# GENOMICS AND POSTOPERATIVE ATRIAL FIBRILLATION

by

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Over 800,000 people undergo Coronary Artery Bypass Graft (CABG) surgery annually for management of their Coronary Artery Disease (CAD) worldwide. Postoperative Atrial Fibrillation (PoAF) is a complication with a 30%-40% incidence after CABG surgery. PoAF is believed to cause additional complications and also possibly increase the total duration of hospital stay in these patients. It is also believed that local and systemic inflammation play a role in the development of this complication and the -174 G/C IL-6 gene polymorphism modulates the inflammatory response. A study is underway to evaluate any plausible association between the -174 G/C IL-6 genotype and PoAF in CABG patients at the Presbyterian University Hospital, Pittsburgh, PA.

As part of an interim analysis of the study with a proposed enrollment of 380 subjects, the -174 G/C –Interleukin 6 genotype variant was determined in 91 CABG patients. Heart rate and rhythm were monitored continuously until discharge. Twenty nine subjects (31.84%) developed PoAF. Multivariate logistic regression analysis included -174 G/C genotype, age, race, sex and other risk factors that are considered to be associated with PoAF. The analysis of the collected data revealed age as a significant predictor of PoAF. Subjects older than 65 years had 2.7 times higher odds of developing PoAF as compared to subjects who were 65 years old or younger. The -174 G/C gene variant or any other predictors were not significantly associated with PoAF in these 91 CABG patients. The length of postoperative hospital stay was not found

to be significantly associated with the presence of PoAF. However, this could be attributed to aggressive management of PoAF by our clinical care team.

With 15.3 million prevalence (USA-yr 2004) and the leading cause of mortality, CAD and its management are of enormous public health importance. PoAF needs to be explored further due to its ill defined etiopathology and the increasingly older patient population that undergo CABG surgery for their CAD. Any further knowledge derived on PoAF would pave way for better anticipation and prevention of this complication.

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## PREFACE

Genomics Competitive Medical Research Fund (CMRF), University of Pittsburgh, for funding support to conduct the genomic analyses. Genomics and Proteomics Core laboratories of the University of Pittsburgh, 3343 Forbes Ave, Pittsburgh, PA-15213, for sample processing and genomic analyses.

Department of Adult Cardiac Surgery, Heart Lung Esophageal Surgery Institute, Presbyterian University Hospital, University of Pittsburgh Medical Center for execution of the study and Dr.Wei for allowing me to turn the interim analysis into my thesis project.

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My family for their understanding and valuable support during the last few years when I learned to balance my full time career and my part time graduate education along with the family responsibilities.

# 1.0 INTRODUCTION

Coronary artery disease (CAD) is a leading cause of mortality in the USA. As per the American Heart Association data for the year 2004, CAD was prevalent in 15.3 million people and was the cause of death for $\simeq$ 2.4 million. Based on the co morbid factors CAD is treated by medical management, percutaneous coronary intervention or surgical revascularization with coronary artery bypass grafting (CABG).

Surgical management of these patients is a complex process due to the high prevalence of co-morbidities in this patient population. CABG surgery is indicated in people with triple vessel disease and seems to offer a survival benefit for patients with impaired left ventricular function (1). More than 800,000 people undergo CABG surgery every year worldwide. Even though it is considered an essential part of the CAD management, CABG surgery is associated with complications such as bleeding, arrhythmias, embolism, stroke and death. One of these complications- atrial arrhythmia known as Atrial Fibrillation (AF) is focused in this study.

## 1.1 CARDIAC SURGERY AND POSTOPERATIVE ATRIAL FIBRILLATION

### 1.1.1 Atrial Fibrillation

Atrial fibrillation (AF) is defined as asynchronous atrial depolarizations without effective atrial contraction. AF is also characterized by the presence of rapid, irregularly timed impulses reaching the Atrio-Ventricular (AV) node from the atrium and is associated with irregularly timed ventricular response. The atrial rate can be between 350 to 600 beats per minute. Due to the low pass filtering properties of atrio-ventricular (AV) node, the ventricular response is much slower.

Atrial fibrillation could be Paroxysmal when the duration of episodes can range from a few seconds to hours but usually terminates spontaneously within 48 hours; Persistent when AF lasts for days and does not terminate spontaneously, but normal sinus rhythm can be restored by electrical cardioversion and/or drugs; Permanent: when patients have a long standing history of AF (greater than one year), despite prior attempts of cardioversion (electrical and/or pharmacologic).

Atrial fibrillation is present in approximately 1% of the American population (2, 3) and in 6% of the population above the age of 65 years (4, 5). These numbers however do not reflect the incidence of AF in post cardiac surgical patients.

### 1.1.2 Postoperative AF (PoAF)

Various studies have shown that Postoperative AF increases the length of hospital stay; markedly increases cost and potentially increases the incidence of post-operative stroke (2, 6, and 7). Nationally, postoperative atrial fibrillation (AF) is a common complication of cardiac surgery, occurring in 25% to 40% of patients and entails total costs of billions of dollars per year to treat (8). At University of Pittsburgh Presbyterian University Hospital, over the past few years, our patients experience AF in the postoperative period at an average rate of 30% -40%. The incidence of postoperative AF increases with the age of the patient. The average age of the patient that undergoes CABG surgery has been on the increase due to the improved longevity of the population. Prevention of postoperative AF holds a significant role in patient care in the cardiac surgery patient population. This prevention can be achieved by learning more about the risk factors that may predispose patients to this complication. In previous literature, several demographic risk factors including senility, diabetes, COPD, have been found to be significantly associated with postoperative AF.

Coronary artery bypass grafting is believed to be associated with local inflammation due to the surgery and also systemic inflammatory response possibly due to the cardiopulmonary bypass (CPB). Several factors are believed to modulate the inflammatory response. Research studies are being done to measure inflammatory markers such as the cytokine interleukin 6 (IL-6) and C - reactive protein in the cardiac surgery patient population. Interleukin 6 is a pro-inflammatory cytokine and major mediator of acute phase inflammatory response. It is also believed that some people are genetically predisposed to have a heightened inflammatory response. There have been studies performed to assess the relationship of genetic polymorphisms

that could affect the expression IL-6. The association between the -174 G/C polymorphism and the high production of IL-6 has enabled some investigators to anticipate and treat the patients prophylactically to reduce the IL-6 levels (9).

