patients with sleep disorders will be excluded as sleep disorders may influence arrhythmogenesis. Those on treatment for SDB will be excluded because treatment would preclude assessment of SDB pathophysiologic effects on atrial

arrhythmogenesis. Those with unstable medical conditions or rapid or uncontrolled heart rate will be excluded due to safety reasons.

Note: Exclusion criteria for positive airway pressure (PAP) intervention: Central Apnea Index≥5 noted on baseline examination sleep study or evidence of Cheyne Stokes Respirations/periodic breathing (cyclical crescendo and decrescendo change in breathing amplitude).

We plan to recruit 10 cases with moderate to severe SDB and paroxysmal AF who are placed on CPAP to participate in an ancillary study to investigate the effect of CPAP on the default mode network, which is a well-established resting state network in the brain that is involved in semantic processing and associated with compromise in cognitive task performance.

Note: Exclusion criteria for the 7T MRI would include ferrous objects in the body (cochlear (ear) implant, brain aneurysm clip, stimulator device, and cardiac pacemaker), severe kidney disease, and history of claustrophobia.

**Controls.** Patients without AF will be recruited from the UHCMC and CCF General Cardiology and Internal Medicine clinics (geographically similar to controls). Selection bias will be minimized as there are a broad range of reasons for patients to present to these clinics.

*Inclusion Criteria for Controls:* 1) Age 18 to 80 years, 2) Individuals in normal sinus rhythm (NSR) with no current AF or history of AF 3) Individuals able to participate in an overnight/daytime sleep and physiologic assessment.

*Exclusion Criteria for Controls:* Current or history of AF, otherwise the same exclusion criteria listed for cases.

We plan to recruit 10 controls without PAF but with moderate to severe SDB to participate in an ancillary study to investigate the default mode network, which is a well-established resting state network in the brain that is involved in semantic processing and associated with compromise in cognitive task performance. The controls will have the cognitive battery and questionnaires only. They will not undergo the 7T MRI.

**MATCHING.** Cases and controls will be individually matched to enhance study efficiency and precision with more stable odds ratio estimates and narrower confidence intervals. Matching will be based upon factors which are biologically strong confounders as follows: *1) age (±5 years), 2) gender, 3) race (white versus non-white) and 4) obesity (BMI +/-2 kg/m$^2$).* Recruitment of controls and cases will be contemporaneous given concurrent assessments of eligibility (AF status and matching criteria). To ensure appropriate individual matching on the factors listed above, a staggered recruitment of controls will occur such that matching parameters will change monthly depending upon the distribution of these factors in the case group. Statistical code will be written to perform matching of cases and controls with these five

variables. If exact control matches are not identified, then unmatched cases will be retained in the cue until the next round of matching.

## 6. RECRUITMENT OVERVIEW

The site's study Research Coordinator will oversee recruitment efforts. Patients with PAF who present to Electrophysiology (EP) Clinic at UHCMC and CCF will be approached for recruitment in the study. Dr. Bruce Lindsay, Director of CCF EP Program and Dr. Judith Mackall (Co-I), Director of the UHCMC EP Center and 6 other electrophysiologists including Dr. Al Waldo (Director of Electrophysiology Research, Co-I) comprise the EP clinical team along with the EP fellowship program/clinic. Approximately 1,680 new patients/year (140/month) with AF are seen in the EP clinics. Of these, ~672 patients/year (~60/month) have PAF. Assuming 50% meet eligibility criteria and 50% of those are interested then ~n=168 patients will be recruited/year (n=14/month). This translates into n=672 potential participants over the 4 year recruitment period which far exceeds the n=150 cases required to meet sample size requirements. As part of clinical care, PAF patients have echocardiograms, may be treated with ablation or anti-arrhythmic medications and often will undergo Holter ECG monitoring. Only those patients with PAF diagnosed within the last 6 months who have not undergone ablation will be approached for enrollment.

A screening form will be designed to identify eligibility criteria. Once a person has been identified as eligible to participate in the study, written informed consent and HIPAA authorization will be obtained. To maintain general descriptive information about the target sample, the screening questionnaires will be entered into a secure research database that does not include any personal identification. All hard copies of this health information will be destroyed about all patients who do not meet study criteria and those who decline participation. All personal health information collected and retained on site prior to obtaining written authorization will be de-linked from identifying personal information. Also, if written authorization is not obtained within 90 days of patients who have agreed to participate in the study, their health information will be destroyed.

**Recruitment Sites and Sources**

At University Hospitals, case participants will be recruited from the Electrophysiology (EP) Clinic affiliated with University Hospitals/Case Medical School (including the practices of Drs. Judith Mackall, Mauricio Arruda, Ivan Cakulev, Bruce Stambler, Robert Goldstein, Mauricio Hong, Jayakumar Sahadevan and Al Waldo along with the EP fellowship program/clinic), the Anticoagulation Clinic (Director, Teresa Carmen and Cardiac Rehab Clinic (Director Richard Josephson) and the Electrocardiogram (event monitor) Lab. Control participants will be recruited from the cardiology and Internal Medicine clinics affiliated with University Hospitals/Case Medical Center (including the practices of Drs. M Cunningham, T Wilson, L Greene, B Effron, S Madan Mohan, Eric Yasinow, Paula Deuley, Claudia Villabona, James Pizzaro, Michael Eckstein, David Cogan, Jyoti Bhatt, and Debra Leizman, George Kilkano, Vanessa Maier, Robert Cirino, A. Halle, C. Orringer, and James O'Donnell.

At Cleveland Clinic, subjects will be recruited from Cardiology, Family Medicine, and Internal Medicine clinical practices, referring physicians, and advertisements. All advertising materials will be approved by the IRB. Study personnel will screen the electronic medical record (EMR) of potential subjects using the adult Cardiology, Family Medicine, and Internal Medicines outpatient schedule.

**A written waiver of consent will be requested (according to 45 CFR46.117) to collect and review health information about prospective subjects prior to obtaining written informed consent.** The research involves no more than minimal risk. The waiver will not adversely affect the rights and welfare of the subjects and the research could not be conducted without the waiver because the health information collected is necessary to ascertain which subjects are eligible to participate in the study.

This is a request for partial waiver of HIPAA authorization" to collect health information" from screening questionnaires or from the medical records of participating electrophysiology clinics. The screening questionnaires will ask health questions to screen for sleeping patterns, and personal health information to determine which patients would qualify for study participation. The medical record screening form identifies eligibility criteria. Once a person has been identified as eligible to participate in the study, they will be scheduled for an in-person study visit at which time, written informed consent and HIPAA authorization will be obtained. (If the patient is available at the time of the screening, as may occur if the screening questionnaire is directly administered, the full screening visit may be conducted at that time in an area sufficiently private to provide for confidentiality. To maintain general descriptive information about the target sample, the screening questionnaires will be entered into a secure research database that does not include any personal identification. All hard copies of this health information will be destroyed about all patients who do not meet study criteria and those who decline participation. All personal health information collected and retained on site prior to obtaining written authorization will be de-linked from identifying personal information. Also, if written authorization is not obtained within 90 days of patients who have agreed to participate in the study, their health information will be destroyed.

**13. Electronic Record System Utilization**

Eligibility will be assessed by a study recruiters (Kathryn Clark (UH) and Rawan Nawabit (CCF)) and the study coordinator (Joan Aylor (CCF)) using the UH and CCF Physician Portal. This will be done by accessing each potentially eligible patient's electronic chart only once in order to review the most recent note from the patient's cardiologist. Information viewed will be used by the study recruiter to check yes or no on the study inclusion and exclusion criteria form with no recording of specific results being made. These forms are de-identified with only the study id listed. No printouts from the Portal will be made. Review of the chart should take no more than 5 minutes per patient. All hard copies of this health information will be destroyed about all patients

who do not meet study criteria and those who decline participation. All personal health information collected and retained on site prior to obtaining written authorization will be de-linked from identifying personal information. Also, if written authorization is not obtained within 90 days of patients who have agreed to participate in the study, their health information will be destroyed.

The reason for requesting access to physician notes on the Portal rather than reviewing paper charts is that paper charts are not stored at each clinic but are instead stored at an off site locality where they are not accessible for review until 24 hours prior to the patient appointment. Because the recruiter is expected to be at a clinic each day, he cannot physically review paper charts until the day of clinic but this is disruptive to patient care. By utilizing the UH Portal, the recruiter can screen for eligible patients without interfering with clinical care.

If questions arise relevant to eligibility (e.g., degree of renal insufficiency, time since free of cancer) that cannot be readily answered by the recruiter, one of the study physicians (at UHCMC, Drs. Kingman Strohl, Kristie Ross, Anna May and Rawad ElGhoul and at the Cleveland Clinic, Drs. Reena Mehra and Harneet Walia) who already has Portal access will use the Physician Portal to access the relevant clinical notes or laboratory information to make a judgment on study eligibility. It should be noted that this study physician may not be a direct caregiver of the patient. The recruiter will get approval from the patient's physician prior to accessing the patient's record for screening. Access to electronic records will be made at most once per patient by a study physician to determine eligibility and only in those cases where staff has been unable to make a clear determination through review records in the clinic and interview with the patient. We anticipate this will be required in less than 5% of screenings. Information viewed will be used by the study physician to check yes or no on the inclusion and exclusion criteria with no recording of specific results being made. These forms are de-identified with only study id listed. No printouts from the Portal will be made.

