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# Gene signatures of postoperative atrial fibrillation in atrial tissue after coronary artery bypass grafting surgery in patients receiving β-blockers

**Miklos D. Kertai, MD, PhD**[1,*], **Wenjing Qi, PhD**[2,*], **Yi-Ju Li, PhD**[2,3], **Frederick W. Lombard, MB, ChB, FANZCA**[1], **Yutao Liu, MD, PhD**[4], **Michael. P. Smith, MS**[1], **Mark Stafford-Smith, MD**[1], **Mark F. Newman, MD**[1], **Carmelo A. Milano, MD**[5], **Joseph P. Mathew, MD, MHSc**[1,*], and **Mihai V. Podgoreanu, MD**[1,*] **for the Duke Perioperative Genetics and Safety Outcomes (PEGASUS) Investigative Team**

[1]Department of Anesthesiology, Duke Perioperative Genomics Program, Duke University Medical Center, Durham, NC 27710, USA

[2]Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC 27710, USA

[3]Molecular Physiology Institute, Duke University Medical Center, Durham, NC 27710, USA

[4]Department of Cellular Biology and Anatomy, Georgia Regents University, Augusta, GA 30912

[5]Department of Surgery, Division of Cardiothoracic Surgery, Duke University Medical Center, Durham, NC 27710, USA

## Abstract

Atrial tissue gene expression profiling may help to determine how differentially expressed genes in the human atrium before cardiopulmonary bypass (CPB) are related to subsequent biologic pathway activation patterns, and whether specific expression profiles are associated with an increased risk for postoperative atrial fibrillation (AF) or altered response to β-blocker (BB) therapy after coronary artery bypass grafting (CABG) surgery. Right atrial appendage (RAA) samples were collected from 45 patients who were receiving perioperative BB treatment, and underwent CABG surgery. The isolated RNA samples were used for microarray gene expression analysis, to identify probes that were expressed differently in patients with and without postoperative AF. Gene expression analysis was performed to identify probes that were expressed differently in patients with and without postoperative AF. Gene set enrichment analysis (GSEA) was performed to determine how sets of genes might be systematically altered in patients with postoperative AF. Of the 45 patients studied, genomic DNA from 42 patients was used for target sequencing of 66 candidate genes potentially associated with AF, and 2,144 single-nucleotide polymorphisms (SNPs) were identified. We then performed expression quantitative trait loci

Corresponding author: Miklos D. Kertai, MD, PhD, Associate Professor, Department of Anesthesiology, Duke University Medical Center, DUMC 3094, Durham, NC 27710; 2301 Erwin Road, 5693 HAFS Bldg, Durham NC 27710. Tel: 919.681.6752. Fax: 919.681.8994. miklos.kertai@duke.edu.

*The first 2 and last 2 authors contributed equally to this work. Members of the Duke Perioperative Genetics and Safety Outcomes (PEGASUS) Investigative Team are acknowledged at the end of the manuscript.

(eQTL) analysis to determine the correlation between SNPs identified in the genotyped patients, and RAA expression. Probes that met a false discovery rate < 0.25 were selected for eQTL analysis. Of the 17,678 gene expression probes analyzed, 2 probes met our prespecified significance threshold of false discovery rate < 0.25. The most significant probe corresponded to vesicular overexpressed in cancer – prosurvival protein 1 gene (*VOPP1*; 1.83 fold change; $P = 3.47 \times 10^{-7}$), and was up-regulated in patients with postoperative AF, whereas the second most significant probe, which corresponded to the *LOC389286* gene (0.49 fold change; $P = 1.54 \times 10^{-5}$), was down-regulated in patients with postoperative AF. GSEA highlighted the role of *VOPP1* in pathways with biologic relevance to myocardial homeostasis, and oxidative stress and redox modulation. Candidate gene eQTL showed a trans-acting association between variants of G protein-coupled receptor kinase 5 gene, previously linked to altered BB response, and high expression of *VOPP1*. In patients undergoing CABG surgery, RAA gene expression profiling, and pathway and eQTL analysis suggested that *VOPP1* plays a novel etiological role in postoperative AF despite perioperative BB therapy.

### Keywords

beta-blocker; gene expression; G protein-coupled receptor kinase 5; human atrial tissue; atrial fibrillation; coronary artery bypass graft; myocardial homeostasis; oxidant stress; redox modulation; vesicular; overexpressed in cancer; survival protein 1

## INTRODUCTION

New-onset postoperative atrial fibrillation (AF) is one of the most common complications after coronary artery bypass grafting (CABG) surgery, occurring in 25% to 40% of patients [1]. Despite advances in surgical techniques and anesthesia management, postoperative AF remains an important risk factor for adverse neurologic events, congestive heart failure, myocardial infarction, and perioperative mortality, and for prolonged hospital length-of-stay, resource utilization, increased costs, and readmission rates [1–4]. Indeed, the impact of postoperative AF on resource utilization and costs per patient is substantial, and includes 48 additional intensive care unit hours, 3 additional hospital days, and $9,000 for other hospital-related costs [1].

Sympathetic activation or an exaggerated response to adrenergic stimulation is an important trigger for postoperative AF [5], and β-blockers (BBs) are a mainstay in the prevention and treatment of postoperative AF. Nevertheless, approximately 20% of patients undergoing CABG surgery develop postoperative AF despite BB use [6, 7], suggesting that genetic variations in genes that code for β-adrenergic receptors and hepatic metabolism of several BBs may play a role in failure of BBs to prevent postoperative AF [8, 9].