It has been suggested that inflammation might play a role in the post cardiac surgery complications and there could be a genetic predisposition to develop postoperative complications. There have been studies performed in Europe to ascertain this association and it has been found that the -174G/C Interleukin-6 promoter gene polymorphism appears to modulate the inflammatory response to surgery and to influence the development of postoperative AF. These data suggest an inflammatory component of postoperative atrial arrhythmias and a genetic predisposition to this complication (10). This polymorphism has also been associated with the development of postoperative renal and pulmonary complications (11). There have been other single nucleotide polymorphisms (SNP) that are also associated with high IL-6 production after CABG surgery (12).

## 1.2 GENOMICS

Genomics is a newly developing field that appears to have a lot of implications in health conditions. Cardiac Surgical procedures are complex and cardiac surgery patients have several co morbidities that affect their postoperative course adversely. There were studies focused on the association of genetic predisposition to surgical outcomes and this new field of perioperative genomics has been gaining significance in the last few years (13).

### 1.2.1 Single Nucleotide Polymorphism (SNP)-174 G/C

In 1998, a functional polymorphism in the promoter region of the IL-6 gene at position –174 (–174G>C) was identified (14) An in vitro study using transfected human cell line cells reported higher baseline IL-6 levels in cells with the G construct compared with those transfected with the *C* allele. Stimulation with lipopolysaccharides or IL-1 resulted in a significantly increased IL-6 transcription rate; this effect, however, was restricted to cells with the *G* allele. In another in vitro study using anti-CD3/CD28–stimulated peripheral blood lymphocytes, IL-6 concentrations were three times higher among carriers of the *G* allele (15). Additionally, some but not all in vivo studies found higher plasma IL-6 concentrations in subjects with the GG genotype than among homozygote for the *C* allele (16).

Recently, the –174G>C gene polymorphism has even been suggested as a risk factor for coronary heart disease, carotid atherosclerosis and stroke (17-20). Research studies focused on probable association between various disease processes and presence of the different genotypes of this polymorphism yielded ambivalent results about the protective effects of genotypes GG and CC (21).

## 1.3 STUDY DESIGN, STATISTICAL CONSDIERATIONS

University of Pittsburgh Institutional Review Board approval was obtained prior to study start. All the enrolled subjects were consented with an IRB approved consent form.

The study is designed as an observational study to investigate the association between the genotypes of a single nucleotide polymorphism (of the IL-6 gene at position 174) and the occurrence of post operative AF in the post CABG surgery patients. Research related tests include genotype analysis for the presence of a -174 GG, CC, GC genotypes of the IL-6 gene. The target population for the study is post Coronary Artery Bypass Graft (CABG) Surgery patients at Presbyterian University Hospital, Pittsburgh. The proposed total enrollment is 380 subjects.

In contrast to a clinical trial where causal effects can be attributed to the intervention due to the randomization, for observational studies such associations could be tentative. In addition to this, an association in itself does not imply causality. However, observational studies yield valuable data in a non-invasive way and can be considered as stepping stones towards randomized clinical trials.

Based on the previous published literature, it is anticipated that of the three genotypes of this polymorphism, the frequency of the GG genotype would be higher in this subjects, due to the fact all of these patients were diagnosed with coronary artery disease and the GG genotype has been suggested as a risk factor for CAD. However there have been a few studies where the GG genotype appears to offer protection against Type II diabetes which renders people more prone for coronary artery disease (21).

## 1.4 REGULATORY AND COORDINATION

This research is supported by the University of Pittsburgh Genomics Competitive Medical Research Fund (CMRF) in which I (Dr.Raghu) am a part of the study's research team. A substantial amount of effort was rendered by me towards the study design and proposal submission for the CMRF. The Institutional Review Board approval process including

preparation of protocol, informed consent document and review board communications were handled entirely by me. The IRB approved protocol is attached in Appendix A.

# 2.0 METHODS

## 2.1 RECRUITMENT PROCEDURE AND SUBJECTS

Patients who underwent the CABG surgery at the Presbyterian University Hospital were approached for participation. Since this study was focused on genomic analysis for the SNP, the subjects were consented during either pre or post operative period. A 6 ml blood sample from each consented subject was collected for the study.

### 2.1.1 Genomic techniques

As per the standard of the Genomics lab, upon receipt in the Core laboratories all blood specimens were assigned a GPCL study number, logged in and then aliquoted. One three ml aliquot was immediately used for DNA extraction using the Gentra Systems Puregene DNA purification protocol and reagents with no deviation from the Gentra protocol. The remainder of the specimen was frozen at -80 ˚C for future use. Upon completion of the DNA extraction the DNA was reconstituted in either TE (10mM Tris pH 8, 1 mM EDTA) or nuclease free water. The rehydrated sample was heated to 65°C for 1 hour and then an aliquot is diluted and the absorbance read at λ260 nm and λ280 nm using a UV spectrophotometer in order to assess DNA

purity and concentration. The concentrated DNA in TE was designated as stock and stored at 4°C or in water at -80°C. Working dilutions in water or TE were stored at 4°C.

The concentrated DNA was diluted in nuclease free water to10 ng/µl. The diluted DNA was arrayed into 96 well plates and frozen at -20°C until use .The Single Nucleotide Polymorphism (SNP) Analysis were done by the TaqMan® method.

## 2.2 DATA ACQUISITION

### 2.2.1 Data collection

The predictor variables of interest were selected based on prior literature review. The data on demographics and variables of interest were collected from medical records of the enrolled subjects. The genomics SNP analysis to determine the genotype was performed as a research related procedure and the results were not added to the clinical medical records of the subjects. The data on the outcome of interest-development of AF during the postoperative period was also collected form the subjects' medical records. This was possible because as part of their routine care the subjects' heart rhythm is always monitored in the intensive care unit and 24 hour telemetry at the step down unit. Any occurrence of AF that resulted in treatment is counted as an incidence of AF.