Similarly, in dealing with potential safety concerns, one of the study physicians at UHCMC, Drs. Strohl, Ross, May, and ElGhoul and at the Cleveland Clinic, Drs. Mehra and Walia) may access the Physician Portal to stratify adverse events. For example, if overnight cardiac monitoring reveals bouts of atrial fibrillation, identifying that the patient has a history of atrial fibrillation will make this finding a low level adverse event requiring only a letter to the patient's study physician. However, if review of the Physician Portal demonstrates the patient has no prior history of atrial fibrillation, this would result in a higher level adverse event where the patient's physician would be contacted within 24 hours to be notified of this important new finding. Again, the Portal would be accessed at most once per adverse event and only if it was unclear if the finding were new (e.g., there would be no need if the patient reported he had a history of atrial fibrillation). This information will be recorded only in terms of grading adverse events, all of which again are on de-identified forms labeled only with a study id and no printouts of Portal information will be made. Using the Portal is necessary to obtain information relevant to patient safety in a timely manner as requesting paper patient outpatient charts can take up to a week.

## 7. OUTLINE OF STUDY VISITS

**Baseline Visit (SA 1 and 2, Table 1).** After informed consent, eligible participants will be scheduled to arrive for an overnight visit in the DCRU/CRU within a standardized time interval (< 2 weeks) from informed consent. During the evening, participants will undergo questionnaire administration, blood pressure measurements, application of the MultiSense™ monitoring device, and anthropometry. They will be provided dinner and undergo polysomnography (PSG). In the morning, participants will undergo fasting venipuncture, overnight urine collection, ECHO, 6 minute walk test and blood pressure measurements. For those with PAF (cases), hook-up for continuous ECG monitoring will be performed along with education regarding how to handle the device and bathing instructions, etc. All measurements in the DCRU/CRU will be performed blinded to PAF status. The only unblinded staff will be a dedicated research assistant who will perform the ECG monitoring hook-up. Blinded staff will collect and process data and perform data entry. After the baseline visit, participants with PAF (n=150) will undergo hook-up of the ECG monitor and an activity monitor at the baseline visit to wear for a 7-21 day period. The monitors will be picked up by a courier service. If eligible for the 7T MRI sub-study (cases - AHI≥15 and agree to CPAP treatment, controls AHI≥15), they will be consented and all questions answered. The individuals who serve as controls who are identified to have sleep apnea (AHI≥5) or for those with PAF who have an AHI ranging from 5-15 on the baseline sleep study in the Clinical Research Unit will be notified of these results and the physician will be contacted if the participant provides permission.

| **Table 1. Timeline of Events for SA 1 and 2** | | | |
|---|---|---|---|
| ***EVENING*** | | ***MORNING*** | |
| 5PM | DCRU/CRU Arrival/Intake | 7AM | Lights On/Blood Pressure Measurements in Triplicate |
| 515PM | Questionnaire Administration | 715AM | Fasting Venipuncture |
| 600PM | Anthropometry | 745AM | Overnight Urine Collection |
| 630PM | Dinner | 8AM | Echocardiography |
| 9PM | PSG Hook-Up | 830AM | Breakfast |
| 10PM | Blood Pressure Measurements in Triplicate | 9AM | Continuous ECG monitoring hook-up and education for cases with PAF |
| 1030PM | Lights Out-Overnight PSG | 930AM | DCRU/CRU Discharge |

**Follow-Up Visit (SA3).** Those with SDB (AHI>15) *without evidence of central apnea (central apnea index>5) or Cheyne Stokes Respirations* via baseline visit PSG and who have PAF will undergo the following:

1. A 5-7 day home-based auto-titration (APAP, Respironics Autopap System One with humidifier) to identify the optimal positive airway pressure (PAP) setting will be performed (with settings 4-20cm H2O).
2. Participants will be educated on the use of PAP and mask fit will be optimized.
3. The research assistant will maintain contact with the participant by telephone during the 5-7 days. The research assistant will contact the participant within 24 hours and then again within the 5-7 period. During the first 24 hours the overnight data will be

reviewed and ascertained for the presence of treatment emergent central apnea. If a central apnea index >5 is noted during the first night initial exposure to CPAP, then the participant will be withdrawn from the study. The participant will be notified about the results of the research study testing and will be encouraged to follow-up with his/her physician. The participant's physician will be contacted provided that the participant provides permission.

4. At the end of the 5-7 day titration, the data on pressure responses and PAP use (with no patient identifying information) are transmitted using a wireless feature from the patient's home to a secure web-site which is accessible by the research staff. If wireless coverage is unavailable, data may be transmitted using a standard phone line or directly retrieving a data card from the machine. The PI will review the data and identify the optimal pressure. If the initial APAP study is unacceptable, a repeat 5-7 day recording will be performed. The goal will be to identify the pressure that results in an AHI<5 events/hour (optimally), however, <10 events/hour would be deemed acceptable. For those participants that have mask leak or interface issues, they will undergo troubleshooting by the coordinator by phone and in-person as needed. If despite addressing these issues, the AHI remains ≥10, then the participant will be withdrawn from the study and will be encouraged to follow-up with his/her physician. The participant's physician will be contacted provided that the participant provides permission. If the participant qualifies for CPAP,, then the subject will approached to assess interest in the MRI sub-study.
5. The research assistant will re-set the device to deliver this optimal fixed pressure identified by the PI through the secure wireless web-site. The web-site will allow the research assistant to measure adherence daily and review with the participant at the scheduled follow up calls to maximize PAP use. Each APAP also contains a "smartcard" which will provide objective adherence data if wireless is not available. The participant contact during follow-up will be as follows: the participant will be called every 2 weeks thereafter during the 3 months period of CPAP usage or more frequently as needed to address CPAP adherence issues. An in-person visit will be scheduled for those participants that have non-adherence due to mask fit/leak noted on more than 2 consecutive nights.
6. An overnight DCRUCRU visit will be scheduled after 3 months of wearing CPAP (*this duration chosen based upon data supporting immediate influences of apnea on PAF[71] and CPAP effects on cardiac function in 1 month [78]) during which the same measures performed at the baseline visit will be collected. If the participant had a 7T MRI at their baseline visit, a follow-up MRI will be scheduled.

As SDB prevalence of ~50% has been noted in those with AF [64], we estimate that ~75 participants will meet criteria to proceed to CPAP. If this goal is not met, then we will supplement recruitment from the sleep disorders program (~1200 studies/year) to identify those with SDB who have PAF to achieve this goal.

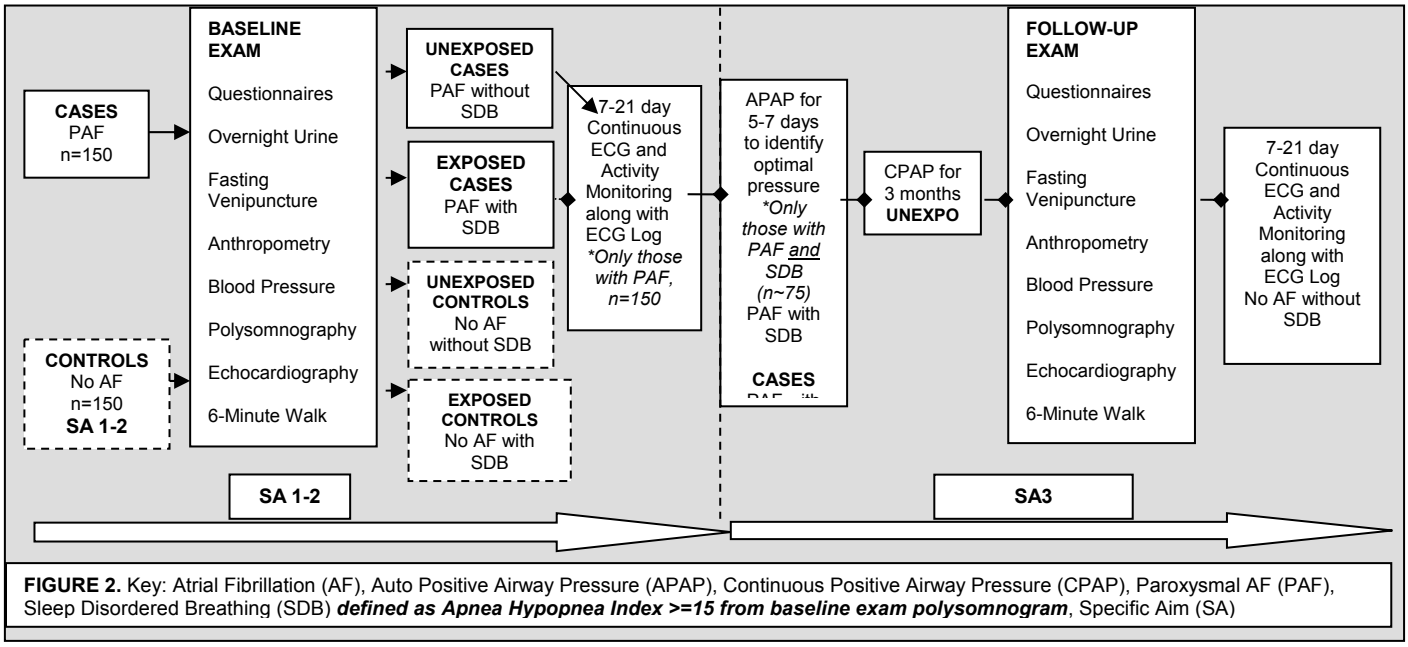



**FIGURE 2.** Key: Atrial Fibrillation (AF), Auto Positive Airway Pressure (APAP), Continuous Positive Airway Pressure (CPAP), Paroxysmal AF (PAF), Sleep Disordered Breathing (SDB) ***defined as Apnea Hypopnea Index >=15 from baseline exam polysomnogram***, Specific Aim (SA)

