Variation in the 4q25 Chromosomal Locus Predicts Atrial Fibrillation after Coronary Artery Bypass Graft Surgery

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The study is registered at ClinicalTrials.gov (http://clinicaltrials.gov/show/NCT00281164)
ABSTRACT

Background: Atrial fibrillation (AF) is the most common adverse event following coronary artery bypass graft (CABG) surgery. A recent study identified chromosome 4q25 variants associated with AF in ambulatory populations. However, their role in postoperative AF is unknown. We hypothesized that genetic variants in the 4q25 chromosomal region are independently associated with postoperative AF after CABG surgery.

Methods and Results: Two prospectively collected cohorts of patients undergoing CABG, with or without concurrent valve surgery at three U.S. centers. From a discovery cohort of 959 patients, clinical and genomic multivariate predictors of postoperative AF were identified by genotyping 45 SNPs encompassing the 4q25 locus. Three SNPs were then assessed in a separately collected validation cohort of 494 patients. After adjustment for clinical predictors of postoperative AF, and multiple comparisons, rs2200733, rs13143308 and five other linked SNPs independently predicted postoperative AF in the discovery cohort. Additive ORs for the seven associated 4q25 SNPs ranged between 1.57 and 2.17 (P value 8.0 x 10^-4 – 3.4 x 10^-3). Association with postoperative AF were measured and replicated for rs2200733 and rs13143308 in the validation cohort.

Conclusions: In two independently collected cardiac surgery cohorts, non-coding SNPs within the chromosome 4q25 region are independently associated with postoperative AF after adjusting for clinical covariates and multiple comparisons.

Keywords: Atrial Fibrillation, Arrhythmia, Postoperative complication, Heart surgery, Genetics
Atrial fibrillation (AF) is the most common adverse event following cardiac surgery, with a reported incidence of 27-40%.\textsuperscript{1,2} Postoperative AF is associated with increased risk of neurological, renal and infectious complications, and increased utilization of healthcare resources.\textsuperscript{1,2} Previously identified clinical predictors of postoperative AF include advanced age, a history of AF or chronic obstructive pulmonary disease, and valve surgery.\textsuperscript{1,3,4} Although much is known of the mechanisms of AF initiation and maintenance, little is known about the molecular basis of AF susceptibility.\textsuperscript{5} Identification of genetic variants associated with postoperative AF may provide important insight into the pathogenesis of AF and may provide new therapeutic strategies for individual patients according to relative risk.\textsuperscript{6,7}

Four high-frequency single nucleotide polymorphisms (SNPs) (rs2200733, rs2200427, rs2634073 and rs10033464) within the 4q25 chromosomal region have been recently associated with AF in ambulatory populations of European and Chinese descent.\textsuperscript{8} These SNPs are in linkage disequilibrium (LD) with each other, precluding implication of a single responsible locus. Although these SNPs currently have no known biological role, they are in proximity to the paired-like homeodomain transcription factor 2 (PITX2) gene which is instrumental in cardiac morphogenesis of the systemic and pulmonary venous inflow tracts.\textsuperscript{9,10}

In contrast to ambulatory populations where clinical and genetic predictors of AF have been validated,\textsuperscript{11} few studies have attempted to identify genetic variants that independently predict new onset postoperative AF in surgical patient populations.\textsuperscript{12} We thus prospectively collected clinical and genomic data from patients undergoing primary coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass (CPB) at three major United States cardiovascular centers, to determine if chromosome 4q25 variants independently predict postoperative AF.
METHODS

Description of Study Cohorts

Patients aged 21-90 years undergoing primary CABG surgery without planned concurrent valve surgery after August 2001 were enrolled at two institutions (Brigham and Women’s Hospital [BWH] and Texas Heart Institute [THI]), within a single study - the CABG Genomics Program (http://clinicaltrials.gov/show/NCT00281164). Patients were excluded from enrollment in the CABG Genomics Program for a preoperative hematocrit < 25% or transfusion of leukocyte-rich blood products within 30 days before surgery. Patients who underwent unplanned concurrent valve surgery after consenting were not excluded.

The Vanderbilt Cardiac Surgery Registry (VCSR) prospectively enrolled patients older than 18 years undergoing CABG with or without valve surgery at Vanderbilt University Medical Center from November 1999 until November 2004. The Registry consists of a repository of clinical and laboratory data for use in outcome studies. Study protocols were approved by respective Institutional Review Boards, and participants were enrolled following informed written consent.