We recently demonstrated that genetic variation in the G protein-coupled receptor kinase 5 gene (*GRK5*) is associated with postoperative AF in patients who underwent CABG surgery and were treated with BBs perioperatively [10]. Unfortunately, this genetic association study could not provide insight into the potential pathophysiological mechanisms associated with postoperative AF in patients treated with BBs. The gene expression pattern in atrial tissue, however, may help us determine the extent to which differentially expressed genes in the

human atrium are associated with an increased risk for postoperative AF in CABG surgery patients, represent activities of certain biologic pathways, or predict altered response to BB therapy [10]. Further, expression quantitative trait loci (eQTL) analysis can determine the cis-/transacting effects of SNPs identified in genetic association studies, on gene expression in atrial tissue. The results of such eQTL analyses can be considered a surrogate to explain the association between genetic variations and AF, which has previously been shown in atrial tissue from discarded hearts of heart failure patients undergoing heart transplantation [11].

To date, only a few studies have attempted to compare patterns of gene expression in cardiac surgery patients with incident or prevalent AF vs patients without AF. However, these studies did not perform eQTL analysis, and thus, could not determine whether SNPs identified in genetic association analyses are linked to atrial gene expression [12, 13]. Furthermore, these prior studies did not investigate the effect of differential atrial gene expression on the pharmacogenetic response to BBs. Therefore, the purpose of the current study was to characterize the gene expression profiles of human atrial tissue, identify biologically relevant pathways, and perform eQTL analysis on atrial tissue in patients who underwent CABG surgery, and were on BB therapy.

## METHODS

The parent studies in our investigation were approved by the Institutional Review Board at Duke University Medical Center, and all subjects provided written informed consent. In the present study, patients were selected from the Perioperative Genetics and Safety Outcomes Study (PEGASUS) and international (i)PEGASUS, longitudinal studies that were conducted at the Duke Heart Center at Duke University Medical Center, Durham, North Carolina.

The parent PEGASUS study enrolled 1004 patients who underwent isolated non-emergent CABG surgery with cardiopulmonary bypass (CPB) between 1997 and 2006 [10]. The iPEGASUS study enrolled 1159 patients to study the effects of cardiothoracic surgery on the proteome, gene expression, and metabolic profile of patients undergoing cardiothoracic surgery between 2004 and 2011 using blood, plasma, and tissue from the perioperative genomics biorepositories stored in the Duke Department of Anesthesiology, Durham, North Carolina. For patients who had more than one cardiac surgery during that period, only data from the first surgery were included.

Patients who met eligibility criteria for the study reported here 1) received perioperative β-blocker (BB) therapy, 2) underwent isolated non-emergent CABG surgery with CPB, and 3) had a right atrial appendange (RAA) tissue sample collected during cardiac surgery at the time of right atrial cannulation before starting CPB. Our findings in study subjects who developed new-onset postoperative AF, were compared to a control group that did not develop new-onset postoperative AF. Perioperative BB therapy was defined as previously described [10], and was characterized as acute or chronic preoperative and postoperative treatment, regardless of BB type, administered before new-onset postoperative AF. Patients with a history of preoperative AF and those who did not receive perioperative BB treatment before new-onset postoperative AF were identified by individual chart and 12-lead ECG

reviews, and excluded. Patients with concurrent valve surgery were also excluded. From the 2 parent datasets, 45 patients met all criteria for postoperative AF or control subjects and were analyzed for RAA tissue gene expression profiling; however, targeted screen eQTL analysis, described below, could be performed only on the 42 patients who had DNA available for genotyping.

Intraoperative anesthetic, perfusion, and cardioprotective management was standardized, as described previously [10]. In brief, general anesthesia was maintained with a combination of fentanyl and isoflurane. Perfusion support consisted of nonpulsatile CPB (30°C–32°C), crystalloid prime, pump flow rates > 2.4 L/min/$m^2$, cold blood cardioplegia, α-stat blood gas management, activated clotting times > 450 seconds maintained with heparin, ε-aminocaproic acid infusion administered routinely, and serial hematocrits maintained at > 0.18.

### Data Collection and End-point Definition

Patient demographics, preoperative and procedural factors, and perioperative medication use, which are components [2, 14] of the postoperative AF Risk Index (Supplementary Table 1), were collected and recorded using the Duke Information System for Cardiovascular Care, an integral part of the Duke Databank for Cardiovascular Disease. The postoperative AF Risk Index is a predictor of postoperative AF in patients undergoing cardiac surgery. Diagnosis of new-onset postoperative AF, as described before [10], was based on postoperative electrocardiogram or rhythm strip, or at least 2 of the following forms of documentation: progress notes, nursing notes, discharge summary, or change in medication.

### RNA isolation and microarray gene expression profiling

Immediately after collection, the RAA tissue samples were flash frozen in a container of liquid nitrogen, placed in a microcassette, and stored in a freezer at −80°C. Total RNA from the RAA tissue samples was isolated using standard methods. Full details of this procedure are given in the Supplementary Methods section. Subsequently, 200 ng of total RNA was amplified and transcribed to cRNA, and hybridized, as described in Supplementary Methods, to Illumina HT-12 Expression BeadChip, per the manufacturer's protocol (Illumina Inc, San Diego, CA), at the Duke Molecular Physiology Institute Molecular Genomics Core [15]. On the chip 29,055 annotated genes with 47,231 probes were targeted.