## 8. STUDY PROCEDURES AND DATA COLLECTION

**2-DIMENSIONAL DOPPLER ECHOCARDIOGRAPHY.** ***Procedure:*** Participants will be studied by one of two senior sonographers during quiet respiration in the left lateral decubitus position with administration of intravenous agitated saline to enhance imaging. Parasternal, apical and subcostal 2-D views and apical 3D views will be obtained with transducer orientation and gain settings adjusted to optimally define endocardial surface of each cardiac chamber. Measurements will be performed by the Director of the ECHO Lab (accredited by the Intersocietal Commission for the Accreditation of Echocardiography Laboratories), Dr. Brian D. Hoit (Co-I) and designate off-line (HeartLabs Inc, Q Labs, Phillips Medical) in a blinded fashion on 3 representative beats and the results averaged. Measurements will be performed according to the American Society of Echocardiography recommendations [79, 80]. Studies will be archived on CD. Rigorous quality control measures will be used including random repeat measurements to calculate inter- and intra-observer reliability.

Approximately 20% of echocardiograms result in suboptimal views in at least 2 of 6 myocardial segments of the left ventricle in apical views. The use of a contrast agent can opacify the sub optimally viewed areas allowing for better analysis. Individuals with obesity, lung disease, or other critical care areas usually require the use of a contrast agent for optimal echocardiogram views. Subjects requiring a contrast dye will be administered an IV dose of Definity ® (perflutren lipid microsphere injectable suspension), a US Food and Drug Administration (FDA) approved contrast dye. Subjects will be screened for known contraindications: known or suspected cardiac shunts or patent foramen ovale, or known hypersensitivity to perflutren. Subjects will also be informed of the potential risks

associated with Definity ® as described in the informed consent document.

***Relevant ECHO Variables:***

1) Left and Right Atrial Parameters: Left atrial volume by using the biplane area-length method [79], right atrial area (from the 4-chamber view), left atrial pressure estimated from the ratio of early diastolic transmitral flow velocity to early annular tissue velocity [81] and right atrial pressure based on the diameter of the inferior vena cava and its response to a "sniff" will be assessed [82]. Peak left atrial systolic strain, a measure of LA remodeling which is inversely related to fibrosis in PAF [83], will also be measured.

2) Left Atrial Appendage (LAA) Parameters: LAA wall motion velocity, a measure associated with AF will be assessed [84]. A value of >40 cm/s, has been noted to be an independent predictor of NSR maintenance in AF[44]. LAA tissue velocity, a more readily obtained and sensitive LAA dysfunction measure will also be measured [84].

3) Left ventricular ejection fraction (LVEF, %): This will be derived from left ventricular end-diastolic volume (LVEDV) and end-systolic volume (LVESV): [(LVEDV-LVESV)/LVEDV x 100] [85].

4) Left ventricular mass index (LVMI): This will be calculated by a validated formula [86] : LVMI = {0.8 x [1.04 x (LVIDd + IVST X LVPWT)$^3$ – (LVIDd)$^3$] + 0.6}/height $^{2.7}$, where LVIDd is LV internal diastolic diameter, IVST is interventricular septal thickness and LVPWT is LV posterior wall thickness, measured in centimeters.

5) Left ventricular hypertrophy (LVH): Using previously published thresholds, we will define LVH as an LVMI of 49.2 g/m$^{2.7}$ for men and 46.7 g/m$^{2.7}$ for women [87]. Subjects with LVH will be classified as having concentric hypertrophy if relative wall thickness [(LVPWT x IVST)/LVIDd)] is >0.41 or eccentric hypertrophy if $\leq$0.41 [88].

**2) POLYSOMNOGRAPHY (PSG).** ***Procedure for PSG:*** Research PSG will be performed using the Compumedics E-series system (Abbottsford, AU) which will include 3 cortical encephalograms, bilateral electro-oculograms, a bipolar submental electromyogram, thoracic and abdominal respiratory inductance plethysmography, airflow (by nasal-oral thermocouple and nasal cannula pressure recording), oximetry (using highly sensitive finger pulse oximeter, sampling frequency 25Hz), electrocardiogram (ECG) at 250Hz (used to derive HRV measures of autonomic function); body position (mercury switch sensor), bilateral leg movements. EEGs are recorded at 125Hz. Sensors will be calibrated, and signal quality/impedance checked by a certified technician, using standardized techniques. PSG scoring criteria are documented in the Case Sleep Research Manual of Procedures (**Appendix B**). ***Relevant PSG Variables:*** Apneas will be classified as "central" or "obstructive" according to the absence or presence of respiratory effort respectively. Hypopneas will be scored as a 50% amplitude reduction in inductance or flow and associated 3% oxygen desaturation or arousal. Periodic breathing will be defined as airflow or inductance channels increasing and decreasing at least 50% from the maximum, in a cyclic waxing and waning or "sinusoidal" manner for a consecutive period of $\geq$10 min.

***Procedure for PSG ECG Signal Extraction:*** ECG monitoring data from the PSG Baseline and Follow Up Exams will be processed and analyzed by the Case Electrophysiology Core (Director: K Loparo, Nord Professor of Engineering). Data will be exported as European Data Format (EDF) ECG files into customized Matlab programs that

will allow application of time and spectral-based algorithms for extracting quantitative ECG measures. Data will be closely inspected and edited using graphical tools. Artifact cleaning will be performed and those periods of NSR in the ECG recording will be identified and scored to perform HRV analyses based upon automated algorithms developed by the Case Engineering group. Random sampling for manual scoring will be performed to confirm agreement and accuracy of artifact scoring and identification of NSR periods performed by automated tools. A dataset of ECG marker output will be generated and merged with the rest of the study data for statistical analyses. HRV analyses (time and frequency domain) will be performed with ECG signal processing tools developed by Dr. Loparo, which are efficient, robust and account for artifact. We will use algorithms and automated methods of feature extraction that have been validated in both synthetic and published data[89, 90], account for varying signal to noise ratio and non-stationary data and have artifact rejection capability. We selected ECG measures as informed by existing literature, AF pathophysiology and reliable extraction; however, other markers will be analyzed for which we have developed algorithms (i.e. non-linear indices such as conditional entropy). ***Relevant PSG ECG Variables:*** **ECG measures will be based upon NSR periods identified by automated analyses with manual over-reading of random segments.* HRV, considered to measure sympathovagal modulation of heart rate, will be quantified using standard time domain methods [time intervals between normal beats (*SDNN*), the root mean square differences between adjacent normal beats (*r-MSSD*), the % of pairs of adjacent beats differing by >50 ms (*pNN50*)], and with frequency spectral based measures which mathematically deconstruct heart beat patterns into underlying rhythmic components using Fast Fourier transforms: low (0.04-0.15 Hz) and high (0.15-0.4 Hz) frequency bands ratio (*LF/HF*). Standardized approaches will be used [91] with close editing for artifact and ectopy. ECG recordings with >20% ventricular ectopic beats will be excluded from analyses [92]. Graphical analysis will include *5-min averaged heart rate (HR) patterns, hourly power spectral analysis, hourly Poincaré plots and beat-by-beat HR tachograms*. As there is no consensus whether respiratory variation reflected in the ECG signal offers useful physiologic information vs. introduction of artifact, we will perform sensitivity analyses with unadjusted and "adjusted" respiration time series analyses [93]. To assess ECG measures independent of respiratory artifact, a 5 min ECG period will be selected and restricted to normal breathing during wakefulness at the beginning, and also at the end of the PSG. ECG markers will be analyzed as continuous outcomes, averaging information from 5 min intervals of NSR periods deriving an aggregate summary of electrophysiological variability. To take into account sleep state, 5-min ECG windows during sleep vs. wake will be selected for analyses. ECG analyses will be performed on concatenated epochs (30 sec windows): 1) Epochs with apneas/hypopneas vs. those without, 2) REM vs. NREM epochs and 3) Epochs with EEG arousals vs. those without.