Only patients undergoing primary CABG surgery utilizing CPB, with or without concurrent valve surgery, were included in the final analyses. Analysis was also restricted to subjects self-reporting four Caucasian grand-parental ancestry, in order to avoid potential population stratification.

Data and End-point Collection

At each site, data were collected during primary hospitalization using study-specific case report forms recording patient demographics, perioperative risk factors, medications, and postoperative outcomes obtained from patient interview and medical records, and staff interviews. Trained medical record data abstractors reviewed the medical records of all subjects. Postoperative AF was defined as
the occurrence of AF identified from nursing, physician and perioperative ECG records, during the postoperative period of the primary hospitalization defined as the duration of contiguous hospitalization in the same institution as the surgery occurred. No formal or informal trials of prophylaxis of AF were conducted during the study period at any institution. Treatment of AF was not specified by this study.

In the discovery cohort comprising patients from the CABG Genomics Program, 1583 subjects recruited from commencement to December 2006. Subjects were excluded if they had one or more of the following exclusions: did not undergo CABG surgery with CPB (n=41), underwent emergency surgery (n=6), were not self-reported Caucasian (n=240), were in AF upon hospital admission or subsequently had a MAZE procedure (n=17) or lacked genotypes or phenotyping data (n=315), yielding 959 analyzed patients.

In the validation cohort comprising patients from the Vanderbilt Cardiac Surgery Registry, 1288 subjects were recruited during the study period. Subjects were excluded if they had one or more of the following exclusions: did not undergo CABG surgery with CPB (n=530), underwent emergency surgery (n=134), were not self-reported Caucasian (n=92), were in AF upon hospital admission or subsequently had a MAZE procedure (n=104) or lacked genotypes or phenotyping data (n=259), yielding 494 analyzed patients.

Genotyping

SNPs between chromosome 4 positions 111,750,000 – 112,075,000 (B36 assembly) encompassing the originally-identified region and PITX2, with a minor allele frequency >5% in the HapMap Caucasian population were identified. Fifteen-one “tagging” SNPs that described 98.2% of the variation within the locus at an $r^2$ of 0.8, in the 276 HapMap-identified SNPs were identified using HaploView software (version 4.0), and were genotyped in the CABG Genomics cohort. Three SNPs identified by deCODE® or
associated with postoperative AF in the discovery cohort (rs2200733, rs10033464 and rs13143308) were validated in the Vanderbilt Cardiac Surgical Registry (VCSR) validation cohort.

DNA was extracted from white blood cells using standard procedures. Genotyping was performed with either the Golden Gate assay using an Illumina Bead Station 500G system (Illumina, San Diego, CA) or the iPLEX Gold assay using a Sequenom MassArray system (Sequenom, San Diego, CA), in accordance with the manufacturers’ standard recommendations. Analysis of the Illumina raw data was done with the clustering algorithm of the Illumina BeadStudio software and individual examination of all intensity plots with manual curation of genotype calls. Sequenom raw data were analyzed with the SpectroTyper 3.4 software (Sequenom, San Diego, CA). Spectra and cluster plots were checked by visual inspection of intensity plots with manual curation of genotype calls.

Statistical Analysis

Categorical and continuous demographic characteristics were compared between groups with Pearson chi-squared and Wilcoxon rank sum tests, respectively. Two clinical models relating perioperative and demographic variables to the occurrence of postoperative AF were used. The postoperative AF risk index of Mathew et al., derived from a large multi-institutional cohort, was used to generate an AF risk score for each patient. Patients did not have records of non-steroidal anti-inflammatory use or potassium supplementation, necessary for calculation of the score, so these variables were removed from the scoring. In addition, we separately derived a multivariable logistic regression model of the occurrence of postoperative AF for VCSR participants and CABG Genomics participants, using forward and backwards stepwise logistic regression with entry and exit P values of 0.20 and 0.05, respectively. Gender and institution were forced into the model in order to maximize non-genetic model performance. There was very high agreement between the AF risk score and the cohort-derived models, for both cohorts, when assessed using Spearman’s rho rank correlation.
PLINK (version 1.01), and SAS (version 9.1.3, SAS Institute, Cary, NC), were used for genetic analysis. Hardy-Weinberg equilibrium was evaluated using an exact test. After application of genotype quality control criteria, univariate analyses were carried out for each SNP to test the null hypothesis of no association between marker polymorphism and postoperative AF, based on allelic, dominant, recessive and trend genetic models. Unadjusted odds ratio (OR) of every SNP marker for postoperative AF were estimated using unconditional logistic regression. Tests of significance are reported as the empirical family-wise P values based on permuting case/control status using permutation methods implemented in PLINK. Heterogeneity in OR estimates was examined across age groups, self-reported Northern vs. Southern European origin, gender, institution, past history of AF, genotyping plate and discovery vs. validation cohort, using Breslow-Day testing and estimating empirical association P values based on stratum-constrained permutation.