### Gene expression analysis

The raw data from gene expression profiling were analyzed using R/Bioconductor (http://cran.at.r-project.org). Various QC criteria were applied to ensure quality of the gene expression profiling. Probe signals with a respective detection p-value (*P)* > 0.05, or a frequency of missing expression data ≥20% across samples were excluded. Quantile normalization was then applied to normalize the average signal intensity generated from the Illumina GenomeStudio program. At this stage, the QC'ed gene expression profiling dataset consisted of 17,678 probes. The association between postoperative AF status and gene expression was evaluated using a linear regression model where gene expression was treated as the dependent variable to regress on the postoperative AF status and the postoperative AF Risk Index. To account for multiple comparisons, false discovery rates (FDR *q* values) [16],

were computed for all qualified probes using the 'qvalue' package in R/Bioconductor (http://genomics.princeton.edu/storeylab/qvalue/). Here, FDR is the estimated probability that a probe represents a false positive finding. The top candidate probes were chosen based on a $q < 0.25$.

To visualize the expression pattern, we selected a larger set of probes with $P < 0.001$, including the significant probes, for heatmap representation and hierarchical clustering analysis using the heatmap function in the gplots package of R (http://cran.at.r-project.org) with Ward's linkage and the Euclidean distance criterion. The results were then displayed in a heatmap, and dendrograms were added to the heatmap figure to represent hierarchical clustering.

## Gene set enrichment analysis

Gene set enrichment analysis (GSEA), developed by the Broad Institute (http://www.broadinstitute.org/gsea/index.jsp), was performed to determine whether *a priori*-defined gene sets showed statistically significant concordant differences in gene expression between study subjects with postoperative AF and controls without postoperative AF [17, 18]. In brief, GSEA combines information from the members of each previously defined set of genes to increase the signal relative to noise, and thereby, improve statistical power [17]. The GSEA method has 3 key steps: 1) calculate an Enrichment Score (ES) for each gene set, (2) estimate significance level of ES, and 3) adjust for multiple hypothesis testing [18]. To create a more reliable ES, we included all probes that met the nominal significance level ($P < 0.05$) from differential gene expression analysis for GSEA. This allowed us to obtain a more stable ranking of pathways to subsequently link these pathways to our top (FDR < 0.25) differentially expressed genes. Since the objective of GSEA analysis is to search for pathways related to the top (FDR < 0.25) differentially genes. The absolute values of $t$ statistics from the gene expression analysis of the selected probes were uploaded to GSEA, and mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database for biologic interpretation of higher-level systemic functions (http://www.genome.jp/kegg/pathway.html). The ES, which reflects the degree to which a gene set is overrepresented at the top (positive ES) or bottom (negative ES) of the ranked list, was then calculated for each gene set containing genes that were mapped to the same pathway in the KEGG pathway database. Subsequently, the normalized Enrichment Score (NES) was calculated for each gene set based on permutation, which is the primary statistics for examining gene-set enrichment results. Finally, to control for the proportion of false positive results, the FDR corresponding to each NES was calculated, where the FDR is the estimated probability that a gene set with a given NES represents a false positive finding. An FDR cutoff < 0.25 was considered appropriate [18].

## Targeted screen expression Quantitative Trait Loci analysis

In earlier work, as part of our ongoing research to explore the role of genetic predisposition for postoperative AF after CABG surgery, we performed target sequencing for a set of candidate genes in a subset of 95 patients from the PEGASUS or iPEGASUS cohorts who were at risk for postoperative AF and who received perioperative BB therapy. Based on the current understanding of genetic predisposition for perioperative complications after CABG

surgery, including postoperative AF, we selected a set of 66 candidate genes (Supplementary Table 2) with a potential for modulating cardiac development, ion channel function, signal transduction, activation and modulation of innate immune responses, modulation of oxidative stress and redox, and pharmacogenetic response to BB therapy [10, 19, 20].

From these 95 patients, 42 patients had genomic data for targeted screen eQTL analysis, and were selected for the current study. Their genomic DNA was isolated from whole blood using standard procedures, and sequenced for the set of 66 candidate genes. Sequencing was performed at the Duke Molecular Physiology Institute Molecular Genomics Core, using the Illumina Truseq® Custom Amplicon Kit (Illumina Inc, San Diego, CA) with an Illumina MiSeq sequencer. The raw Illumina sequencing data were analyzed using the Illumina MiSeq Reporter. Variant calling followed GATK best practice (https://www.broadinstitute.org/gatk/guide/best-practices), where Picard (v 1.111) was used to reorder .bam files from MiSeq, GATK (v 3.1.1) for sequence realignment and recalibration, HaplotypCaller for variant calling with hard filters, and ANNOVAR for annotation.

At this stage, 2,144 SNPs were identified and available for analysis. After applying additional QC criteria, we excluded 293 SNPs that were missing in > 10% of samples, 19 SNPs that significantly deviated from Hardy-Weinberg equilibrium ($P \leq 10^{-6}$), and 1097 SNPs with minor allele frequency (MAF) < 5%. For the top probes ($q < 0.25$), eQTL analysis was performed using a linear regression model to regress the probe expression on each SNP, which was coded by a dominant genetic model. SNPs were considered significant if $P < 0.05$. All eQTL (expression/SNP association) analyses were performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). Finally to control for multiple comparisons, FDR was computed using $q$ value [16], and a $q$ value cutoff < 0.25 was used as threshold.

## RESULTS

Demographics and clinical characteristics for study subjects were stratified according to the actual documented presence or absence of postoperative AF (Table 1). The mean age was 58.8 ± 11.2 years; 36 (80%) of the subjects were men; and the median (interquartile range) postoperative AF risk score was 6 (0 to 12). Of the 42 patients in the current study, 13 (28.9%) developed postoperative AF. These subjects had a significantly higher median postoperative AF risk score compared to controls without postoperative AF (10 [5 to 17] vs 2.5 [−7 to 7]; odds ratio [OR], 1.13; 95% confidence interval [CI], 1.03 to 1.24; $P = 0.009$).