**3) 7-21 Day CONTINUOUS ECG MONITORING.**

***Procedure:*** A 3-lead (2 channel), wireless, lightweight (3.2 ounce) ECG monitoring device (Heartrak External Cardiac Ambulatory Telemetry ®) will be used to collect the ECG data over a 7-21 day period in those with PAF after the baseline and follow-up DCRU/CRU visits. The device is: 3x7", bandwidth: 0.05-30 Hz, sampling rate=205 Hz, stores 30 days of

data, 30 feet transmission mode and memory hold of 10 years with a <1% data transmission failure and data loss in field testing. 7-21 day was chosen given the variability of PAF and because ECG monitoring >48 hours is reasonable to capture AF paroxysms [94].
*ECG Sensor Application.* This will be performed by a trained technician. Participants will be asked to remove clothing from the waist up to attach the sensors to the chest. Staff will ensure privacy by covering the participant with a sheet or gown. The staff will shave necessary areas on the chest prior to sensor application to ensure that the sensors stick closely to the skin and will provide the participant with the ECG monitor/holster/clip, leads, communicator and charger, electrodes and a handbook with simple pictorial/written educational instructions.
*At-home ECG Monitoring Logistics.* The ECG monitor box may be clipped to a belt or pocket, and the communicator may be placed in a pocket (much like a cell phone). The participant will be instructed to engage in usual activities and instructed to remove the monitor before bathing and reapply the sensors after bathing. An ECG technician will be available by phone 24-7 to address issues with device malfunction, lead placement and replacement of equipment. Research staff will contact the participants every 3-4 days to assess for symptoms and help troubleshoot any mechanical or logistical issues. ECG quality grade data will be collected: (excellent: >90% of ECG data without artifact, good: 70-90% without artifact, fair: 50-70% without artifact, poor <50% artifact free).
***Relevant ECG Variables:*** AF paroxysm frequency will be scored by a certified ECG technician and categorized by sleep vs. wake states based upon self-report from the ECG log. This will allow for assessment of AF burden temporal patterns via 7-21 day ECG monitoring relative to SDB. These ECG data will also be analyzed by Dr. Loparo's group, including Anupam Basuray and Kanna Posina, if there is insufficient NSR data from the PSG ECG to perform HRV analyses.

**4) ACTIVITY MONITORING. Procedure:** An accelerometer (GT3X+, Actigraph Co., Ft Walton Bch, Fl) will be used for 7-21 days, coincident with the time of continuous ECG monitoring, to provide objective measurement of the duration and intensity of physical activity and sleep. Subjects will be instructed to secure the accelerometer on their wrist with an elastic band, wearing it through each day, other than while in the water.

**5) FASTING VENIPUNCTURE (52cc).** ***Procedure:*** Phlebotomy will be performed the morning of the baseline and follow-up visits using standard techniques by trained research staff following written procedures (e.g., pre-labeled bar coded tubes, minimizing trauma, etc.). An intravenous catheter will be placed by the nurse in the morning to perform the blood draw and to facilitate performing the morning ECHO study with agitated saline. The sample will be divided into tubes for the varied analyses (20 mL clot for serum, 20 mL EDTA, 4.5 mL special anticoagulant tube, 5 mL citrate, 2.5 PAXgene). Clots will be centrifuged and the serum removed within 1 hr of venipuncture. Assays will be stored in dedicated, alarmed freezers at -80°C in the DCRU/CRU Core Lab until shipped to the UVM Biochemistry Lab. Extra aliquots designated for future studies will be prepared and stored at -80 °C.
***Relevant Biomarker Variables:*** Assays were selected based upon literature or our own preliminary data. We will investigate the following biological pathways: systemic inflammation [C-reactive protein (hs-CRP), interleukin-6 (IL-6), and soluble IL-6 receptor

(sIL-6R)], oxidative stress (endothelin-1 and oxidized LDL). hs-CRP will be measured using the BNII nephelometry utilizing a particle enhanced immunoassay with intra-assay and inter-assay CVs of 2.1%-5.7%. Circulating IL-6 and sIL-6R will be measured by ultra-sensitive ELISA (R&D Systems with inter-assay CVs 6.3%-15%). Endothelin-1 will be measured by an ELISA test (R&D Systems with inter-assay CV of 6.5% and intra-assay CV of 4.6%). Oxidized LDL will be measured by competitive ELISA (Mercodia Oxidized LDL Competitive ELISA) with intra-assay and inter-assay CV's < 10%. Samples will be run in batch and in duplicate; if they do not agree within pre-specified levels, they are re-run. Because additional aliquots will be saved, other assays will be possible as emerging science identifies their value.

**6) DNA collection.** Blood will be collected to extract RNA, which will be stored to test future hypotheses regarding genetic determinants of treatment response.

**7) OVERNIGHT URINE.** ***Procedure:*** An overnight urine will be obtained and 3 -1.0 ml, 1 – 9.0 ml plain urine and 1 – 9.0 ml acetic acid aliquots will be stored at -80 °C.
***Relevant Biomarker Variable.*** The proposed measurement for urinary isoprostane 8-epi-prostaglandin F2a, a stable nonenzymatic oxidation product of arachidonic acid [95, 96] (expressed as ng/mmol urinary creatinine), is an inexpensive ELISA validated against gas chromatography-mass spectroscopy [97] and used in the Framingham Heart Study [98], with unpublished data showing its elevation in SDB.

**8) ANTHROPOMETRY.** *Height (inches, cm)* is measured with the subject in stocking feet, using a wall-mounted stadiometer; *weight (pounds, kg)* with a calibrated digital scale. *Neck circumference* (cm) is determined with a non-stretchable measuring tape (nearest 0.5 cm) while the subject is seated with the head in the Frankfort horizontal plane, measuring below the thyroid prominence, perpendicular to the horizontal axis of the neck. *Waist circumference* (cm) is measured at the smallest area between the lower ribs and iliac crest. *Hip circumference* (cm)
is measured at the widest area around the buttocks. The wrist, abdominal, thigh, calf, and mid upper arm circumferences will also be measured using a non-stretchable measuring tape. *Skinfolds* are measured with calibrated metal calipers from 7 sites (chest, calf, thigh, calf, subscapular, midaxillary, suprailiac, abdominal), using a rotational order, obtaining additional measures if variation >10%.

**9) RESTING BLOOD PRESSURE (BP).** BP will be measured after the participant has been sitting quietly for at least 5 minutes following standardized guidelines using a calibrated sphygmomanometer. Cuff size will be determined by measuring the circumference of the upper arm, measured at the midpoint and identifying the appropriate bladder size from a standard chart. Measurements will be repeated three times and recorded.

**10) QUESTIONNAIRES (Appendix B)**. The following will be collected: a) The *Berlin Sleep Questionnaire*, a simple 10-item questionnaire that categorizes sleep apnea risk [99], b) *Epworth Sleepiness Scale* [100], a simple 8-item questionnaire to assess sleepiness,

c) The *Sleep Habits and Medical Condition Questionnaire* [101], which includes comorbidities, medication use (including anti-arrhythmic medications), caffeine use, smoking, alcohol use, etc., d) The *Medical Outcomes Survey-SF 36 (MOS-SF)* [102], a standardized tool for assessing general health status, physical and social functioning, and fatigue, e) *Patient Health Questionnaire-9 (PHQ-9),*which assesses depression symptoms. It has ten questions where the answers will be added to indicate severity of depression. The Research Assistant will score depression questionnaires and if the score is indicative of depression (10 or greater) the Research Assistant will then contact a study physician who will determine appropriate referrals and responses and contact the participant to advise him to seek medical/psychiatric consultation. If there was any indication that a participant was in imminent danger to himself or others, the participant would be advised to go to the nearest emergency room for evaluation by a psychiatrist. If signs of depression, anxiety or other psychological disorders were evident without evidence for immediate risk, the participant would be advised to contact a psychologist for further evaluation. f) *Daily ECG/Activity Log,* which will be completed during 7-21 day ECG monitoring including data on sleep/wake times including naps, periods of vigorous activity, caffeine use, alcohol use, smoking, monitor removal time and symptoms (e.g. palpitations, dyspnea, chest pain) g) *Atrial Fibrillation Effect on Quality-of-life,* a 20-item questionnaire that assesses the impact of atrial fibrillation on quality of life.

**11) 6 Minute Walk Test.** Baseline oxygen saturation and heart rate will be recorded. If oxygen saturation >90%, then proceed with protocol. The patient will walk for 6 minutes down the hall at usual pace. Early termination criteria to stop the test if O2 sat drops to ≤80% for 6 consecutive seconds and/or signs and symptoms of dyspnea, chest discomfort, palpitations or any other concerning symptoms. If the test is terminated before 6 minutes is complete, the distance the patient walked should be documented as the 6-minute walk distance for that test. Heart rate should be recorded concurrently with O2 saturation throughout the entire test. Heart rate and oxygen saturation should be recorded before the start of the study, at the end of the six minute walk and for each minute during the post 3-minute recovery period. Using the BORG Scale record rate the symptoms of breathlessness and leg fatigue. Any medications taken prior to the 6 minute walk will be recorded along with dose and time.