Potential population stratification between Northern vs. Southern European origins was examined using SNPs within the lactase gene (LCT) known to vary in frequency along a north-south cline (rs182549, rs2322659, rs3754689, rs3769005, rs4954490, rs4988235). The results did not show significant heterogeneity; therefore, we performed a pooled analysis across these factors. Family-wise error rates were estimated for 45 SNP comparisons within each model by permutation.

LD blocks were first derived in Haploview (version 4.1) using default criteria for the 4q25 region surrounding the most significant seven SNPs. Phased haplotypes for individuals were assigned by PLINK directly and Omnibus tests were conducted to assess the overall association between haplotype block and the postoperative AF phenotype. OR for risk of postoperative AF for each haplotype were estimated using logistic regression, referred to the major haplotype. Specific haplotypes within the blocks were then assessed for association with postoperative AF, based on dominant, recessive and additive genetic models. Tests of significance are reported as the empirical family-wise P values as in SNP association.
Improvement in risk prediction by addition of genotype information to the cohort-specific model was assessed using the area under the receiver operating characteristic; the U-statistic was then estimated to significance of change in area. In addition, improvement in prediction was also assessed by reclassification between risk classes using integrated discrimination improvement$^{17}$ implemented in the R package Hmisc (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/Hmisc).

RESULTS

Discovery Cohort

Characteristics of the discovery and validation cohorts, stratified by the occurrence of postoperative AF, are shown in Table 1. In the CABG Genomics discovery cohort (N=959), postoperative AF was observed in 289 subjects (30.1%) without inter-institutional difference in AF rate. Postoperative AF was associated with older age, a prior history of AF, hypertension and low left ventricular ejection fraction. Operative characteristics associated with postoperative AF were the use of intraoperative inotropes, but not the number of coronary artery bypass grafts performed, the site of venous cannulation or the operation performed. The modified AF Risk index was significantly higher in the patients with postoperative AF than those not experiencing AF (20 ± 9 vs. 16 ± 10; P<10$^{-6}$). A multivariable logistic model of the occurrence of postoperative AF, derived from these data, confirmed older age and prior AF to be liabilities for postoperative AF, and postoperative “statin” use to be associated with reduced risk of postoperative AF (Table 2). Postoperative AF most commonly had its onset on postoperative days 2 (17.9%), 3 (33.9%), 4 (21.9%) or 5 (10.6%). Only 5.6% of patients had their first onset of AF beyond postoperative day 7.

One SNP failed genotyping; all remaining SNPs passed filtering by genotyping call rate >95% and manual curation and the absence of significant deviations from Hardy-Weinberg equilibrium (P < 0.001
in controls) or non-random missingness. Five SNPs had an observed minor allele frequency <2.5% and were excluded from further analysis, thus a total of 45 SNPs were used (Table 3). Thirty samples with >10% genotyping failure were excluded. Thus, 45 SNPs in the 4q25 chromosomal region were evaluated in the CABG Genomics cohort (Table 3). Significant pair-wise linkage disequilibrium was present in the region (Figure 1), consistent with LD structure observed in the HapMap Caucasian cohort and the European populations used in the original report.8 Within the discovery cohort, seven 4q25 SNPs (rs4626276, rs2634073, rs2634071, rs212998, rs2200733, rs13143308 and rs2220427) were associated with postoperative AF while accounting for clinical covariates, using the modified AF risk index1 and the cohort-derived clinical models, specifically including age, a past history of AF and surgical procedure and other covariates (Table 3). However, the previous association of SNP rs10033464 with AF in ambulatory populations, was not seen for postoperative AF. These associations were consistent with both additive and dominant genetic models. Additive odds ratios (ORs) for the seven associated 4q25 SNPs ranged between 1.57 and 2.17 (P value 8.0 x 10⁻⁴ to 3.4 x 10⁻³) in the discovery cohort. Demographic variables, notably age and a prior history of AF did not stratify ORs for SNP effects. Specifically, the effect of rs2200733 was not stratified by age (P= 0.41) or a prior history of AF (P= 0.30). The observed associations survived correction for family-wise error. Observed associations for all SNPs examined are given in the Supplementary material.