### Differential expression associated with atrial fibrillation

Results of the RAA tissue gene expression analysis are depicted in a volcano plot in Supplementary Figure 1, representing the distribution of the fold changes and $P$ values of the 17,678 probes analyzed. Twenty-eight of the probes analyzed met our prespecified threshold of $P < 0.001$ for hierarchical clustering analysis (Table 2). The heatmap in Supplementary Figure 2 shows the different expression patterns of these top probes in subjects who developed postoperative AF vs those who did not develop postoperative AF. Two of the top 28 probes met our prespecified significance threshold of $q < 0.25$. The first probe ($P = 3.47 \times 10^{-7}$, $q = 0.0051$; Table 2) corresponds to the vesicular overexpressed in cancer – prosurvival protein 1 gene (*VOPP1*) – and was up-regulated in patients who developed

postoperative AF; whereas the second probe ($P = 1.54 \times 10^{-5}$, $q = 0.1129$; Table 2) corresponds to *LOC389286*, which is analogous to family with sequence similarity 126, member B (*FAM126B*), based on the chromosome location of *FAM126B* (chromosome 2: 201838441–201936392), was down-regulated in patients who developed postoperative AF.

### Gene set enrichment analysis

The top 1270 probes ($P < 0.05$) were selected and ranked based on the absolute values of the t statistics from the gene expression analysis for gene set enrichment analysis (GSEA). Based on the results of the GSEA, 4 statistically significant pathways with a $q < 0.25$ were identified (Table 3). The most significant of these pathways, based on the KEGG pathway maps, is the retinoic acid-inducible gene-I (RIG-I) -like receptor signaling pathway (Supplementary Figure 3), which is associated with signaling crosstalk with TLR-related and NF-κB–related signaling pathways, and is the most significant pathway with potential relevance to myocardial homeostasis and pathophysiology. [21] The other 3 significant pathways identified in the GSEA with potential relevance to AF were the glutathione pathway, associated with oxidative stress and redox modulation, [22] the ribosome pathway, associated with translation and protein accumulation during cardiac remodeling [23, 24], and the peroxisome pathway, associated with significant changes in the intracellular and extracellular redox milieu [25, 26].

### Targeted screen expression quantitative trait loci (eQTL) analysis

Of the 45 patients selected for our study, 42 had genomic data for targeted screen eQTL analysis. The expression levels of the 2 top probes which map to *VOPP1* and *LOC389286* were tested for association with SNPs generated from MiSeq for the 66 candidate genes. Top SNPs ($P < 0.01$) associated with expression levels of probes ILMN_2226955 (*VOPP1*) and ILMN_2170625 (*LOC389286)* are listed in Table 4. AF risk variants rs10228436 and rs10277413 in epidermal growth factor receptor (*EGFR*) were the significant SNPs ($q < 0.25$) associated with expression levels of *VOPP1.* They were associated with an increased expression of *VOPP1* in quantile-normalized expression of *VOPP1* (both $P = 0.0004$ and $q = 0.1404$; Table 4). We also found a statistically significant association between variants of *GRK5*, which was also identified in our previous candidate gene association study and linked to postoperative AF in patients who were treated with BB therapy [10], and an increased quantile-normalized expression of *VOPP1* (rs148960146, $P = 0.0088$, and rs12721552, $P = 0.0088$; Table 4).

Further, eQTL analysis also showed that several AF risk variants are associated with an increase in *LOC389286* (*FAM126B*) expression (Table 4). Interestingly, we found that the AF risk alleles “A” of rs10228436 and “G” of rs10277413 in *EGFR* are associated with increased expression of *VOPP1* (beta = 0.6) but with decreased expression of *LOC389286* (*FAM126B*) (beta = −0.6; $P = 0.0074$ and $P = 0.0074$; Table 4).

## DISCUSSION

Using a comprehensive approach with genome-wide gene expression analysis and target eQTL analysis on human RAA tissue, we identified novel genes and pathways that are

significantly associated with an altered RAA gene expression profile in patients who underwent CABG surgery with CPB and subsequently developed postoperative AF in spite of perioperative BB therapy.

The most significant gene identified in our study that is differentially expressed in RAA tissue in these patients was *VOPP1,* a pro-survival gene that when up-regulated, increases resistance to oxidative stress-induced inflammatory responses to prevent apoptotic cell death [27]. Previously, *VOPP1* is up-regulated in several solid tumors such as glioblastoma [28] and gastric adenocarcinoma [29]. Several investigators have found VOPP1 regulates cell proliferation and migration, and suppresses apoptosis [30, 31]. It is also a potential regulator of NF-κB signaling, and participates in regulating the intracellular redox state [27]. Indeed, Baras et al [27] found that *VOPP1* overexpression in cancer cells plays a significant role in controlling the intracellular redox state. Loss of this control results in oxidative cell injury, which in turn, leads to cell death via the intrinsic apoptotic pathway.

Currently, the mechanisms that connect atrial *VOPP1* expression with the development of AF in cardiac surgery patients remain unclear. Several signaling cascades that induce myocyte hypertrophy in the adult heart also function to enhance myocyte survival in response to pleiotropic death stimuli [32]. Therefore, *VOPP1* overexpression may represent a novel pathway to cardiomyocyte hypertrophy as well as atrial fibrosis and atrial remodeling, a maladaptive process resulting from pro-survival preconditioning responses to persistent cell death stimuli such as oxidative stress [33].