### 2.2.2 Data set

Data were collected from 91 subjects who consented for study participation. The data collection included demographics such as age, race, gender, medical history on chronic diseases including presence of diabetes, hypertension, dyslipidemia, smoking history, chronic obstructive pulmonary disease (COPD), previous occurrence of AF, treatment with B blockers prior to the surgery, preoperative left ventricular ejection fraction (LVEF), previous myocardial infarction, redo sternotomy, usage of cardio –pulmonary bypass (CPB), number of coronary artery bypass grafts, PoAF and the genotype of the -174 G/C SNP. Table 1 presents a description of the major variables included in this study.

## Table 1. Description of the Variables

| Name | Predictors |
|---|---|
| ID | Subject ID |
| PoAF | Postoperative Atrial Fibrillation (outcome variable) |
| Age | Age in years at diagnosis time of surgery |
| Race | Subject Race |
| Gend | Gender |
| Dbts | Presence of Diabetes |
| Hyprtnsn | Presence of Hypertension |
| Dyslpdmia | Presence of Dyslipidemia |
| COPD | Presence of Chronic Obstructive Pulmonary Disease |
| RemteSmk | Past Smoking |
| Currntsmk | Current Smoking |
| PreAF | Previous AF episode |
| PrevMI | Previous Myocardial Infarction |
| PreBetaBlkr | Treatment with B blockers prior to surgery |
| SNP | Single Nucleotide Polymorphism at the -174 G/C location |
| Redo | Redo Sternotomy |
| CPB | CardioPulmonaryBypass |
| Grafts | Number of grafts used |
| Hospital Stay | Number of days of Hospital Stay |
| Other adverse effects | Other adverse events during the hospital stay |

## 2.3 STATISTICAL ANALYSIS

This is an interim analysis performed on the data from 91 subjects. It is anticipated that this analysis would provide us with insight on the prevalent genotypes of the polymorphism studied and also a profile of the enrolled subject population.

The analysis plan consisted of evaluating variables of interest, computing descriptive statistics, and fitting logistic regression model to assess the association between predictor variables and the outcome variable- PoAF. The significance level (α) for all analyses was 0.05 unless otherwise stated. Analyses were carried out using SPSS® software version 15.0. A secondary analysis included a linear regression analysis between the length of hospital stay and PoAF.

### 2.3.1 Statistical Models

Logistic regression was selected to analyze the study data. The outcome variable is dichotomous- presence or absence of PoAF. This model estimates odds ratio which is the probability of developing PoAF over probability of not developing PoAF. The mathematical equation for the logistic regression model is given by

$$\text{logit}[\theta(x)] = \log\left[\frac{\theta(x)}{1-\theta(x)}\right] = \alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i$$

Where θ is the probability of the presence of PoAF, x represents a vector of risk variables and β represents the log odds ratio associated with the $i^{th}$ risk variable.

Linear regression analysis was performed to assess any significant association between the duration of hospital stay and PoAF.

### 2.3.2 Evaluation of Variables

The data consisted of categorical predictor variables exclusively, except for the age and LVEF of the subjects. Due to the fact that increased age seem to predispose the surgery patients for postoperative complications, a new variable 'Age Category' was created and 'age' was dichotomized into two categories, > 65 years and 65 years and younger. Since it is believed that people with better left ventricular function (LVEF $\geq$ 50%) would have better outcomes after the surgery, the LVEF was categorized into two groups, < 50% and $\geq$50%. Categorical variables were binary except for SNP and 'Number of grafts' which had 3 and 6 values respectively.

# 3.0 RESULTS

## 3.1 DESCRIPTIVE STATISTICS

Descriptive statistics were computed for all relevant variables. The study subject population was predominantly male (69%), Caucasian (95.6%), current non-smokers (87.91%), hypertensive (82.42%) and first cardiac surgery (92%). Only about 46% were diabetics. The mean age was 65.5 years with a minimum of 41 and a maximum of 86 years. Patients $\leq$ 65 yrs and > 65 years were about equal (47.25%/ 52.75%) as was the history of previous MI present in 47.25%. PoAF was observed in 29 of the 91 subjects (31.84%) falling within the expected range of incidence (30-40%). There were 16 missing values for variable LVEF.

Tables 2, 3 and 4 and Figures 1-6 present these descriptive statistics.

## Table 2. Categorical Outcome/ Predictors

| Predictors | Value | Count – percentage |
|---|---|---|
| Post Operative AF (Outcome) | No | 62 (68.13%) |
| | Yes | 29 (31.87%) |
| PreOperative AF | 0 | 86 (94.51%) |
| | 1 | 5 (5.49%) |
| 174 G/C SNP | CC | 41 (45.05%) |
| | GC | 39 (42.86%) |
| | GG | 11 (12.09%) |
| Age Category | < = 65 years | 43 (47.25%) |
| | > 65 years | 48 (52.75%) |
| Male | Female | 28 (30.77%) |
| | Male | 63 (69.23%) |
| | 2 | 5 (5.49%) |
| | 3 | 32 (35.16%) |
| Number of grafts | 4 | 36 (39.56%) |
| | 5 | 16 (17.58%) |
| | 6 | 2 (2.2%) |
| Preop Beta Blockers | No | 35 (38.46%) |
| | Yes | 56 (61.54%) |
| Previous MI | No | 48 (52.75%) |
| | Yes | 43 (47.25%) |
| Race | Other | 4 (4.4%) |
| | White | 87 (95.6%) |
| Remote Smoking | No | 41 (45.05%) |
| | Yes | 50 (54.95%) |
| Repeat Sternotomy | No | 84 (92.31%) |
| | Yes | 7 (7.69%) |
| Current Smoking | No | 80 (87.91%) |
| | Yes | 11 (12.09%) |
| Diabetes | No | 49 (53.85%) |
| | Yes | 42 (46.15%) |
| Hypertension | No | 16 (17.58%) |
| | Yes | 75 (82.42%) |
| Dyslipidemia | No | 15 (16.48%) |
| | Yes | 76 (83.52%) |
| COPD | No | 77 (84.62%) |
| | Yes | 14 (15.38%) |
| CPB | No | 16 (17.58%) |
| | Yes | 75(82.42%) |
| LVEF | <50% | 25 (33.3%) |
| | > 50% | 50 (66.6%) |