Within the discovery cohort, the addition of rs2200733 genotype status improved the area under the receiver operating characteristic (ROC) from 0.702 to 0.720 (P = 2.7x10⁻¹¹). The addition of rs13143308 genotype status improved the area under the ROC from 0.702 to 0.719 (P < 6.1x10⁻⁵). Furthermore, correct classification of AF status was improved by the addition of rs2200733 genotype (P = 5.3x10⁻⁵) and by the addition of rs1314408 genotype (P = 6.6x10⁻⁵) when assessed using integrated discrimination improvement. Further, we examined for reclassification of individuals between risk classes by the addition of genotype. Defining risk classes as those with cohort-specific model risk less
than or greater than the overall observed risk of AF in the discovery cohort, we observed 70 individuals who were correctly reclassified from the incorrect risk group to the correct risk group, and 52 individuals who were incorrectly reclassified from the correct risk group to the incorrect risk group, by the addition of genotype to the model, for a net gain of 18 correct reclassifications.

Validation Cohort

In the VCSR validation cohort (N=494), postoperative AF was observed in 151 subjects (30.6%). VCSR patients who developed postoperative AF were more likely to be older, be of male gender and have a prior history of AF or pulmonary disease and undergo combined valve and CABG surgery. The male gender risk was driven by a higher incidence of valve surgery in males. Preoperative diuretic use and intraoperative inotrope use were significant associated with postoperative AF, perhaps reflecting more severe myocardial disease. Postoperative β-blocker use was not significantly different between patients who developed postoperative AF and those who remained in sinus rhythm. The modified postoperative AF Risk index\textsuperscript{1} was significantly higher in the patients with postoperative AF than those not experiencing postoperative AF (20 ± 10 vs. 11 ± 8; P<0.0001). After adjustment for multiple comparison and clinical covariates, specifically including age, a past history of AF and surgical procedure, rs2200733 and rs13143308 were significantly associated with postoperative AF (rs2200733, OR = 1.97, 95% CI = 1.24-3.15; rs13143308, OR=1.76, 95% CI = 1.23 – 2.52). There was no evidence for heterogeneity of odds ratios between the two cohorts.

Within the validation cohort, the addition of rs2200733 genotype status improved the area under the ROC from 0.731 to 0.740 (P = 1.3x10\textsuperscript{-7}). The addition of rs13143308 genotype status improved the area under the ROC from 0.731 to 0.743 (P = 7.6x10\textsuperscript{-5}). Correct classification of AF status was improved
by the addition of rs2200733 genotype (P = 0.027) or by the addition of rs1314408 genotype (P = 0.005) when assessed using integrated discrimination improvement.

There was a strong haplotype dependence of the associations, consistent with regional block structure (Figure 1) and the previously described haplotype structure. A single haplotype described the risk alleles of all seven associated SNPs in the discovery cohort (Table 4); with observed haplotype frequencies of 15.3% in affected individuals and 9.0% in unaffected individuals. Haplotype associations were present after accounting for clinical covariates using both clinical models and survived correction for family-wise error. There was no observed association between postoperative AF and SNPs within 91Kbp of the PITX2 gene.

**DISCUSSION**

AF is the most frequently occurring arrhythmia both in ambulatory and post-cardiac surgical patients, and is associated with significant morbidity. There is strong evidence for heritability of ambulatory AF, and variation in potassium and sodium channel genes has been associated with AF in ambulatory populations. However, these mutations are rare and are predominantly limited to family kindreds. Two common SNPs, rs2200733 and rs13143308, associated with the 4q25 chromosomal locus define a haplotype that has recently been associated with AF. The haplotype identified by rs2200733 was found to confer a relatively higher risk (OR=2.14) of AF or atrial flutter, whereas the haplotype identified by rs13143308 conferred a more modest risk increase (OR=1.75) compared to the common sequence. The functional role of the 4q25 variants in postoperative AF have yet to be elucidated.

We found that variants in the 4q25 locus are independently associated with postoperative AF after cardiac surgery. This finding supports the original findings of Gudbjartsson and his colleagues in
ambulatory patients and extends their findings to the postoperative period, with its marked pro-
inflammatory milieu. This is perhaps surprising, given the numerous perturbations of electrolyte and
organ homeostasis and necessary surgical pericardotomy. However, it argues strongly for a common
biological mechanism in both populations. Furthermore, the association between the 4q25 locus and
new onset postoperative AF remains significant even after accounting for patients with a prior history of
AF. The addition of genotype to the clinical model derived risk class, improves overall prediction by
correctly reclassifying 7.3% of patients, but at cost of incorrectly reclassifying 5.4% of patients. The
overall value of genotype information to clinicians, by reclassification of individuals to risk classes,
remains to be determined.