Our eQTL analysis revealed a significant *cis*-acting association between the risk-associated SNP alleles of *EGFR* (genotyped from peripheral white blood cells) and up-regulation of *VOPP1* (in the RAA). EGFR has been implicated in regulating electrical excitability of the heart, and is thought to play an important role in the pathogenesis of cardiac arrhythmias induced by ischemia/reperfusion injury [34]. Indeed, experimental inhibition of EGFR is associated with reduced incidence and duration of cardiac arrhythmias triggered by ischemia/reperfusion injury [34].

We also observed a novel *trans*-acting association between the risk-associated alleles of *GRK5* and increased *VOPP1* expression. The encoded protein for the *GRK5* gene, GRK5, is abundant in the normal heart, and regulates cardiac inotropic and chronotropic actions of catecholamines, which bind and activate β-adrenergic receptors. When these receptors become activated, GRK5 regulates their activity through desensitization via agonist-dependent phosphorylation [35]. GRK5 also enhances nuclear activity in cardiomyocytes, which could contribute to heart failure progression via maladaptive cardiac growth, [36] and inhibits of transcriptional activity of NFκB [37]. Nuclear factor-κB mediates transcriptional changes seen in AF [38], and its inhibition may provide protection against ischemia/reperfusion injury, a hallmark of CABG surgery [39]. Nevertheless, we do not yet know how the genetic variants in *GRK5* identified in the current study may influence gene expression of *VOPP1* and play a role in maladaptive cardioprotective mechanisms, nor how they may influence the myocardial transcriptional activity of NF-κB, and thus, modulate responses to BB therapy or increase the risk for postoperative AF after CABG surgery.

In our gene expression analysis, we found that the second most significant probe corresponding to *LOC389286*, which is analogous to *FAM126B,* was down-regulated in patients who developed postoperative AF. *FAM126B* is a putative paralog of *FAM126A*, a gene that encodes the membrane protein hyccin, and is involved in the formation of myelin in the central and peripheral nervous system [40]. Hyccin is also expressed in several adult tissues including the heart [40]. *FAM126A* is downregulated by β-catenin [41], and thus appears to be receptive to the Wnt/β-catenin signaling pathway, which plays a role in both repair and remodeling mechanisms of the adult heart [42]. Indeed, in an experimental model of myocardial ischemic injury, β-catenin-dependent activation of the Wnt signaling pathway was associated with myocardial neovascularization and myocardial fibrosis [43]. However, the role of *FAM126B* and its protein in the pathophysiology of ischemic heart disease and/or AF remains unknown.

In our study, GSEA also identified several biologic pathways that were significant and potentially relevant to postoperative AF. Interestingly, the most significant of these was the "RIG-I-like receptor signaling pathway." The RIG-I-like receptor family (RIG-I, MDA5, and LGP2) belongs to the group of so-called pattern recognition receptors, which include the family of toll-like receptors, and its specific role is to recognize pathogen-associated molecular patterns in microbes. The potential role of these receptors in noninfectious activation of innate immune responses has been described in liver ischemia/reperfusion injury [44], but no studies have yet examined the role of RIG-I-like receptors and their signaling pathway in myocardial ischemia/reperfusion injury or in noninfectious myocardial inflammatory processes. Nevertheless, these receptors interact intimately with the toll-like receptor-related and NF-κB-related signaling pathways, which were previously described in myocardial inflammatory signaling [21] and local inflammatory responses in AF [38].

The second most significant and potentially relevant pathway identified by GSEA was the glutathione (GSH) metabolism pathway, which was previously linked to the pathogenesis of AF. [22] GSH depletion renders cells vulnerable to oxidative insults, and is associated with many diseases [45] including diabetes, obesity, and heart failure, which are common among patients with ischemic heart disease. In fact, Carnes et al found that left atrial GSH levels are significantly lower in patients with paroxysmal or persistent AF [22]. Increasing evidence also supports a link to systemic oxidative stress [22]. Cargnoni et al reported that oxidative insults such as myocardial ischemia/reperfusion, result in acute loss of cardiac GSH, and that intracellular oxidized GSH accumulation correlates with NF-κB activation [46]. Taken together, these findings suggest that patients at risk for AF may have impaired or depleted redox defense mechanisms at baseline, rendering them more vulnerable to perioperative oxidative and nitrosative insults, and subsequent inflammatory responses.

Our analysis also showed that the ribosomal protein pathway may be relevant in the pathogenesis of AF. Since ribosomal gene sets are normally expressed at low levels in non-proliferative tissues (such as normal cardiac tissues) this observation could be interpreted that the observed pro-survival oncogenic genotype described above may indeed be associated with an increase in protein translation – the primary mechanism of myocyte hypertrophy and atrial fibrosis [23, 24].

The fourth pathway identified by GSEA is the peroxisome pathway, which drives peroxisome biogenesis. Peroxisomes facilitate a range of tightly regulated oxidative reactions in response to changes in the intracellular micro-environment as well as various external environments [26]. They serve as intracellular hubs for a range of redox-, lipid-, inflammatory-, and nucleic acid-mediated signaling pathways [25]. The molecular mechanisms that regulate peroxisome biogenesis are diverse and extensive, and are the focus of ongoing investigations [25, 26].

Several potential genetic factors contribute to the development of postoperative AF in the setting of cardiac surgery, and to the response and efficacy of BBs. The most extensively studied genetic factors implicated in the development of postoperative AF [20], or in symptomatic responses to antiarrhythmic drug therapy for chronic AF [47], are the noncoding polymorphisms near *PITX2* in the chromosome 4q25 region. A recent study of patients who underwent cardiac surgery [48] showed *cis*-acting associations between risk SNPs at 4q25 and increased *PITX2a* isoform expression in atrial tissue. However, in our study we did not observe differential expression of *PITX2* in RAA tissue, and variants in the gene showed only nominally significant *trans*-acting associations with *VOPP1*. This discrepancy may be due to differences in sample size, study design and patient population, or allele frequencies.