## Table 3. Continuous Predictors

| Predictor | Mean | Max | Median | Min | Mode |
|---|---|---|---|---|---|
| Age | 65 | 86 | 66 | 41 | 65 |
| Hospital Stay- Number of days (Outcome) | 8 | 50 | 7 | 3 | 5 |

## Table 4. Descriptive for Age in PoAF subgroups

| | PoAF | N | Mean | Std Dev | Std error |
|---|---|---|---|---|---|
| Age(≤65) | No | 62 | 63.60 | 10.534 | 1.338 |
| (>65) | Yes | 29 | 69.55 | 8.724 | 1.620 |

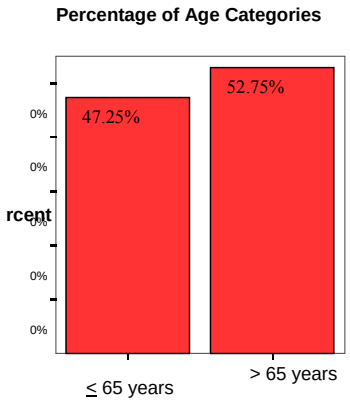


**Figure 1. Bar chart of Age categories**

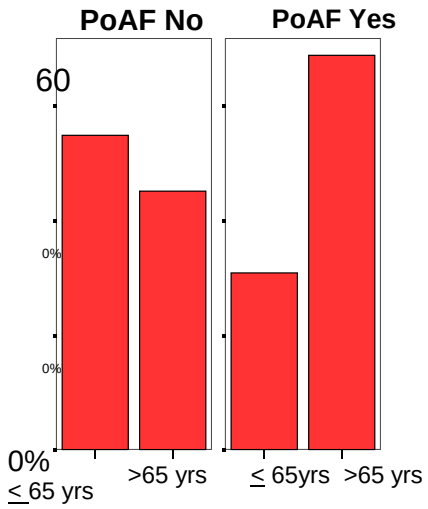


**Figure 2. Bar chart of Age categories subset based on PoAF**

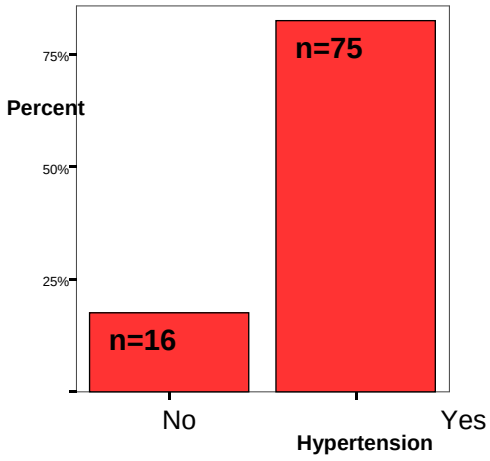


**Figure 3. Bar chart of Hypertension**

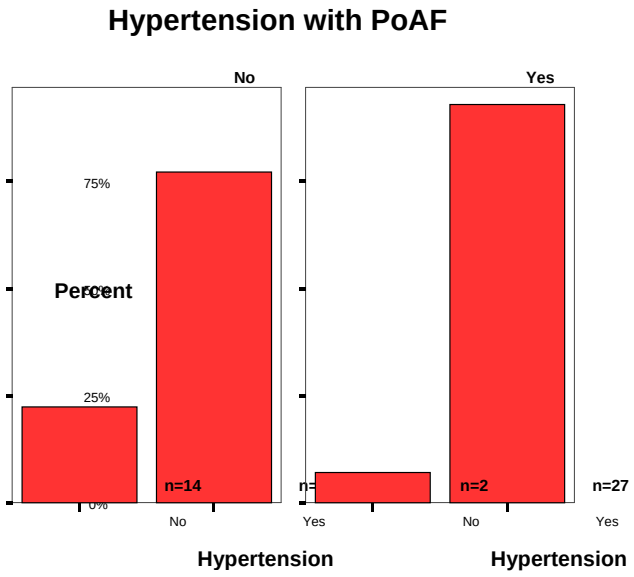


**Figure 4. Bar chart of Hypertension subset based on PoAF**

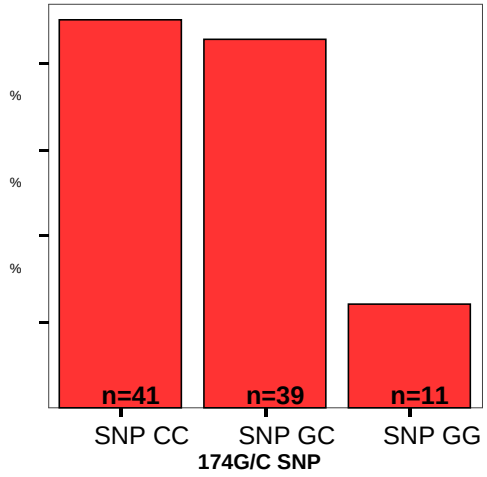


**Figure 5. Bar chart of SNP genotypes**

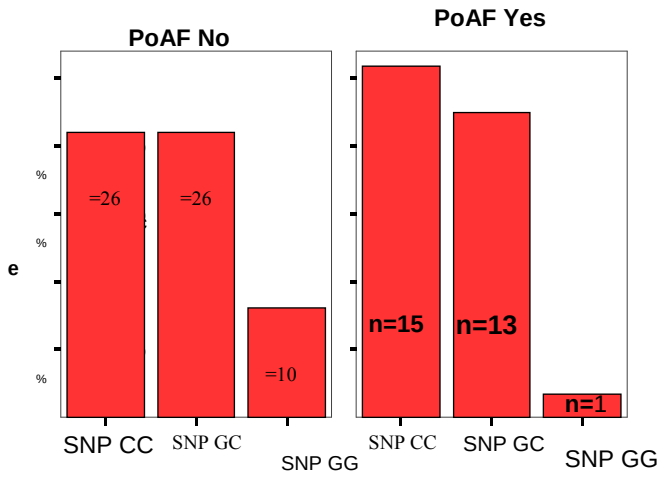


**Figure 6. Bar chart of SNP frequency- subset based on PoAF**

Both age categories are about equal proportions however there seem to be a predominance of age group >65 years in the subset that developed PoAF. The t –test to compare mean age between the PoAF group (69.55 yrs) and no PoAF group (63.60 yrs) was not significant (p-value of 0.10).