Although the potential mechanism of action of the genetic locus identified by the two non-coding
SNPs is unknown, and may be mediated through effects of distant genes, it is interesting to note that
the closest gene, located approximately 90 kb centromeric, is the transcription factor, PITX2. Mouse
PITX2 knockouts have demonstrated a critical role for one isoform, PITX2c, in left-right asymmetry,
specificaly the development of the left atrium. Loss of PITX2c leads to right atrial isomerization and
a failure to suppress a default pathway for sinus node formation in the left atrium of the embryo.

PITX2c has been demonstrated to be necessary for the development of the pulmonary myocardium, or
the sleeve of cardiomyocytes extending from the left atrium into the initial portion of the pulmonary
vein. Clinical and animal studies have demonstrated that ectopic foci of electrical activity arising from
within the pulmonary veins and posterior left atrium play a substantial role in initiating and maintaining
AF. Furthermore, electrical isolation of the pulmonary veins and left atrial region is the goal of catheter
ablation procedures that increasingly have been used to treat AF in the last decade. Additionally, the
candidate gene ENPEP, lying ~300kb centromeric encodes glutamyl aminopeptidase that metabolizes
angiotensin II, an important regulator of blood pressure implicated in the genesis of AF. An alternative
positional candidate is the 99 amino acid hypothetical protein, LOC729065 that lies within the
associated haplotype block, but to-date has not been demonstrated to be expressed in human or animal cardiac tissue. SNPs with the best association with postoperative AF lie in the near upstream region and first estimated intron of LOC729065. Our findings do not directly support an association of postoperative AF with PITX2 or other gene. The associated 4q25 SNPs are not in LD with coding or identified regulatory SNPs of the PITX2 gene (Figure 1). Furthermore, a strong recombination point lies between the PITX2 gene and the AF-associated SNPs. It is possible that one or more of the identified, or as-yet unidentified, SNPs regulate transcription of a gene instrumental in the genesis of AF, but this is currently unknown.

This study is limited by the relatively small sizes of the surgical populations we used. Although the cohorts are likely underpowered for rigorous identification of associations by genome-wide genotyping techniques, surgical populations with detailed phenotyping of AF or other adverse outcomes are rarely available. However, they examine important public health issues in an increasingly aged population.

In conclusion, genetic variants in the 4q25 region are independently associated with an increased risk of postoperative AF. These findings delineate an important genetic role in the etiology of postoperative AF and provide a detailed genomic landscape in which to examine biological mechanisms.

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Disclosures: None
References:


Table 1. Demographic characteristics of the Discovery and Validation cohorts.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Discovery Cohort</th>
<th>Validation Cohort</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Atrial Fibrillation [N= 289]</td>
<td>No Atrial Fibrillation [N= 670]</td>
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</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>7 (2.4)</td>
<td>61 (9.1)</td>
<td></td>
</tr>
<tr>
<td>50 – 59</td>
<td>39 (13.5)</td>
<td>198 (29.6)</td>
<td></td>
</tr>
<tr>
<td>60 – 69</td>
<td>93 (32.2)</td>
<td>228 (34.0)</td>
<td></td>
</tr>
<tr>
<td>70 – 79</td>
<td>107 (37.0)</td>
<td>152 (22.7)</td>
<td></td>
</tr>
<tr>
<td>≥ 80</td>
<td>43 (14.9)</td>
<td>31 (4.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (N; % female)</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>62 (21.5)</td>
<td>128 (19.1)</td>
<td>0.40</td>
</tr>
<tr>
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<td>29.1 ± 5.2</td>
<td>29.5 ± 5.5</td>
<td>0.12</td>
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<tr>
<td>Institution</td>
<td>240 (83.0)</td>
<td>537 (80.2)</td>
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</tr>
<tr>
<td>Medical History (N; %)</td>
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<td></td>
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<tr>
<td>Prior atrial fibrillation</td>
<td>26 (9.0)</td>
<td>14 (2.0)</td>
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<tr>
<td>Hypertension</td>
<td>231 (80.2)</td>
<td>485 (72.5)</td>
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<tr>
<td>Diabetes – IDDM</td>
<td>19 (6.6)</td>
<td>70 (10.3)</td>
<td>0.17</td>
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<td>Diabetes – NIDDM</td>
<td>57 (19.7)</td>
<td>121 (18.1)</td>
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<tr>
<td>Prior myocardial infarction</td>
<td>135 (46.1)</td>
<td>282 (42.2)</td>
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<td>Pulmonary disease</td>
<td>51 (17.8)</td>
<td>109 (16.4)</td>
<td>0.58</td>
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<td>Creatinine &gt; 1.5mg/dL</td>
<td>21 (7.3)</td>
<td>43 (6.4)</td>
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<tr>
<td>LV ejection fraction &lt; 40%</td>
<td>50 (19.1)</td>
<td>77 (13.4)</td>
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<tr>
<td>Preoperative Medications (N; %)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ACE inhibitor</td>
<td>140 (48.4)</td>
<td>307 (45.9)</td>
<td>0.47</td>
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<tr>
<td>Antiarrhythmic</td>
<td>14 (4.8)</td>
<td>12 (1.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Aspirin</td>
<td>209 (72.3)</td>
<td>508 (75.8)</td>
<td>0.33</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>224 (77.5)</td>
<td>520 (77.7)</td>
<td>0.94</td>
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<tr>
<td>Calcium channel blocker</td>
<td>47 (16.3)</td>
<td>88 (13.2)</td>
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<td>Diuretic</td>
<td>72 (24.9)</td>
<td>145 (21.7)</td>
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</tr>
<tr>
<td>HMG-CoA reductase inhibitor</td>
<td>206 (71.3)</td>
<td>513 (76.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>Operative Characteristics</td>
<td></td>
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</tr>
<tr>
<td>CPB duration (min)</td>
<td>101 ± 43</td>
<td>96 ± 36</td>
<td>0.24</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>270 (29.5)</td>
<td>645 (70.5)</td>
<td>0.054</td>
</tr>
<tr>
<td>Valve/CABG</td>
<td>19 (43.2)</td>
<td>25 (56.8)</td>
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</table>
Intraoperative inotrope use