This study has some potential limitations. First, the RAA tissue used for gene expression profiling was sampled at the time of venous cannulation before starting CPB, but a second RAA tissue sample was not collected after terminating CPB. Thus, potential acute changes in the pre-existing gene expression patterns that may result from myocardial ischemia/reperfusion injury, could not be studied. Second, AF usually originates in the left atrium, specifically from the pulmonary veins, while only a small fraction originates from the superior vena cava or the inferior vena cava, or in the right atrium [49]. Therefore, the gene expression profile of the RAA tissue may not fully reflect the gene expression patterns that could contribute to the development of postoperative AF [50]. However, taking left atrial tissue in CABG patients would increase the risk for complications, and it is our opinion that such sampling for research purposes only, cannot be justified ethically. Third, given the exploratory nature of our analysis, we used expression microarray for RAA tissue gene expression profiling after considering the relatively higher cost of RNA sequencing. Fourth, after correcting for multiple comparisons in our eQTL analysis only the top 2 risk SNPs for *EGFR* (rs10228436 and rs10277413; FDR q values of 0.14) remained associated with the expression levels of *VOPP1*. The other SNPs did not reach the prespecified significance threshold of $q < 0.25$ likely due to the small sample size of our study. Finally, the RAA tissue samples analyzed in the current study were obtained from Caucasian patients, and therefore, our findings cannot be generalized to other ethnic groups.

In conclusion, in a cohort of patients treated with perioperative BBs who underwent CABG surgery and subsequently developed postoperative AF, we identified gene expression patterns and activation of molecular pathways that were unique compared to patients who did not develop postoperative AF. These genetic associations may shed some light on the molecular mechanisms that lead to electrical and structural atrial remodeling, creating a substrate for AF. In this patient population with known significant ischemic heart disease, it

appears that repetitive myocardial ischemic insults cause changes in myocardial homeostasis and pathophysiology [21], as well as oxidant stress and redox modulation [22], which could lead to the development of postoperative AF. Low persistent levels of oxidative stress may induce preconditioning responses to protect cells from apoptosis. However, while pro-survival responses protect the cells from apoptosis, they also lead to maladaptive responses resulting in hypertrophy in adult cardiomyocytes, and fibrosis in fibroblasts. Future animal studies directed at characterizing the molecular pathways that underlie the observed gene expression patterns reported here may facilitate the development of perioperative strategies to prevent this potentially devastating complication.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **AF** | atrial fibrillation |
| **BB** | β-blocker |
| **CABG** | coronary artery bypass grafting |
| **CPB** | cardiopulmonary bypass |
| **EGFR** | epidermal growth factor receptor |
| **ES** | enrichment score |
| **eQTL** | expression quantitative trait loci |
| **FDR** | false discovery rate |
| **FAM126B** | family with sequence similarity 126, member B |
| **GSH** | glutathione |
| **GSSG/2GSH** | glutathione disulfide-glutathione couple |
| **GSEA** | Gene Set Enrichment Analysis |
| **GRK5** | G protein-coupled receptor kinase 5 |

| | |
|---|---|
| **KEGG** | Kyoto Encyclopedia of Genes and Genomes |
| **NES** | normalized enrichment score |
| **NF-κB** | nuclear factor-kappa beta |
| **PEGASUS** | Perioperative Genetics and Safety Outcomes Study |
| **QC** | quality control |
| **PITX2** | Paired-Like Homeodomain 2 |
| **RAA** | right atrial appendage |
| **RIG-I-like** | retinoic acid-inducible gene-I-like receptor |
| **SNP** | single nucleotide polymorphisms |
| **TGF-β1** | transforming growth factor beta 1 |
| **VOPP1** | vesicular overexpressed in cancer, prosurvival protein 1 |

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## Table 1

Demographic, clinical, and procedural characteristics of the study populations based on postoperative Atrial Fibrillation Risk Index.

| Predictor | No postoperative AF (n=32) | Postoperative AF (n=13) | *P*-value* |
|---|---|---|---|
| **Age, y** | 55.81±9.48 | 66±12.1 | 0.0118 |
| **Medical History** | | | |
| Atrial fibrillation | 0 (0) | 0 (0) | |
| Chronic obstructive pulmonary disease | 2 (6.25) | 2 (15.4) | 0.344 |
| **Concurrent valve surgery** | 0 (0) | 0 (0) | |
| **Withdrawal of Postoperative Treatment** | | | |
| Beta-blocker | 0 (0) | 0 (0) | |
| ACE inhibitor | 18 (56.25) | 7 (53.9) | 0.883 |
| **Beta-blocker Treatment** | | | |
| Preoperative and postoperative | 32 (100) | 11 (84.6) | 0.0788 |
| Postoperative | 32 (100) | 13 (100) | |
| **Preoperative and Postoperative ACE Inhibitor Treatment** | 5 (15.63) | 0 (0) | 0.301 |
| **Preoperative and Postoperative Statin Treatment** | 26 (81.25) | 8 (61.5) | 0.171 |
| **Postoperative Treatment** | | | |
| Potassium supplementation | 30 (93.75) | 12 (92.3) | 0.861 |
| Non-steroidal anti-inflammatory drugs | 10 (31.25) | 3 (23.1) | 0.585 |

Continuous variables are presented as means ± standard deviation, and categorical variables as percent frequencies. ACE, angiotensin converting enzyme inhibitor; AF, atrial fibrillation.

*$P$-values were derived from the Wald tests, or from Fisher's Exact Test as appropriate.