Table of chi-square statistics between the outcome and predictor variables revealed age category as a significant predictor (p-value 0.034). None of the other predictors seem to be significantly associated. Predictor variable hypertension had a p-value of 0.067. The chi-square values are presented in Table 5.

**Table 5. Pearson Chi-Squares for the association between predictors and the outcome**

| Variable | Chi-square | p-value |
|---|---|---|
| **Age Category** | **4.492** | **0.034** |
| **Hypertension** | **3.354** | **0.067** |
| **Repeat Sternotomy** | **2.231** | **0.135** |
| **Preoperative AF** | **1.928** | **0.165** |
| **174 G/C SNP (2 df)** | **3.087** | **0.214** |
| **LVEF** | **0.784** | **0.376** |
| **Race** | **0.634** | **0.426** |
| **CPB** | **0.422** | **0.516** |
| **Male** | **0.202** | **0.653** |
| **Remote Smoking** | **0.178** | **0.673** |
| **Current Smoking** | **0.116** | **0.733** |
| **COPD** | **0.113** | **0.737** |
| **Previous MI** | **0.1** | **0.751** |
| **Diabetes** | **0.03** | **0.862** |
| **Dyslipidemia** | **0.018** | **0.894** |
| **Preop Beta Blockers** | **0.005** | **0.943** |
| **Number of grafts** | **0.492** | **0.974** |
| **SNPGG** | **2.990** | **0.084** |

## 3.2 REGRESSION ANALYSES

### 3.2.1 Logistic regression

Logistic regression analysis with a full model revealed 'age category' as a borderline significant predictor (p-value 0.055, OR-3.41, 95% CI for OR (0.97, 11.98)) for PoAF. There were 16 missing values for LVEF resulting in removal of these data points from the analysis.

The analysis was repeated without LVEFCAT (p-value 0.652) and revealed 'age category' as a significant predictor (p-value 0.031, OR-3.38, 95% CI for OR (1.12, 10.22)). Backward stepwise analysis was also performed to determine the significant predictors. The significance level was set to 0.10 for variable removal, which revealed 'age category' as a significant predictor (p-value 0.037, OR-2.7, 95% CI for OR (1.06, 6.85)) as well. Model fit were assessed by Hosmer and Lemeshow Test. Table 6 present the variables with p-values under 0.10 derived from the analyses.

**Table 6. Results from Logistic Regression Analyses**

| MODELS | Predictors with <0.10 p-value | | | | |
|---|---|---|---|---|---|
| | Age (continuous instead of categorical) | Age Category (categorical instead of continuous) | Hypertension | PreOpAF | SNPGG |
| **Full Model** [a] | **0.019** | **0.055** | **0.062** | | |
| **Models Without LVEF** | | | | | |
| **Full Model** | **0.014** | **0.031** | **0.081** | **0.069** | |
| **Full Model SNPGG instead of SNP** [b] | **0.015** | **0.031** | **0.081** | **0.066** | |
| **Full Model SNP CC instead of SNP** [c] | **0.015** | **0.036** | | **0.087** | **0.079** |
| **Full Model SNPGC instead of SNP** [d] | **0.011** | **0.032** | **0.10** | | |
| **Single predictor- Age** | **0.013** | | | | |
| **Singlepredictor-AgeCategory** | | **0.037** | | | |

a- SNP with 2 df, GG, GC and CC , b- SNP GG vs nonGG , c- SNP CC vs nonCC, d- SNP GC vs nonGC.

Because the study is designed to focus on the association between the genotypes and PoAF, additional variables SNPHMZGG, SNPHMZCC and SNPHTZGC were created. SNPHMZGG was coded 1 for GG homozygote and 0 for the GC heterozygote and the CC homozygote. SNPHMZCC was coded 1 for CC homozygote and 0 for the rest. SNPHTZGC was coded 1 for the heterozygote and 0 for the homozygote. Logistic regression with the full model was repeated with each new SNP variable substituting variable SNP.

Age Category still remained as a single significant predictor. None of the SNP categories- homozygote or heterozygote was significant predictors. The SNPGG homozygote was the least frequent of the polymorphisms in the 91 study subjects and had the following values- pvalue of 0.079, OR 0.12, 95% CI for OR (0.01, 1.28). Table 6 presents the variables with p-values under 0.10 derived from the analyses.