<table>
<thead>
<tr>
<th>Postoperative Medications (N; %)</th>
<th>137 (47.4)</th>
<th>254 (37.9)</th>
<th>0.006</th>
<th>117 (78.0)</th>
<th>204 (60.5)</th>
<th>0.0001</th>
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<td>HMG-CoA reductase inhibitor</td>
<td>209 (72.6)</td>
<td>569 (84.9)</td>
<td>&lt;0.0001</td>
<td>28 (18.5)</td>
<td>58 (16.9)</td>
<td>0.66</td>
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<tr>
<td>Beta blocker discontinued</td>
<td>8 (2.8)</td>
<td>27 (4.0)</td>
<td>0.33</td>
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</tbody>
</table>

BMI, body mass index; LV, left ventricle; NIDDM, non-insulin dependent diabetes mellitus; IDDM, insulin dependent diabetes mellitus; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA reductase; ACE, angiotensin converting enzyme.

Continuous data are portrayed as mean ± standard deviation.

P values are Kruskal-Wallis one-way analysis of variance by ranks for continuous data and $\chi^2$ distribution for nominal and ordinal data.
Table 2. Multivariate clinical predictors of postoperative atrial fibrillation.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Discovery Cohort</th>
<th>Validation Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% confidence interval)</td>
<td>P value</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>50 – 59</td>
<td>1.72 (0.72—4.07)</td>
<td></td>
</tr>
<tr>
<td>60 – 69</td>
<td>3.35 (1.46—7.67)</td>
<td></td>
</tr>
<tr>
<td>70 – 79</td>
<td>6.21 (2.69—14.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥ 80</td>
<td>12.0 (4.73—30.4)</td>
<td></td>
</tr>
<tr>
<td>Prior atrial fibrillation</td>
<td>3.42 (1.69—6.93)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Valve/CABG</td>
<td>1.07 (0.52—2.18)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Models are additionally adjusted for gender, institution, CPB duration and HMG-CoA reductase use in the discovery cohort, and additionally adjusted for gender and CPB duration in the validation cohort.
Table 3: Logistic regression-based model association with covariate adjustment in the *CABG Genomics* validation cohort for selected 4q25 SNPs odds ratios with 95% confidence interval and asymptotic p-values, accounting for the cohort-specific clinical model.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome 4 position</th>
<th>MAF No AF</th>
<th>MAF AF</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
<th>FWER permuted P value ‡</th>
<th>MAF No AF</th>
<th>MAF AF</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
<th>FWER permuted P value ‡</th>
<th>Heterogeneity between cohorts P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4626276</td>
<td>111,869,438</td>
<td>0.10</td>
<td>0.16</td>
<td>2.10 (1.52 – 2.91)</td>
<td>7.6 x 10⁻⁶</td>
<td>0.0003</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs2634073</td>
<td>111,885,232</td>
<td>0.17</td>
<td>0.23</td>
<td>1.57 (1.21 – 2.04)</td>
<td>0.0008</td>
<td>0.023</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs2634071</td>
<td>111,888,669</td>
<td>0.17</td>
<td>0.23</td>
<td>1.58 (1.22 – 2.06)</td>
<td>0.0006</td>
<td>0.017</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs2129982</td>
<td>111,923,592</td>
<td>0.19</td>
<td>0.27</td>
<td>1.75 (1.36 – 2.25)</td>
<td>1.6 x 10⁻⁵</td>
<td>0.0005</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs2200733</td>