**Table 2**

Most significant probes and their corresponding genes differentially expressed in patients with atrial fibrillation and without atrial fibrillation.

| Probe ID | Chr | Gene Symbol | Gene Name | log2 fold change | Fold change | *P*-value* | *q*-value |
|---|---|---|---|---|---|---|---|
| ILMN_2226955 | 7 | VOPP1 | Vesicular, overexpressed in cancer, prosurvival protein 1 | 0.8715 | 1.8295 | $3.47\times10^{-7}$ | 0.0051 |
| ILMN_2170625 | 2 | LOC389286 | Similar to FAM126B | −1.0428 | 0.4854 | $1.54\times10^{-5}$ | 0.1129 |
| ILMN_3246634 | 5 | LOC100134108 | Similar to succinate dehydrogenase complex, subunit A, flavoprotein | −0.5004 | 0.7069 | $5.94\times10^{-5}$ | 0.2905 |
| ILMN_3235704 | 22 | GGT3 | Gamma-glutamyltransferase 3 | −0.8081 | 0.5711 | $1.27\times10^{-4}$ | 0.3925 |
| ILMN_1712386 | 21 | C21orf45 | MIS18 kinetochore protein homolog A (S. pombe) | 0.6562 | 1.5759 | $1.55\times10^{-4}$ | 0.3925 |
| ILMN_1800420 | 11 | RNF214 | Ring finger protein 214 | 0.6071 | 1.5232 | $2.13\times10^{-4}$ | 0.3925 |
| ILMN_1684114 | 7 | LOC286016 | Triosephosphate isomerase 1 pseudogene | −0.5781 | 0.6698 | $2.19\times10^{-4}$ | 0.3925 |
| ILMN_1898691 | 3 | | BX103476 NCI_CGAP_Lu5 Homo sapiens cDNA clone IMAGp998C053946 | −0.8486 | 0.5553 | $2.21\times10^{-4}$ | 0.3925 |
| ILMN_1812091 | 17 | FAM20A | Family with sequence similarity 20, member A | −0.4831 | 0.7154 | $2.43\times10^{-4}$ | 0.3925 |
| ILMN_1783026 | 1 | RNPC3 | RNA-binding region (RNP1, RRM) containing 3 | 0.6118 | 1.5281 | $2.67\times10^{-4}$ | 0.3925 |
| ILMN_1783627 | 5 | CAST | Calpastatin | −0.5208 | 0.6970 | $4.03\times10^{-4}$ | 0.4785 |
| ILMN_1671871 | X | ITGB1BP2 | Integrin beta 1 binding protein (melusin) 2 | −0.6795 | 0.6244 | $4.03\times10^{-4}$ | 0.4785 |
| ILMN_3214893 | 13 | LOC100132761 | Hypothetical protein | −1.1581 | 0.4481 | $4.39\times10^{-4}$ | 0.4785 |
| ILMN_3240962 | 20 | DDRGK1 | DDRGK domain containing 1 | −0.4731 | 0.7204 | $5.01\times10^{-4}$ | 0.4785 |
| ILMN_3273946 | 7 | CRCP | CGRP receptor component | −0.5344 | 0.6904 | $5.54\times10^{-4}$ | 0.4785 |
| ILMN_1814204 | 21 | C21orf55 | DnaJ (Hsp40) homolog, subfamily C, member 28 | −0.3233 | 0.7992 | $6.22\times10^{-4}$ | 0.4785 |
| ILMN_1908989 | 10 | | PT2.1_10_D05.r tumor2 Homo sapiens cDNA 3, mRNA sequence | −0.4098 | 0.7527 | $6.23\times10^{-4}$ | 0.4785 |
| ILMN_1691048 | 11 | SLC22A18AS | Solute carrier family 22 (organic cation transporter), member 18 antisense | 0.6960 | 1.6200 | $6.26\times10^{-4}$ | 0.4785 |
| ILMN_1724309 | 10 | FAM35A | Family with sequence similarity 35, member A | −0.7141 | 0.6096 | $6.58\times10^{-4}$ | 0.4785 |
| ILMN_1819640 | 2 | | 602501296F1 NIH_MGC_75 Homo sapiens cDNA clone | 0.6172 | 1.5339 | $6.68\times10^{-4}$ | 0.4785 |
| ILMN_1696469 | 1 | LOC648509 | Similar to plakophilin 4 isoform a | −0.9688 | 0.5109 | $6.94\times10^{-4}$ | 0.4785 |
| ILMN_2317348 | 9 | APTX | Aprataxin | −0.3858 | 0.7654 | $7.83\times10^{-4}$ | 0.4785 |
| ILMN_1753712 | 19 | STX10 | Syntaxin 10 | 0.4549 | 1.3707 | $8.36\times10^{-4}$ | 0.4785 |
| ILMN_1764628 | 1 | LYPLA2 | Lysophospholipase II | 0.4736 | 1.3886 | $8.42\times10^{-4}$ | 0.4785 |
| ILMN_1788135 | 1 | APITD1 | Apoptosis-inducing, TAF9-like domain 1 | 0.5296 | 1.4435 | $9.37\times10^{-4}$ | 0.4785 |
| ILMN_2358783 | 2 | ASB3 | Ankyrin repeat and SOCS box containing 3 | −0.5281 | 0.6934 | $9.42\times10^{-4}$ | 0.4785 |
| ILMN_1661174 | 13 | LOC731640 | Similar to 60S ribosomal protein L21, transcript variant 2 | −0.3024 | 0.8109 | $9.72\times10^{-4}$ | 0.4785 |

| Probe ID | Chr | Gene Symbol | Gene Name | log2 fold change | Fold change | *P*-value* | *q*-value |
|---|---|---|---|---|---|---|---|
| ILMN_1750011 | 17 | EXOC7 | Exocyst complex component 7 | 0.5260 | 1.4399 | $9.80\times10^{-4}$ | 0.4785 |

* Adjusted for postoperative AF risk index as continuous variable; Chr: chromosome.