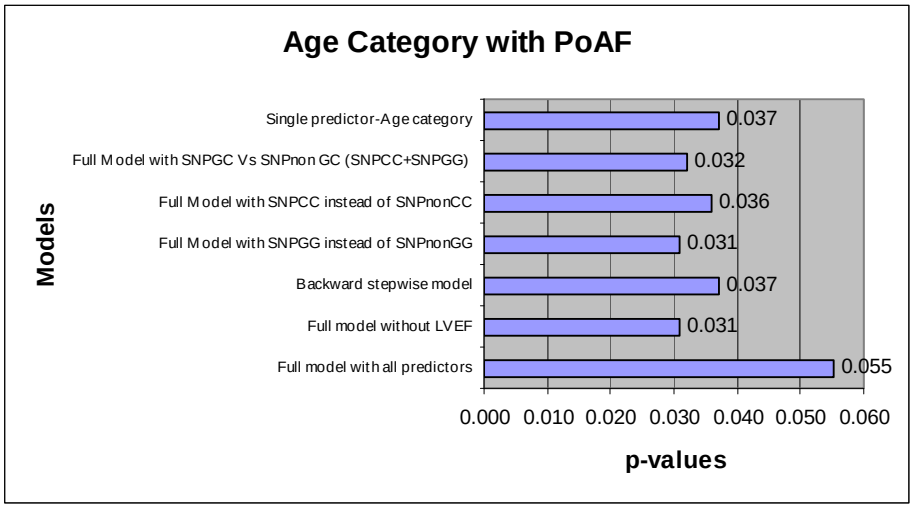


**Figure 7. Bar chart of p-values - Age Category**

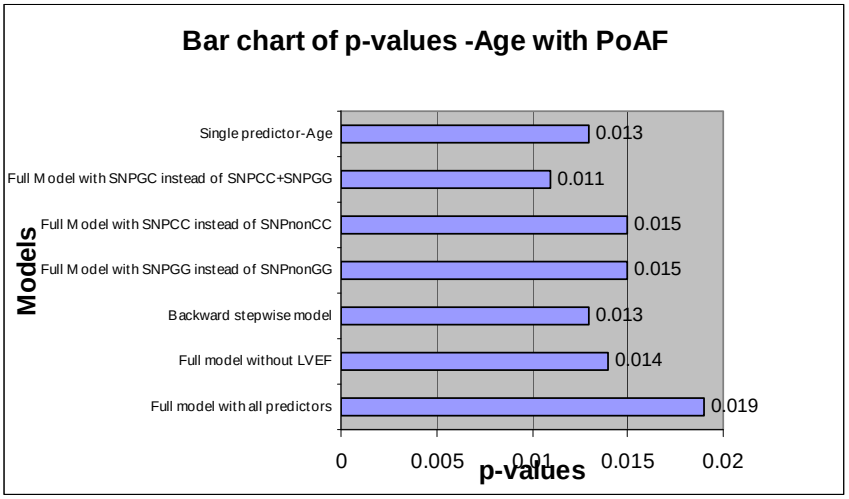


**Figure 8. Bar chart of p-values -Age with PoAF**

Figures 7 and 8 present Bar Charts of p-values for the age variable, obtained from different logistic regression models both as a categorical and as a continuous variable.

### 3.2.2 Linear Regression

Linear regression was selected to analyze the association between PoAF, other postoperative complications and the total duration of hospital stay. The outcome was the duration of stay and the predictors were PoAF and other complications. Analysis with all 91 subjects revealed other postoperative complications (p-value 0.025) to be significantly associated with the postoperative stay but not PoAF. However, review of the histogram of the duration of hospital stay revealed an outlier with a value of 50. The analysis was repeated without the outlier, which revealed neither PoAF (p-value 0.278) nor other complications (p-value 0.075) to be significant predictors of duration of postoperative hospital stay.

# 4.0 DISCUSSION

Postoperative AF is a common complication after the Cardiac surgery with an incidence of 30-40%. The fiscal and clinical ramifications of this complication are substantial. AF plays a major role in neurological complications after surgery and also may compromise the cardiac hemodynamics. Discovering the etiological factors of this complication can pave the way towards prevention which may lead to further reduction in the postoperative morbidity in this patient population. There have been several studies aimed at elucidating the pathophysiology and etiology behind PoAF during the recent years. The pre and intra operative factors that are thought to predispose the patients to AF after surgery are quite a few. This in itself indicates the lack of clear understanding on the mechanism behind PoAF which further need to be explored. The current study that is under progress is informative as it would yield critical information on genotypes of the Coronary Artery Bypass Graft surgery (CABG) patients at the Presbyterian Hospital, UPMC.

Previous publications cited are ambiguous, with few articles suggesting association of the -174GG SNP with higher levels of circulating Interleukin 6 (IL-6) and coronary heart disease (CHD) and another few suggesting the association of high IL-6 production and CHD with the C allele. All subjects in this study had been diagnosed with coronary artery disease. The genotype pattern revealed 45% of CC, 43% of GC and 12% of GG. Although not significant with a p-value of 0.079, the GG genotype has a negative coefficient (β -2.15) as compared to GC and CC

together, points towards a probable inverse association of the GG genotype with PoAF. However this needs to be explored further with a larger sample size.

The limitations of this analysis are small sample size and the unavailability of IL-6 levels for the subjects which might also offer insight on the inflammatory process in these patients. This study is ongoing and further data collection and the final analysis might reveal additional information.

In concordance with the previous published literature, age emerged as the most significant predictor variable associated with PoAF. The following variables might be probable predictors that need to be explored further with a larger sample size: presence of hypertension, SNPGG and pre-operative AF. The linear regression analysis revealed no significant association between the postoperative hospital stay and PoAF. This could be attributed to aggressive monitoring and management of PoAF in this hospital.

# 5.0 CONCLUSION

Age emerged as a significant predictor of PoAF in the 91 subjects that underwent CABG surgery during the last yea. If the patient is > 65 years old, the odds of AF after the bypass surgery increased by a factor of 2.7 (Odds Ratio 2.7). With one year increase in age, the odds of PoAF increases by 1.06 times (OR 1.06). These data do not reveal any significant association between PoAF and the length of the hospital stay after the bypass surgery.

The SNP -174G/C polymorphism does not seem to be associated with PoAF. However, in contrary to the anticipated genotype distribution, the GG genotype is the least frequent of the three and it would be prudent to continue the study to further explore the genotype distribution in this population.

# **APPENDIX A-** IRB PROTOCOL

## GENOMICS AND POSTOPERATIVE ATRIAL FIBRILLATION

## **A.1** OBJECTIVE AND SPECIFIC AIMS

- To ascertain a plausible association between a single nucleotide polymorphism: -174 G/C of the interleukin 6 gene and postoperative Atrial fibrillation in Coronary artery bypass
- To ascertain a plausible association of the above said polymorphism to other post-operative adverse events including renal and pulmonary complications during the postoperative period.