<td>111,929,618</td>
<td>0.09</td>
<td>0.16</td>
<td>2.14 (1.55 – 2.96)</td>
<td>4.3 x 10⁻⁶</td>
<td>0.0001</td>
<td>0.09</td>
<td>0.14</td>
<td>1.97 (1.24—3.15)</td>
<td>0.004</td>
<td>0.012</td>
<td>0.75</td>
</tr>
<tr>
<td>rs13143308</td>
<td>111,933,868</td>
<td>0.19</td>
<td>0.27</td>
<td>1.75 (1.36 – 2.25)</td>
<td>1.6 x 10⁻⁵</td>
<td>0.0005</td>
<td>0.19</td>
<td>0.27</td>
<td>1.76 (1.23—2.52)</td>
<td>0.002</td>
<td>0.004</td>
<td>0.88</td>
</tr>
<tr>
<td>rs2220427</td>
<td>111,934,338</td>
<td>0.09</td>
<td>0.16</td>
<td>2.17 (1.56 – 3.01)</td>
<td>3.4 x 10⁻⁶</td>
<td>0.0001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs10033464</td>
<td>111,940,210</td>
<td>0.10</td>
<td>0.11</td>
<td>1.21 (0.86 - 1.71)</td>
<td>0.28</td>
<td>1.0</td>
<td>0.10</td>
<td>0.13</td>
<td>1.41 (0.87—2.26)</td>
<td>0.16</td>
<td>0.34</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Models are adjusted for age, gender, prior atrial fibrillation, institution, surgical procedure, CPB duration and HMG-CoA reductase use in the discovery cohort, and adjusted for age, gender, prior atrial fibrillation, surgical procedure and CPB duration in the validation cohort.

‡ Family-wise empirical P value is corrected for multiple comparisons.
Table 4: Distribution and asymptotic P-values for haplotype association, including haplotype-specific tests and omnibus association test for the discovery and validation cohorts. Odds ratios and tests of association are adjusted for the cohort-specific clinical model, using an additive genetic model.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Discovery cohort</th>
<th>Validation Cohort</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AF patients haplotype frequency</td>
<td>No AF patients haplotype frequency</td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>CGG</td>
<td>0.728</td>
<td>0.809</td>
<td>1.83 (1.88—3.00)</td>
</tr>
<tr>
<td>TTG</td>
<td>0.160</td>
<td>0.093</td>
<td>1.33 (0.94—1.87)</td>
</tr>
<tr>
<td>CTT</td>
<td>0.113</td>
<td>0.098</td>
<td>2.18 (1.98—3.00)</td>
</tr>
</tbody>
</table>

The haplotype encompasses the following SNPs: rs2200733, rs13143308, rs10033464. Global haplotype association P value = 4.2 x 10⁻⁵

‡ Family-wise empirical P value corrected for multiple comparisons. Haplotypes with overall cohort frequencies of <1% were not considered.
Figure 1 Legend: Haplotype block structure of the 45 successfully typed 4q25 SNPs in the *CABG Genomics* discovery cohort. Increasing correlation ($r^2$) between intersecting SNPs is reflected in increasing darker shading within each intersecting square. Correlations and block structure were estimated using HaploView 4.0. Negative log P value of the association of each SNP with postoperative AF for an additive genetic model adjusted for clinical covariates is shown.
Variation in the 4q25 Chromosomal Locus Predicts Atrial Fibrillation after Coronary Artery Bypass Graft Surgery

Simon C. Body, Charles D. Collard, Stanton K. Shernan, Amanda A. Fox, Kuang-Yu Liu, Marylyn D. Ritchie, Tjörvi E. Perry, Jochen D. Muehlschlegel, Sary Aranki, Brian S. Donahue, Mias Pretorius, Juan-Carlos Estrada, Patrick T. Ellinor, Christopher H. Newton-Cheh, Christine E. Seidman, Jonathan G. Seidman, Daniel S. Herman, Peter Lichtner, Thomas Meitinger, Arne Pfeufer, Stefan Kääb, Nancy J. Brown, Dan M. Roden and Dawood Darbar