**Table 3**

Top pathways identified in our study.

| Pathway name | Size | Enrichment Score | Normalized Enrichment Score | Nominal *P*-value | False Discovery Rate *q*-value |
|---|---|---|---|---|---|
| KEGG_RIG-I-like receptor signaling pathway | 6 | 0.7222 | 2.1581 | 0.0068 | 0.0478 |
| KEGG_Glutathione metabolism | 5 | 0.7295 | 1.9830 | 0.0043 | 0.1186 |
| KEGG_Ribosome | 6 | 0.6277 | 1.8305 | 0.0289 | 0.2227 |
| KEGG_Peroxisome | 5 | 0.6447 | 1.7743 | 0.0193 | 0.2427 |

KEGG: Kyoto Encyclopedia of Genes and Genomes; Size: the number of genes in the input list that were included in the pathway.

**Table 4**

Results of the target expression quantitative trait loci analysis.

| Gene Expression | | | Expression Quantitative Trait Loci | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Probe ID** | **Chr** | **Gene Symbol** | **SNP** | **Chr** | **BP** | **Gene Symbol** | **Gene Location** | **Minor allele** | **Major all ele** | **MAF** | **Beta** | ***P*- value** | ***q*-value** |
| ILMN_2226955 | 7 | VOPP1 | rs10228436 | 7 | 55238268 | EGFR | UTR3 | A | G | 0.3333 | 0.5988 | 0.0004 | 0.1404 |
| | | | rs10277413 | 7 | 55238464 | EGFR | UTR3 | G | T | 0.3333 | 0.5988 | 0.0004 | 0.1404 |
| | | | rs2275845 | 13 | 110823178 | COL4A1 | intronic | A | G | 0.08333 | −0.6839 | 0.0029 | 0.5349 |
| | | | rs10492497 | 13 | 110831866 | COL4A1 | intronic | C | T | 0.08333 | −0.6839 | 0.0029 | 0.5349 |
| | | | rs1000989 | 13 | 110827303 | COL4A1 | intronic | C | T | 0.3571 | −0.4762 | 0.0066 | 0.6672 |
| | | | rs2017000 | 7 | 55242609 | EGFR | intronic | G | A | 0.25 | 0.4915 | 0.0079 | 0.6672 |
| | | | rs148960146 | 10 | 121190174 | GRK5 | intronic | A | G | 0.07143 | 0.6584 | 0.0088 | 0.6672 |
| | | | rs12721552 | 10 | 121199381 | GRK5 | intronic | A | G | 0.07143 | 0.6584 | 0.0088 | 0.6672 |
| | | | rs143258813 | 21 | 35832052 | KCNE1 | intronic | GC | G | 0.05952 | −0.8223 | 0.0088 | 0.6672 |
| | | | rs6599213 | 3 | 38617126 | SCN5A | intronic | A | G | 0.07143 | 0.6554 | 0.0091 | 0.6672 |
| ILMN_2170625 | 2 | LOC389286 | rs36018953 | 20 | 1411595 | FKBP1A- SDCBP2, NSFL1C | intergenic | G | A | 0.08333 | 1.056 | 0.0007 | 0.5004 |
| | | | rs1830519 | 6 | 161132830 | PLG | UTR3 | G | A | 0.3214 | 0.6561 | 0.0041 | 0.6380 |
| | | | chr18:77228132 | 18 | 77228132 | NFATC1 | intronic | A | T | 0.2619 | 0.6026 | 0.0070 | 0.6380 |
| | | | chr18:77228137 | 18 | 77228137 | NFATC1 | intronic | G | GC | 0.2619 | 0.6026 | 0.0070 | 0.6380 |
| | | | chr18:77228152 | 18 | 77228152 | NFATC1 | intronic | A | T | 0.2619 | 0.6026 | 0.0070 | 0.6380 |
| | | | chr18:77228154 | 18 | 77228154 | NFATC1 | intronic | GA | G | 0.2619 | 0.6026 | 0.0070 | 0.6380 |
| | | | rs10228436 | 7 | 55238268 | EGFR | UTR3 | A | G | 0.3333 | −0.5993 | 0.0074 | 0.6380 |
| | | | rs10277413 | 7 | 55238464 | EGFR | UTR3 | G | T | 0.3333 | −0.5993 | 0.0074 | 0.6380 |
| | | | rs2289566 | 11 | 20117232 | NAV2 | exonic | C | T | 0.3452 | −0.6057 | 0.0078 | 0.6380 |

COL4A1: collagen, type IV alpha 1; EGFR: epidermal growth factor receptor; FAM126B: family with sequence similarity 126, member B; FKBP1A-SDCBP2, NSFL1C: FK506 binding protein 1A-syndecan binding protein (Syntetin) 2, NSFL1 (P97) Cofactor (P47); GRK5: G protein-coupled receptor kinase 5; KCNE1: potassium channel, voltage-gated subfamily E regulatory beta subunit 1; NAV2: neuron navigator 2; NFATC1: nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1; PLG: plasminogen; SCN5A: sodium channel, voltage gated, Type V alpha subunit; VOPP1: vesicular, overexpressed in cancer, prosurvival protein 1. (BP, base pair; Chr, chromosome; MAF, minor allele frequency; SNP, single-nucleotide polymorphism)