**12) Arterial Applanation Tonometry:** Arterial Applanation Tonometry is a noninvasive technique that provides the basis for high fidelity analysis of central and peripheral pulse pressures. Waveforms of radial artery recordings will be calibrated with sphygmomanometric brachial artery mean and diastolic pressure measurements. Radial measurements will be performed on the same arm using the SphygmoCor device after sphygmomanometric pressure is obtained with use of an applanation tonometry probe containing a solid state high fidelity Millar transducer over the radial artery with a minimum of two consecutive measurements to obtain pulse wave analysis results. For pulse wave velocity, lead II ECG (LL, LA, RA) will be performed along with cardotid and femoral applanation tomometry. Orientation and pressure applied to the transducer will be adjusted to optimize applanation of the artery between the transducer and the

underlying tissue. The pulse wave velocity and pulse wave analysis will be assessed and provide measures of arterial stiffness and indices of subclinical atherosclerosis, and correlated with changes in oxidative stress markers. Waveforms will be processed using the SphygmoCor software (model EM3, version CvMS 9.0, Atcor Medical Pty, West Ryde, Australia).) The applanation tonometry measurements will be obtained the morning of the baseline and 3-month follow-up visits using a standardized protocol.

**13) MultiSense™ Monitoring:** The MultiSense™ Monitor is a self-contained, reusable, rechargeable, battery-powered, flexible band-aid-like strip, measuring 10.2x3.0 cm (4x1.2 inches), that is capable of detecting and recording a number of correlated physiological parameters. The monitor is taped to the participant's chest using a FDA approved, disposable, double-backed adhesive tape. The device provides a continuous detection and recording, up to 10 days, of high quality ECG signals. The device also provides pulse synchronous measures of blood oxygen saturation and concomitant indices of physical activity and body position (eg. Upright, or lying on back, stomach, right side, or left side). The double-backed adhesive tape insures motionless, waterproof contact between the skin, the electrodes and other sensors of the MultiSense™ Monitor. At the end of the monitoring period, the accompanying software enables a PC to download the recorded data, display, and analyze the data.

The software mentioned above includes an industry-proven arrhythmia-recognition package provided by a 3rd party (Monebo Technologies). It has been thoroughly tested using standard (MIT-BIH/AHA/NST) arrhythmia databases, and has earned FDA 501(k) approval. The software allows automated ECG analysis and interpretation by providing callable functions for ECG signal processing, QRS detection and measurement, QRS feature extraction, classification or normal and ventricular ectopic beats, heart rate measurement, measurement of PR and QT intervals, and rhythm interpretation.

**14) Magnetic Resonance Imaging (MRI):** An MRI is an electronic picture of your brain created using a strong magnet instead of x-ray energy. The MR scan time will be 60 minutes. The MR scanner makes loud knocking noises. The participant will be provided with earplugs to dull the sounds. A dental impression may be taken of their teeth in order to fashion a bite plate for use during the scan. After the impression is taken, the plate will be fashioned to a device fixed to the table in order to assist in keeping their head from moving during the scan session.

The MRI scan will be performed for research purposes and it is not a complete diagnostic imaging examination. It should not be considered a substitute for a standard clinical MRI evaluation. Because the research MRI cannot exclude abnormalities, no report from the scan will be generated. However, if during the process of reviewing the research MRI an unexpected abnormality is seen, it will be reported to the participant and their physician.

**15) Cognitive Battery and Questionnaires (Only for participants with MRI)**

A complete comprehensive evaluation of memory and thinking skills will be performed on those that participate in the Magnetic Resonance Imaging sub-study. The cognitive battery includes the Mini Mental State Examination (MMSE), an 11-question measure that tests five areas of cognitive function: orientation, registration, attention and calculation, recall, and language., the Rey Auditory Verbal Learning Test (RAVLT), evaluates verbal learning and memory, the Brief Visuospatial Memory Test (BVMT-R), assesses visual memory, the Symbol Digit Modalities Test (SDMT), evaluates cerebral dysfunction, Trails Making Test A and B, a test of visual attention and task switching, Delis-Kaplan Executive Function System (DKEFS) which measures a variety or verbal and nonverbal executive functions, and the Ruff 2 & 7 Selective Attention Test which measures sustained attention and selective attention.

There will be two questionnaires that the participant will be asked to complete. The Beck Depression Inventory, a 21 question multiple choice questionnaires used for measuring the severity of depression and the Edinburgh Handedness Inventory which is a measurement scale to assess the dominance of a person's right or left handedness in everyday activities.

## 9. ANALYTIC PLAN

EXPOSURES: *Primary:* 1) SDB defined by AHI, i.e. number of apneas + hypopneas/hour of sleep. *Secondary:* 1) Hypoxia: % sleep time with SaO2 < 90%, 2) Obstructive Sleep Apnea [obstructive apneas and hypopneas/hour of sleep, obstructive apnea hypopnea index (OAHI)], 3) Central Sleep Apnea [central apneas/hour of sleep, Central Apnea Index (CAI)], 4) Periodic Breathing and 5) EEG arousals [per hour of sleep, Arousal Index (AI)].
OUTCOME: 1) PAF (SA1) and 2) AF burden defined as frequency of AF paroxysms overall and based on self-reported sleep vs. wake (SA2 and 3)
COVARIATES: Age, race, body mass index (BMI, $kg/m^2$), waist circumference (cm), anti-arrhythmic/beta blocker/calcium channel blocker/ACE inhibitor/angiotensin receptor blocker medications, pacemaker, defibrillator, heart failure (NYHA class), BP, diabetes mellitus, left atrial volume, LVEF, caffeine use, alcohol use and smoking (current versus never smoker or pack years) and activity level. When assessing hypoxia as the relevant exposure, pulmonary disorders will be included as potential confounders.

**Table 2. Mediator (SA1) or Outcome (SA3)**

| Domain | Primary | Secondary | Rationale |
|---|---|---|---|
| **Echocardiographic Measures** | Left Atrial Volume ($ml/m^2$) | LA Pressure, LA Systolic Strain, RA area, RA Pressure, LAA Peak Anterograde Flow Velocity (cm/s), Right Atrial Pressure (mmHg), LVEF, LVMI, LVH (concentric LVMI>0.41, eccentric ≤0.41). | Left Atrial Volume predicts risk of AF and PAF [103-106]. Secondary measures also associated with AF. |
| **Autonomic Function (Heart Rate Variability)** | SDNN (ms) (Time Domain HRV Measure) | Time Domain (r-MSSD (ms), pNN50), Frequency Domain [LF (Hz), HF (Hz), LF/HF)], Graphical Parameters (HR patterns, power spectral analysis, Poincaré plots and beat-by-beat HR tachograms) and Novel non-linear measures (e.g. Conditional Entropy [107]). | Reduced SDNN and LF/HF have been identified as AF triggers [40]. |
| **Systemic** | hs-CRP (mg/L) | IL-6 (pg/ml), sIL-6R (ng/mL) | hs-CRP and IL-6 associated with SDB and AF[47-49]. s-IL6R varies with overnight |

| | | | |
|---|---|---|---|
| **Inflammation** | | | hypoxia[46] |
| **Oxidative Stress** | Endothelin-1 (pg/ml) | Urinary isoprostane 8-epi-prostaglandin F2a (ng/mmolCr), Oxidized LDL (U/L) | Endothelin-1 implicated in AF [57-59]. Isoprostane [60-62] and oxidized LDL sensitive to SDB [76]. |

**ANALYTIC METHOD BY AIM.**

**SA1. Quantify the extent that SDB and its subtypes are associated with PAF.** This aim will be addressed by fitting conditional logistic regression models that condition on the matched pair. We will separately analyze each SDB variable (primary variable: AHI) to obtain an estimate and 95% CI of the odds ratio of PAF for a 1-unit increase in the SDB exposure. Non-linear effects between SDB and the log odds of PAF will be assessed using restricted cubic splines. Knots for the spline will be based on the SDB threshold at which PAF risk increases. Unadjusted and hierarchical adjusted models will be performed using the covariates listed above.

The secondary exposure variables (e.g. hypoxia, OAHI, CAI, AI) will be considered in separate models.

***Exploratory Aim 1a.*** **Cardiac structure, autonomic and biochemical variables (Table 2) will explain a proportion of the SDB and PAF relationship.** This aim will be addressed by applying Pearl's mediation formula [108] to the conditional logistical regression model. This approach will allow a causally sound definition of the mediation effect of a given intermediate variable in the relationship between SDB (defined by AHI) and PAF. The approach involves a model for each mediator (e.g. a general linear model for a continuous mediator) and possibly a random effect to account for clustering due to matching and can be thought of as a generalized structural equations model. This approach will allow us to simultaneously assess multiple mediators and will also allow us to consider AHI as a continuous variable. Each model will be fit using likelihood methods and model fit will be checked using appropriate information criteria. After fitting the overall model, we will obtain, for each mediator, an estimate of the indirect effect (e.g. the portion of the overall effect of SDB on PAF mediated through the intermediate variable). A bootstrap resampling technique will be used to obtain a 95% CI for each indirect effect. If the CI does not contain zero, this will indicate statistically significant mediation.

***Exploratory Aim 1b.*** **Explore age interactions in the SDB-PAF relationship.** Each conditional logistic regression model will include an interaction between age and SDB to investigate if age is a modifier of the effect of SDB on PAF, i.e. whether the odds ratio of PAF for a 1-unit increase in SDB varies by age.