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## SUPPLEMENTAL MATERIAL

**Table 1:** Logistic regression-based model association with covariate adjustment in the CABG Genomics discovery cohort: 4q25 SNPs odds ratios with 95% confidence interval and asymptotic p-values, accounting for the patients’ individual calculated modified AF risk index (Mathew et al. 1) and the cohort-specific clinical model. Odds ratios refer to the minor allele.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome 4 position</th>
<th>Individual calculated modified AF risk index</th>
<th>Cohort-specific clinical model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Additive genetic model</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dominant genetic model</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>FWER permuted P value†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Odds ratio (95% confidence interval)‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Odds ratio (95% confidence interval)‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>FWER permuted P value†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Odds ratio (95% confidence interval)‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>FWER permuted P value†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Odds ratio (95% confidence interval)‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>rs4834295</td>
<td>111754655</td>
<td>1.14 (0.92-1.42)</td>
<td>1.16 (0.95-1.49)</td>
</tr>
<tr>
<td>rs2278782</td>
<td>111761603</td>
<td>0.96 (0.73-1.25)</td>
<td>1.20 (0.96-1.49)</td>
</tr>
<tr>
<td>rs2595110</td>
<td>111764772</td>
<td>0.79 (0.63-0.98)</td>
<td>0.97 (0.82-1.14)</td>
</tr>
<tr>
<td>rs17041972</td>
<td>111766204</td>
<td>0.97 (0.74-1.27)</td>
<td>0.88 (0.75-1.04)</td>
</tr>
<tr>
<td>rs7668322</td>
<td>111768045</td>
<td>1.05 (0.79-1.40)</td>
<td>0.88 (0.72-1.08)</td>
</tr>
<tr>
<td>rs976568</td>
<td>111770170</td>
<td>1.24 (1.01-1.51)</td>
<td>1.06 (0.87-1.26)</td>
</tr>
<tr>
<td>rs17554590</td>
<td>111782351</td>
<td>0.93 (0.48-1.81)</td>
<td>0.76 (0.58-1.00)</td>
</tr>
<tr>
<td>rs2595098</td>
<td>111782931</td>
<td>0.93 (0.62-1.41)</td>
<td>0.74 (0.57-1.01)</td>
</tr>
<tr>
<td>rs1448818</td>
<td>111789672</td>
<td>1.14 (0.92-1.42)</td>
<td>0.98 (0.82-1.14)</td>
</tr>
<tr>
<td>rs2723291</td>
<td>111793640</td>
<td>1.21 (0.99-1.48)</td>
<td>0.88 (0.71-1.09)</td>
</tr>
<tr>
<td>rs12498374</td>
<td>111803868</td>
<td>1.27 (1.00-1.61)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs2122076</td>
<td>111809693</td>
<td>1.21 (0.99-1.49)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs2723330</td>
<td>111810457</td>
<td>1.22 (1.00-1.50)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs11932280</td>
<td>111810979</td>
<td>1.19 (0.95-1.47)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs1448822</td>
<td>111820547</td>
<td>1.22 (1.00-1.50)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs13120244</td>
<td>111823793</td>
<td>0.92 (0.68-1.26)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs6854883</td>
<td>111826764</td>
<td>1.39 (1.06-1.82)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs2723307</td>
<td>111837645</td>
<td>1.14 (0.93-1.39)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs1448798</td>
<td>111842634</td>
<td>1.21 (0.98-1.49)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs17625509</td>
<td>111846843</td>
<td>0.90 (0.65-1.26)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs10222783</td>
<td>111854275</td>
<td>0.79 (0.53-1.18)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs10032150</td>
<td>111866067</td>
<td>1.33 (1.07-1.66)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs4626276</td>
<td>111869438</td>
<td>1.89 (1.40-2.55)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
<tr>
<td>rs17631468</td>
<td>111871231</td>
<td>1.50 (1.01-2.25)</td>
<td>0.88 (0.69-1.11)</td>
</tr>
</tbody>
</table>
The cohort-specific clinical model consisted of Institution, age, gender, a history of prior AF, CPB duration and the use of a statin in the in-hospital postoperative period.

* Significance is described as having a Bonferroni-adjusted P value <0.05.
† Permutation test based on the original allelic or model association result, compared to the association result for that SNP for every replicate.
‡ Family-wise empirical p-value corrected for multiple comparisons.
§ Calculated AF risk index does not include potassium supplementation or use of non-steroidal anti-inflammatory agents.