## **A.2** BACKGROUND AND SIGNIFICANCE

### **A.2.1** Background

#### **Atrial Fibrillation:**

Atrial fibrillation (AF) is a condition in which there are highly asynchronous atrial depolarizations without effective atrial contraction. AF is also characterized by the presence of rapid, irregularly timed impulses reaching the AV node from the atrium and is associated with irregularly timed ventricular response. The atrial rate can be between 350 to 600 beats per minute. Due to the low pass filtering properties of atrio-ventricular (AV) node, the ventricular response is much slower.

Atrial fibrillation falls within these 3 categories:

Paroxysmal: The duration of episodes can range from a few seconds to hours but usually terminates spontaneously within 48 hours.

Persistent: Persistent AF lasts for days and does not terminate spontaneously, but normal sinus rhythm can be restored by electrical cardioversion and/or drugs.

Permanent: Includes those patients with a long standing history of AF (greater than one year), despite prior attempts of cardioversion (electrical and/or pharmacologic) or in whom cardioversion is not considered indicated.

AF is present in approximately 1% of the American population and in 6% of the population above the age of 65 years. While this points to AF as the most common of all of the sustained arrhythmias, it says little about its incidence after cardiac surgical procedures.

**Postoperative AF:**

Postoperative AF has been shown to increase the length of hospital stay; markedly increase cost and potentially increases the incidence of post-operative stroke. Nationally, postoperative atrial fibrillation (AF) is a common complication of cardiac surgery, occurring in 25% to 40% of patients and total costs of billions of dollars per year to treat At University of Pittsburgh Presbyterian University Hospital, over the past few years, our patients experience AF in the postoperative period at an average rate of 30% -40%. The incidence of postoperative AF increases with the age of the patient. The average age of the patient that undergoes CABG surgery has been on the increase due to the improved longevity of the population.

Coronary artery bypass grafting is associated with systemic inflammatory response. There have been studies performed to assess the relationship of genetic polymorphisms that could affect the expression of cytokines such as interleukin 6 (IL-6). Interleukin 6 is a pro-inflammatory cytokine and major mediator of acute phase response. The correlation between the -174 G/C polymorphism and the high production of IL-6 has enabled some investigators to anticipate and treat the patients prophylactically to reduce the IL-6 levels.

It has been suggested that inflammation can have a role in the development of atrial arrhythmias after cardiac surgery and that genetic predisposition to develop postoperative complications exists. There have been studies performed in Europe to ascertain this association and it has been found that the -174G/C Interleukin-6 promoter gene variant appears to modulate the inflammatory response to surgery and to influence the development of postoperative AF. These data suggest an inflammatory component of postoperative atrial arrhythmias and a genetic predisposition to this complication, this polymorphism has also been correlated with the development of postoperative renal and pulmonary complications.

There have been other single nucleotide polymorphisms (SNP) that are associated with high IL-6 production after CABG surgery which could also play a role in the development of atrial fibrillation and other complications.

### A.2.2 Significance

The focus of this pilot study is to find out if a similar association between the -174 G/C polymorphism and postoperative atrial fibrillation exists in our CABG patient population. The data collected can either provide further support to the above study results or provide contradicting information. If supportive, this study can assist us in providing an insight on a genetic marker that could help us anticipate complications and could enable us to treat them prophylactically.

The study would also provide us with a biological sample bank to work on when additional funding is available to do further analyses on other polymorphisms that could potentially influence the expression of inflammatory markers on postoperative complications.

## A.3 RESEARCH DESIGN AND METHODS

**A.3.1** Drug/Device Information: There are no drugs / devices involved in this study.

**A.3.2** Research Design and Methods:

This pilot study focuses on ascertaining the frequency of -174G/C SNP and to find out the association of this SNP with the postoperative complications of the patient, specifically on postoperative Atrial Fibrillation. When further information on other polymorphisms is available, the biological sample could be a source for further association analyses.

Patients of the investigators that are scheduled for an elective CABG surgery at the Presbyterian University hospital will be approached for participation. A total of 380 patients, male and female, of age $\geq$ 18 years that undergo routine isolated CABG surgery will be consented and enrolled. Enrolled patients will have a single blood sample of 6 ml drawn when routine standard care blood samples are drawn –thus eliminating the need for additional needle prick.

Any personal identifiers on the blood sample tube will be removed and code numbers specific for this research study will be affixed on to the samples by the research coordinator

before the samples are sent to the Genomics laboratory for analyses. The samples would be stored and transported at 4°C to be received by the lab within 4 days of the blood draw.

The samples will be processed and the DNA extracted as per the techniques mentioned below. The DNA will be analyzed to ascertain a single nucleotide polymorphism (SNP) at the -174 G/C location. The remaining extracted DNA will be stored with the code numbers at the Genomics laboratory for potential analyses of other polymorphisms at a later time. The samples will be under the control of the investigators. If the subject expresses agreement on the consent form to provide their identifiable (SSN, name, dates of service) information along with their stored genetic sample, the samples would be provided to other investigators with an approved IRB protocol for their genetic research or other research studies This part of the study is optional and the subject may choose not to provide their saved samples to other investigators.

The study participants' medical records will be reviewed for collection of data on demographics, past medical history, postoperative course and any adverse events during their postoperative hospital stay. Prospective data will be collected until the patient is discharged after the CABG surgery. However, based on the statistical recommendations for the purpose of comparability, the data will be analyzed at specific time points -3rd postoperative day, $7^{th}$ postoperative day.

The data collected will be stored with research codes and not with the identifiable personal information. The linkage codes will be maintained in a password protected file by the research coordinator.

**Source of the tissue:**

Patients that undergo isolated CABG surgery will have a single sample of 6 ml drawn either prior to their CABG surgery or prior to their discharge after the CABG surgery. The samples will have the personal identifiers removed and marked with the research study code numbers. The samples will be forwarded to the Genomics laboratory for DNA extraction and genotype analyses.