**SA2. Examine patterns of PAF burden via 1-week continuous ECG monitoring relative to SDB.** We will model the relationship between mean frequency AF paroxysm variation and SDB (e.g. AHI considered as a continuous variable). A linear regression model will be used to assess possible quadratic and threshold effects of SDB on variation. These models may also include sleep state by SDB interaction terms (along with the state indicator) to examine if the effect of SDB on variation differs by sleep state. Maximum likelihood (based on a normal distribution for variation if appropriate) will be used to fit models, and the Akaike Information Criterion (AIC) will be used to select the best fitting model. Estimates of the effect of SDB on variation (and corresponding 95% CI) will be obtained based on the selected model.

**SA3. Examine the effects of SDB (AHI≥15) treatment for 3 months on AF paroxysm frequency and also measures of autonomic function, cardiac morphology, biochemical measures in those with SDB and PAF.** For this subgroup of SDB (AHI≥15) and PAF, we will perform paired t-tests to compare pre- and post- treatment mean outcomes (Primary outcome: AF paroxysm frequency from 1-week ECG monitoring) and also ECHO and biochemical measures. For any outcome for which a departure in normality is found (based on the Shapiro-Wilk test) but a transformation of the outcome cannot be found to adequately address the issue,

we will use the Wilcoxon signed rank sum test to detect a difference in the distribution of the pre- and post- responses. This assessment is not intended to be confirmatory, but rather to provide estimates of variability and effect sizes to inform future studies/randomized trials. Linear regression models will then be developed to model PAF burden at the 3 month visit by conditioning on baseline PAF burden, baseline SDB, and time from baseline to 3 month visit. Additional adjusted models will carefully consider the covariates list above.

**POWER BY AIM.**

**SA1.** A sample size of 300 will be able to detect an odds ratio of 1.50 with 80% power (and 1.60 with 90% power), assuming a 2-sided alpha level of 0.05, a correlation in AHI between cases and controls of zero and a probability of PAF of 0.20 when AHI is set at its mean value. Note that the odds ratio is interpreted as the odds ratio for PAF for a 1 standard deviation increase in AHI. Alternatively assuming that the probability that AHI≥15 for patients without PAF is 30% [64], a sample size of 150 matched pairs will be able to detect an odds ratio of PAF of 2.13 with 90% power assuming a 2-sided alpha-level of 0.05. Note that this calculation assumes that the correlation for SDB between subjects with and without PAF is 0.20, as suggested by Dupont (1988)[109] when the correlation is unknown. Our prior cross-sectional work demonstrating SDB-AF odds ratio point estimates of 2-4 [1, 2] supports sufficient power to address this aim.

**Exploratory Sub-Aim 1a.** To calculate the power to detect mediation, a difference in coefficients approach will be used [110] which involves the fitting of two logistic regression models that model the association between SDB (AHI≥15) and the log odds of PAF (one with and the other without the potential mediator in the model) to estimate the proportion of exposure effect that is mediated. Assuming a correlation between the adjusted and unadjusted effect estimates of 0.9 and that the probability of PAF in SDB and no SDB is 0.07 and 0.01 respectively (based upon prior data [2]), a sample size of 150/group will be able to detect mediation proportions of 0.5 with 84% power assuming a 2-sided 0.05 alpha-level test. Although power may be reduced depending on the observed imbalance in sample sizes for the SDB and no SDB groups, power will be increased due to matching (not taken into account here to be conservative).

**SA2.** Using a two-sample t-test (2-sided 0.05 alpha-level) to test for a difference in mean variation between the SDB (AHI≥15) and no SDB groups, a sample size of 150 (75 with SDB and 75 without SDB) will detect mean differences within 0.46 standard deviations with 80% power and 0.53 standard deviations with 90% power.

**SA3.** For a log-normally distributed outcome, assuming a correlation between pre- and post-PAF burden of 0.8 and a coefficient of variation of 1.5 (based upon prior data [111]), a sample size of 75 will detect a mean ratio between post and pre PAF burden of 0.83 with 83.1% power based on a paired t-test and a two-sided significance level of 0.05.

## 10. DATA ANALYSIS

EXPOSURES: *Primary:* 1) SDB defined by AHI, i.e. number of apneas + hypopneas/hour of sleep. *Secondary:* 1) Hypoxia: % sleep time with SaO2 < 90%, 2) Obstructive Sleep Apnea [obstructive apneas and hypopneas/hour of sleep, obstructive apnea hypopnea index (OAHI)], 3) Central Sleep Apnea [central apneas/hour of sleep, Central Apnea Index (CAI)], 4) Periodic Breathing and 5) EEG arousals [per hour of sleep, Arousal Index (AI)].
OUTCOME: 1) PAF (SA1) and 2) AF burden defined as frequency of AF paroxysms overall and based on self-reported sleep vs. wake (SA2 and 3)
COVARIATES: Age, race, body mass index (BMI, kg/m$^2$), waist circumference (cm), anti-arrhythmic/beta blocker/calcium channel blocker/ACE inhibitor/angiotensin receptor blocker medications, pacemaker, defibrillator, heart failure (NYHA class), BP, diabetes mellitus, left atrial volume, LVEF, caffeine use, alcohol use and smoking (current versus never smoker or pack years) and activity level. When assessing hypoxia as the relevant exposure, pulmonary disorders will be included as potential confounders.

**Table 2. Mediator (SA1) or Outcome (SA3)**

| Domain | Primary | Secondary | Rationale |
|---|---|---|---|
| **Echocardiographic Measures** | Left Atrial Volume (ml/m$^2$) | LA Pressure, LA Systolic Strain, RA area, RA Pressure, LAA Peak Anterograde Flow Velocity (cm/s), Right Atrial Pressure (mmHg), LVEF, LVMI, LVH (concentric LVMI>0.41, eccentric ≤0.41). | Left Atrial Volume predicts risk of AF and PAF [103-106] Secondary measures also associated with AF. |
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## 11. POTENTIAL RISKS

There are no serious risks to study participation. The study procedures do not place subjects at greater risk of harm or discomfort than those encountered in routine medical care. Intravenous catheter placement and venous sampling is associated with temporary pain at the time of needle insertion, may result in bruising at the needle insertion site and occasional fainting. Blood pressure measurements may cause some discomfort associated with cuff inflation. Sleep monitoring may disturb sleep. The anthropometry uses a measuring tape and metal calipers, and the pinch from the measuring tape and calipers may cause some mild discomfort related to tugging on your skin. This disappears almost immediately after the measurement is made. The attachment of the sensors during sleep monitoring, the MultiSense™ sensor and ECG monitoring may cause skin irritation. However, the latter is usually minor and temporary. The participant may feel some pressure at the site of the probe that is used to perform echocardiography. If you require an echocardiogram with a contrast dye, you will receive an intravenous (IV) administered contrast dye that contains microscopic bubbles during your echocardiogram. This type of contrast dye may, in rare cases, cause severe and potentially life-threatening allergic reactions, including a drop in blood pressure, difficulty breathing, fainting, and internal organ failure. This agent may also cause temporary minor back pain, a headache, and/or itchy skin all of which usually goes away within 30 minutes. You will be closely monitored for about 30 minutes after your echocardiogram is complete by a doctor. There are no significant risks associated with 6-minute walk testing.

Accidental breaching of confidentiality is a recognized risk to all studies that collect patient-level data; however, as described below there are multiple levels of safety measures that are taken to avoid this.

CPAP is the most commonly prescribed treatment for SDB. The use of CPAP can be associated with a number of minor side effects, including nasal congestion, runny nose, dry nose or mouth, eye irritation from mask leaks, skin irritation from the mask, or discomfort from the mask or air pressure. PAP therapy may rarely cause nose bleeds and can cause worsening of a sinus condition or abdominal bloating. The potential for skin and eye irritation is minimized by proper mask fit and use of a humidifier and application of appropriate pressure levels.

The risks of the 7T MRI sub-study are the same as the risks of a routine MRI of the brain. MRI has no known long term risks. There are no known biological risks associated with MR imaging. An MRI may cause possible anxiety for people due to the confined space of the testing area resulting in feelings of claustrophobia and the loud banging made by the machine. Protective headphones that minimize the noise inside the MRI and allow the technologists to talk to you directly to the participant will be used.. Some people feel discomfort associated with lying still within the MR scanner. The use of a bite block may assist in keeping still. There may be local pain and bruising during the placement of the IV. Patients with severe kidney disease are at risk for developing a serious systemic fibrosing disease, NSF. Anyone with severe kidney disease will not be

placed in this study.

If a bite bar is used, there is a possibility of some discomfort from this device including fatigue of the jaw muscles or muscle tiredness of the neck. In addition, a small percentage of people have a very sensitive gag reflex, which may prevent the use of a bite bar.

Since the MRI is a powerful magnet, participants cannot be scanned if they have certain metal devices in their body such as a cochlear (ear) implant, brain aneurysm clip, implanted stimulator device, or cardiac pacemaker. If any devices or implants or if they have a history of metal in their eye or body, the MR technologist will be notified. There is also a risk of injury if metal is brought into the imaging room, which may be pulled into the magnet. No magnetic objects (pocket knives, key chains, necklaces, earrings) from clothes and body will be allowed in the scanner area to prevent any injuries. A Safety zone is established around the MR scanner to prevent objects containing iron from coming into contact with the scanner.