**Genomics techniques:**

DNA extraction from whole blood:

Blood for DNA extraction should be drawn into blood collection tubes containing EDTA (Purple top). As per the standard of the Genomics lab, upon receipt in the Core laboratories all blood specimens are assigned a GPCL study number, logged in and then aliquoted. One three ml aliquot is immediately used for DNA extraction using the Gentra Systems Puregene DNA purification protocol and reagents with no deviation from the Gentra protocol. The remainder of the specimen is frozen at -80 ˚C for future use. Upon completion of the DNA extraction the DNA is reconstituted in either TE (10mM Tris pH 8, 1 mM EDTA) or nuclease free water. The rehydrated sample is heated to 65°C for 1 hour and then an aliquot in diluted and the absorbance read at λ260 nm and λ280 nm using a UV spectrophotometer in order to assess DNA purity and

concentration. The concentrated DNA in TE is designated as stock and stored at 4°C or in water at -80°C. Working dilutions in water or TE are stored at 4°C.

The concentrated DNA will be diluted in nuclease free water to 10 ng/µl. The diluted DNA will be arrayed into 96 well plates and frozen at -20°C until use .The Single Nucleotide Polymorphism (SNP) Analysis will be done by the TaqMan® method.

### **A.3.3** Data Collection and Statistical Considerations

The recommendation of the consulting group at the Department of Biostatistics, University of Pittsburgh is to use logistic regression analysis. Univariate models will be computed for each of the independent variables to determine significance (and also used for subgroup analysis), with complication status as the outcome. A multivariate model adjusting for covariates can then be determined and used to find the adjusted overall odds ratio.

## **A.4** HUMAN SUBJECTS

### **A.4.1** General Characteristics - Minority Inclusion and Non-Discriminatory Statements

The racial, gender and ethnic characteristics of the proposed subject population reflects the demographics of Pittsburgh and the surrounding area and/or the patient population of the University of Pittsburgh Medical Center. We shall attempt to recruit subjects in respective proportion to these demographics. No exclusion criteria shall be based on race, ethnicity, gender, or HIV status**.**

### **A.4.2** Inclusion/Exclusion Criteria - Pregnancy and Birth Control Statements

**Inclusion Criteria:**

- Patients that are about to have a Coronary Artery Bypass Grafting Surgery done.

**Exclusion Criteria**

- Inability or unwilling to provide an informed consent to the study.
- Pregnancy and Birth Control Statements:
- This study does not have any impact on pregnancy or birth control.

**A.4.3** Recruitment Procedures

Patients of the investigators that undergo Coronary Artery Bypass Grafting Surgery will be approached for participation. A total of 380 subjects both male and female of age ≥ 18 years will be enrolled in the study.

**A.4.4** Risk/Benefit Ratio

**Risks:**

There are no risks associated with the study as the study requires a one time blood sample of 6ml (1 teaspoonful) from the participant. This sample will be taken along with the routine samples and so does not require a separate venipuncture.

There is a possibility that if the results of the research studies involving your genetic material were to become generally known this information could impact future insurability, employability, or reproduction plans, or have a negative impact on family relationships.

**Benefits:**

The information derived from the data collected on the genomics and postoperative atrial fibrillation might benefit future surgery patients.

**DATA SAFETY MONITORING BOARD:**

The investigators will serve as a monitoring group for this study. Issues reviewed include recruitment, data analysis, and confidentiality to ensure there have been no breeches in subject confidentiality and that subject information is only shared with investigators working with the protocol. Scientific literature or abstracts that may have an impact on the safety of study participants or the ethics of the research study will be reviewed.

## A.5 COSTS AND PAYMENTS

**A.5.1** Research Study Costs:

The subject and/or the insurer will not be billed for the research-only procedures, which include the single blood sample genomics analysis and data collection from medical records. The study will pay for the research-only services.

The subject/ third party payer will be billed for routine care services such as their CABG surgery, including any applicable co-pays, coinsurance and deductibles.

**A.5.2** Research Study Payments

There will not be any payments to the subjects for participation in the study.

## A.6 QUALIFICATIONS OF INVESTIGATORS

Lawrence Wei, M.D. is a Clinical Associate Professor of Surgery, and will be the principal investigator on this study. He has had over 14 years of experience with Cardiac Surgeries and is interested in research activities to reduce complications of Cardiac surgeries.

Ronald Pellegrini, M.D. is a Clinical Professor of Surgery with over 30 years of experience .He routinely performs CABGs, valve surgeries and other cardiac surgeries. He will be a co investigator on this study.

Daniel Pellegrini, M.D. is a Clinical Assistant Professor at the Division of Cardiothoracic Surgery at UPMC. He will be a co investigator in this study.

Kenton Zehr, MD is the Chief of Cardiac Surgery at UPMC. He has extensive experience in cardiac surgery and his interests focus on valve and aneurysm surgeries.

Amit Patel, MD is an Assistant Professor of Surgery with the Division of Cardiac Surgery at UPMC. He is also the Director of Cardiac Cell Therapy. He will be a co-investigator in this study.

Giovanni Speziali, MD is an Assistant Professor of Surgery with the Division of Cardiac Surgery at UPMC. He was a fellow at Mayo Clinic and joined the division recently. He will be a co-investigator in this study.

Christian Bermudez MD is an Assistant Professor of Surgery with the Division of Cardiac Surgery at UPMC. He will be a co-investigator in this study.

Yoshiya Toyoda MD PhD. is an Assistant Professor of Surgery with the Division of Cardiac Surgery at UPMC. He has extensive experience in cardiothoracic surgical procedures. He will be a co-investigator in this study.

Thomas Gleason MD Director of the Center for Thoracic Aortic Disease, Co-Director of the Center for Heart Valve Disease, Associate Professor of Surgery, Division of Cardiac Surgery, He will be a co-investigator in this study.

Michael Siegenthaler MD is Associate Director of the Artificial Heart Program, Director of Thoracic Endovascular Therapy, Associate Professor of Surgery, Division of Cardiac Surgery. He has extensive experience in cardiothoracic procedures.

Jennifer Gabany CRNP CCRC is a research nurse practitioner with the Division of Cardiac Surgery. She will be a co-investigator in this study.

**Sujatha Raghu MBBS** is a research coordinator with the Division of Cardiothoracic Surgery and she will serve as the coordinator and help with the analyses for the study.

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