Repeated evaluations of mood and mental status may be slightly frustrating or produce fatigue and boredom. Rest breaks will be provided during cognitive testing, and the study staff members are trained to offer assistance and to discontinue testing if necessary.

**Protection Against Risks**.

***A. RECRUITMENT AND INFORMED CONSENT***

Trained research personnel who have completed necessary certification for human subject research will screen Electrophysiology (EP) Clinic referrals for cases (those with PAF), Cardiology/Internal Medicine clinics for controls (those without AF) and supplementary recruitment from the Echocardiography Database for controls (those without AF). Participants will be screened when presenting for a routine EP clinic visit (or Cardiology/Internal Medicine visit for controls), after the caring physician has ascertained that the patient may be interested in learning more about the study and meeting study staff. When meeting with the research staff (at the clinic visit or another scheduled time), full written informed consent will be obtained. Alternatively, the charts of potentially eligible cases with PAF will be marked to inform the EP physician of eligibility. The physician will be asked to briefly discuss the study with the patient and assess interest. Patients who express interest will be contacted by study staff to further review the study protocol and if interested, a face to face meeting will be arranged to fully review the protocol and obtain informed consent. Consent forms will explain in a lay persons' terminology the nature of all procedures. It will be stressed that participation is voluntary. The research assistant and PI will be available to answer questions. The investigator will also be available to answer questions about the study then or at other times. Participants are also informed that at any time that can request that any stored blood be discarded and not used for research purposes. All activities will be compliant with local IRB and HIPPA guidelines, such as obtaining physician permission to initiate

contact, destroying all pre-screening forms, and obtaining verbal permission, as appropriate for phone interviews. At the beginning of the study, and then periodically, any IRB, HIPAA or privacy requirements that may be in effect at that time also will be checked to assure that plans are compliant with current rules.
Although the clinics will be the primary source of recruitment, controls we will also assess eligibility of individuals from the UHCMC/CCF Echocardiography Database (>10000 ECHOs/year). Dr. Hoit will identify patients who meet matching criteria (left ventricular function, age, sex, race) based upon the cases who have been enrolled in the study. Referring physicians will be notified through emails or phone calls when eligible patients are identified. A letter signed by Dr. Hoit, the PI and the physician who referred the patient for the ECHO will then be sent to these patients describing the research study and providing study contact information. A time will be arranged for the patient to meet the research staff in a face to face meeting fully review the protocol and obtain informed consent.

## *B. PROTECTION AGAINST RISKS*

All participant information will be kept strictly confidential and used for research purposes only. Records will be identified by a subject ID number rather than the subjects' name, and all will be secured in locked files in secured rooms. Computers are located in restricted access research suites; computer information is protected by use of passwords and a firewall. Computer files do not permit linking individual data with medical or other data collected for research purposes. The list of names and identification numbers will be stored separately from other data in locked files to which project staff only will have access. Informed consents explicitly state that information on non-paternity is never revealed. All presentations and publications will only utilize aggregate data.

Data transmission between the Electrophysiology Core and Echocardiography Laboratory and the Administrative Center-CC will be accomplished through a secure, password protected website using a Virtual Private Network (VPN), an established method for secure transfer. Implementation of this method will prevent unauthorized viewing of participant information during data transmission. The Administrative Center-CC and Cores will not receive any information allowing participant identification. Participant data received by the Administrative Center-CC will be identified only by the participant ID.

Risks will be minimized by using hypoallergenic materials, appropriate fitting of nasal CPAP masks and use of a humidifier with the PAP devices. If nasal symptoms are experienced, a study physician will contact the participant and determine if an over-the-counter or prescription decongestant is needed. Visits will be scheduled during a time when the individual is stable and free of acute illnesses. Staff will be trained and certified to perform phlebotomy and other research procedures.

Abnormal findings requiring urgent evaluation may also be identified at the time of the DCRU/CRU visits. All such findings will be labeled as adverse events (AEs) or serious adverse events (SAEs) as appropriate. A blood pressure finding of systolic greater than

180 or diastolic greater than 120 will result in evaluation of the patient in the DCRU/CRU to assess for evidence of end organ dysfunction. Such a finding will lead to immediate transfer to the emergency room for acute blood pressure treatment. Asymptomatic hypertension without evidence of end organ dysfunction will result in contact being made with the patient's primary care physician to notify them of the finding or if the patient does not have a primary care physician, referral to a clinic to provide antihypertensive treatment. Similarly, participants with an increased heart rate (i.e. >120bpm) will undergo medical evaluation by a physician (PI or physician designate) in the CRU. If the participant is deemed unstable, then the participant will be sent to the emergency department.

It is also possible the concerning arrhythmias or concerning symptoms may arise during the 7-21 day continuous ECG monitoring. Cardiology Diagnostic Services has certified ECG technicians who are monitoring the heart rhythms in real-time and also are the contact source for the participants to call 24-7 if there are symptoms during the monitoring. If the technician identified a serious arrhythmia (e.g. high degree atrioventricular block, ventricular tachycardia, atrial fibrillation with rapid rate (>120bpm))or concerning symptoms, then the PI will be contacted directly and the patient will sent to the emergency room for evaluation.

Intravenous catheter and blood sample collection may cause a small bruise that is temporarily uncomfortable, but will disappear in several days. A topical anesthetic will be used to minimize discomfort when necessary. To minimize the possibility of fainting, the intravenous catheter and blood sample collection will take place while the participant is sitting.

A medical release will be given to the participants to sign to authorize release of their health information to their physician if there is any urgent health information that requires immediate attention. Letters outlining results of the sleep study will be sent to the participant and with the permission of the participant will also be sent to the primary care or caring physician to arrange for appropriate treatment and follow-up of sleep disordered breathing.

***Data Safeguarding Plan:*** Data security is addressed at many levels. The database administrator will be responsible for providing and documenting appropriate user access to the study database, preventing such data security problems as: unauthorized internal access to data, external access to data, and malicious intent to destroy data and systems. Data audit software and systems will be used that document data modification, user access associated with modification, data associated with modification, and values prior to modification.
The database administrator will also be responsible for optimizing database performance, reliability and back-up of data. Data transmission amongst the Administrative Center-CCI and the Cores and University of Vermont will be accomplished through a secure, password protected website using a Virtual Private Network (VPN), an established method for secure data transfer, and use of encryption.

Study investigators and staff will be provided with user accounts and passwords. All presentations and publications will only utilize aggregate data. All UHCMC and CCI staff will be trained and certified in the ethical conduct of research.

De-identified study data will be hosted on a server in a private network only accessible through VPN. The computer server will be hardened according to the data security guideline and check-list by the UHCMC Data Security division, with the study database environment reviewed by the UHCMC Data Security team before implementation. Additional security measurements such as 128-bit encryption for SSL, auditing trail, and data encryption at rest will be implemented as appropriate.

## SOURCES OF MATERIALS

The research data will be collected strictly for research and include polysomnography, echocardiography, electrocardiography (from polysomnography and 7-21 day ECG monitoring), anthropometry, blood pressure and blood or urine assayed for markers of inflammation and oxidative stress. Questionnaires addressing medical conditions, medications, sleep habits and symptoms will be administered. Participants will be asked to provide permission to obtain health records for medical encounters in order to adjudicate adverse events if needed. Other data that will be reviewed to ascertain eligibility will be from the echocardiography database and medical records. In addition, for those placed on CPAP, CPAP compliance and residual apnea hypopnea index using CPAP will be ascertained using data electronically stored on the CPAP units participants will be using.

*Linkages to subjects and access to research data*: All research data will be collected on standardized research forms with de-identified ID numbers, but without personal identifiers. Any data collected from medical records will be entered onto case report forms with only visit ID numbers, excluding personal identifiers. Only study personnel will have access to research data which is kept in either locked cabinets or password computers in locked buildings. All personal data will be stored separately from de-identified research data. The primary data files are stored in locked cabinets in secured rooms in this research facility. Bloods will be stored using de-identified bar codes at the Core Laboratory at Case until shipped to University of Vermont. All data transmitted to the Administrative Center-CCI will include no personal identification data. Data received at Administrative Center-CCI will be stored in a dedicated MySQL system in a server within a private network. Access to such data will be through VPN and a dually-authenticated client computer. Access to the query, exploration and exchange interface of the customized Physio-MIMI system will be permitted only to authorized team members using the resident secure, Role-Based Access Control mechanism.

## 12. POTENTIAL BENEFITS TO THE SUBJECTS AND OTHERS AND IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

There are a number of potential benefits to participants. All participants that undergo sleep apnea screening will be provided information on the results of their sleep studies which may be useful in further health management. Participants started on CPAP may further experience direct benefit related to the direct treatment of underlying sleep apnea.
At completion of the study, participants will receive summary results of their sleep patterns from polysomnography, CPAP usage data plus review of their symptoms in the context of normative data (e.g., Epworth sleepiness score) as well as blood pressure findings. If approved by the subject, these results will also be sent to the subject's physician. Participants will also receive a monetary compensation of $75 for each overnight visit to the DCRU/CRU for their time. To receive payment the participant must agree to complete a W-9 form which requires them to provide an address and social security number to the accounting department. This payment may be considered taxable income by the IRS. The participant will be issued a 1099-Misc form only if payment exceeds $600 from all studies in which they are participating, in a fiscal year. Parking costs will be covered and participant will receive dinner and breakfast during the DCRU/CRU stay. We also believe that the gains in understanding of the relationship between SDB and an important outcome, atrial fibrillation, will outweigh any potential risk to the participants. Since the risks of participation for individual subjects are extremely low, and the benefits to society are potentially large, the risk-benefit ratio is more than acceptable. Participants who choose not to participate in this study may seek evaluation and treatment for possible SDB from their physician.

SDB is very common, and may have serious adverse health consequences such as increased risk of clinically significant arrhythmias such as AF and associated increase in morbidity and mortality. The proposed study will further explore linkages between SDB and PAF, as well as help to elucidate the pathophysiology of these associations. Given that SDB is treatable, improved understanding of these interrelationships of SDB and AF could have very significant clinical impact.

### Research Injury Language:

If injury occurs as a result of your involvement in this research, medical treatment is available from University Hospitals and the Cleveland Clinic or another medical facility but you/your medical insurance will be responsible for the cost of this treatment. There are no plans for payment of medical expenses or other payments, including lost wages, for any research related injury.

## 13. SAFETY MONITORING

### Data and Safety Monitoring Plan

***Database Design, Data Management and Data sharing:*** The Center will integrate the collected PSG, ECHO and ECG (from PSG and 7-21 day ECG monitoring) raw signal data in a customized instance of Physio-MIMI system developed by Dr. Zhang (PI) as an NCRR-funded Multi-CTSA-site project for federated access to PSG data in sleep labs. Our approach will allow the reuse of several Physio-MIMI components: (1) its built-in user-friendly query interface called VISAGE with a web-based case-control exploration engine; (2) its Sleep Domain Ontology (SDO) for semantic interoperability and data quality control; and (3) its flexible interface with a relational database system. To achieve this, we will (1) design a study-specific data model for storying the rich study data in MySQL tables; (2) extending the data dictionary to account for additional terms not covered in SDO; (3) interfaces for data collection, data conversion and data exchange. Data sharing will be facilitated by Physio-MIMI's extensible Role-Based Access Control mechanism, which allows for fine-controlled access to data: based on specified source tables, specified data fields, and counts limit. Curated data and application tools will be made available for long-term data sharing and dissemination.

**Adverse Event Reporting.** Reporting of adverse events will follow standard reporting guidelines. Each event will be classified according to its severity, whether it was expected or unexpected, and its likelihood of relatedness to the study.

**Immediate and Urgent Medical Referrals** are medical conditions that require timely identification and possible treatment. Once identified either at the DCRU/CRU, the PI will be contacted within 24 hours (for Urgent Referrals) or before discharge from the DCRU/CRU (for Immediate Alerts). If confirmed as meeting Referral criteria, the Immediate/Urgent Referral form is completed which indicates which follow-up activities occurred, including whether the participant and or his/her physician was contacted, whether contact was by telephone and/or letter, and what recommendations were made.

A Safety Monitor will be assigned to the study (UHCMC, Kristie Ross MD and at CCF, Harneet Walia MD), who will review adverse events and data targeted by the research coordinator to assist with adverse event adjudication and also to ensure that no physiological (e.g., blood pressure) findings warrant immediate intervention. If so, the participant/family will be contacted and advised of the findings and offered assistance with appropriate referrals. Adverse events (classified by severity and unexpected/expected) will be defined and reported to the IRB, NIH, and the Safety Monitor in accordance with the rules regulating each severity class (expected vs. unexpected, serious vs. other; related vs. unrelated).

On a weekly basis, the P.I. will review all data collected, including logs of phone calls between the coordinator and participant, ECG data and biochemical levels from blood tests.

If any serious adverse events occur, these will be reported to the IRB and NIH. All data from clinical assessment, physiological measurements, and biochemical measurements will be transferred to a computerized database in a dedicated instance of Physio-MIMI. Data completeness and accuracy are monitored by: 1) technician review of data at the time of the study; 2) review of raw and processed data by the coordinator prior to data entry; 3) data entry with form-configured screens and on-screen logic/range checks; 4) double data entry; 5) review of outliers and additional logic checks with statistical software. Discrepant entries will be verified by reviewing the raw data. Manuals will detail the protocol and all procedures, including recruitment, data collection, data transfer, specimen collection, and data management.
The P.I. will assure that research staff (research coordinator, research assistant, polysomnologist, sleep technologist and CRU nurses) undergo rigorous training, undergo re-training and re-certification periodically (at least every 6 months or more frequently if excessive variation is seen). Sleep studies are scored and processed by certified polysomnologists blinded to the identity of the participant using standardized procedures.

Confidentiality will be maintained during the trial including monitoring, preparation of interim results, review, and response to monitoring recommendations. The P.I. will be responsible for the close review and evaluation of the progress of this clinical trial, including periodic assessments of data quality, participant recruitment, accrual and retention, participant risk versus benefit, validity and integrity of the data, and other factors that can affect study outcome. The P.I. will protect the confidentiality of the study data and the results of monitoring.

## Data and Safety Monitoring Board

The DSMB will consist of three independent scientists: a statistician (Dr. Lester Kirchner, Geisinger Health System, Danville PA), electrophysiologist/cariologist (Dr. Joseph Marine, John Hopkins University School of Medicine), cardiovascular epidemiologist (Dr. Susan Heckbert, University of Washington, Seattle WA) and sleep specialist (Dr. Ulysses Magalang, Ohio State University Medical Center) and Executive Chair (Dr. Sean Caples, Mayo Clinic, Rochester MN) , all of whom are external to the PI's institution and will convene via biannual conference calls (and more often if warranted by safety considerations) to ensure the establishment of a plan for data and safety monitoring for the continuous positive airway pressure intervention, conduct ongoing monitoring and ensure that monitoring is timely and effective. Monitoring will also consider factors external to the study when interpreting the data, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study. A Medical Monitor will also be assigned (UHCMC - Dr. Kristie Ross, and CCF – Dr. Harneet Walia, Sleep Medicine specialists) to assess and categorize adverse events as they arise.

A DSMB will be established to ensure the safety of all participants involved in the study and to ensure the validity and integrity of the data. The P.I. will ensure that the research team responds to recommendations that emanate from monitoring activities. The members of the DSMB will not be associated with the research aspects of the trial, and will be entirely independent of the investigators involved in the study. The DSMB will also

protect the confidentiality of the trial data and the results of monitoring. The DSMB will evaluate the progress of this study, including periodic assessments of data quality and timeliness, participant recruitment; accrual and retention, participant risk versus benefit and monitor adverse events.

The three independent scientists will be voting members and will be appointed for a 2 year renewable term by the PI in consultation with the other study investigators and NHLBI staff. The statistician (Dr. Kirchner) will serve as the Board's Executive Secretary and will have primary responsibility for preparing a written summary of the Board's discussions and recommendations. Prior to the first meeting, the Executive Chair will document that the members do not have any conflicts of interest. The PI will be responsible for coordinating activities of the DSMB including: arranging DSMB meetings and communications and identifying and reviewing materials to be presented to the DSMB. The primary responsibility of the DSMB will be to monitor the progress of the study and recommend modifying the trial or terminating the trial as appropriate. Concerns that might dictate modification or termination of the study include participant safety, outcome data, data quality, integrity, intervention efficacy, and recruitment performance.

The DSMB Executive Chair and PI as well as the study staff will prepare the interim reports issued to the DSMB. Those reports prepared by the PI and the study staff will include reports of adverse incidents, reports of trial participant recruitment and follow-up, and reports from related studies. The purpose of the first meeting will be to evaluate the protocol and establish guidelines for monitoring the study. Subsequent meetings in the first year and following years will be for the purpose of data and safety monitoring. If any Board member feels it is necessary to meet on a face-to-face basis in the subsequent years, a meeting at the study site will be convened. All reports to be discussed at the meeting will be mailed to all Board members and NHLBI Program Scientist at least one week prior to the meeting. The format of the meetings will be such that they consist of an open session and a closed session. Trial investigators, trial staff, staff of the institution and NHLBI staff may attend the open session.

The PI and study staff will be responsible for identifying, reviewing, and reporting *adverse events* and *unanticipated problems* to the IRB and NHLBI. The PI will report the following within one week of occurrence: 1. Unanticipated problems or unexpected serious adverse events that may be related to the study protocol, 2. IRB-approved protocol or consent form revisions that indicate a change in risk for participants, 3. A summary of recommendations made by the DSMB or other monitoring entity and (if applicable) the action plan for response and 4. Notice of any actions taken by the IRB or regulatory bodies regarding the research and any responses to those actions. All personal identifiers must be removed from any documents sent to NHLBI. In the annual progress reports, the PI will address the adherence to the DSM plan, provide a summary of any DSM issues that have occurred during the previous year and describe any changes in the human subjects research or to the DSM plan that affect risk. Modifications to the human subjects research or DSM plan will be submitted to the NHLBI Medical Officer prior to implementation of the change in study practice.

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