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Usefulness of Single Nucleotide Polymorphism in Chromosome 4q25 to Predict In-Hospital and Long Term Development of Atrial Fibrillation and Survival in Patients Undergoing Coronary Artery Bypass Grafting

Salim S. Virani, MD^{a,b}, Ariel Brautbar, MD^b, Vei-Vei Lee, MS^d, MacArthur Elayda, MD, PhD^d, Shehzad Sami, MD^d, Vijay Nambi, MD^b, Lorraine Frazier, RN, PhD^c, James M Wilson, MD^d, James T Willerson, MD^d, Eric Boerwinkle, PhD^c, and Christie M Ballantyne, MD^b

^a Michael E. DeBakey Veterans Affairs Medical Center Health Services Research and Development Center of Excellence, Houston, TX

^b Section of Cardiovascular Research, Baylor College of Medicine and Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart and Vascular Center, Houston, TX

^c University of Texas Health Science Center at Houston, Houston, TX

^d Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, TX

Abstract

We aimed to determine whether polymorphisms in chromosome 4q25 are associated with postoperative atrial fibrillation (AF), long term AF, postoperative or long term stroke, and long term survival after coronary artery bypass grafting (CABG). We performed genotyping for rs2200733 and rs10033464 in Caucasian participants (n=1,166) of TexGen genetic registry. Development of postoperative or long term AF, postoperative or long term stroke, and long term mortality were ascertained. Both rs2200733 and rs10033464 were associated with postoperative AF (odds ratio [OR]=1.41, 95% confidence interval [CI]; 1.04–1.91, and OR=1.47, 95% CI; 1.05–2.06, respectively). Carriers of the risk allele (T) had an increased risk of postoperative AF with preoperative beta blocker (BB) (for rs2200733: OR=1.47, 95% CI; 1.004–2.16 for those on BB, and OR=1.13, 95% CI; 0.73–1.73 for those not on BB; for rs10033464: OR=1.89, 95% CI; 1.22–2.93 for those on pre-operative BB, and OR=1.04, 95% CI; 0.65–1.65 for those not on BB). Both rs2200733 and rs10033464 were also associated with long term AF (hazards ratio [HR]=1.32, 95% CI; 1.05–1.67, and HR=1.28, 95% CI; 1.00–1.66, respectively). Carriers of rs2200733 had increased long term mortality (HR=1.57, 95% CI; 1.10–2.24). These variants were not associated with postoperative or long term stroke. In conclusion, variants in 4q25 are associated with an increased risk of postoperative or long term AF, and possibly mortality in Caucasians undergoing CABG, and could potentially affect the choice of therapy used to decrease postoperative AF.

Address of the Corresponding Author: Salim S. Virani, MD, Health Services Research and Development (152), Michael E. DeBakey Veterans Affairs Medical Center, 2002 Holcombe Blvd., Houston, TX 77030, Phone: 713-794-8517, Fax: 713-748-7359, virani@bcm.edu.

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Keywords

atrial fibrillation; single nucleotide polymorphisms; coronary artery bypass grafting

INTRODUCTION

Recently, several epidemiologic cohorts¹⁻⁶ have shown an association between single nucleotide polymorphisms (SNPs) in the chromosome 4q25 region (rs2200733 and rs10033464) and the development of atrial fibrillation (AF). The SNPs in the same region have also been associated with an increased risk of ischemic strokes⁷ as well as an increased risk of AF recurrence after catheter ablation.⁸ Although, a recent study showed that polymorphisms at 4q25 locus are also associated with in-hospital AF development after CABG,⁹ these findings have not been replicated in other studies. It is also not known whether the association between these variants and postoperative AF could be modulated by the use of pre-operative therapies such as beta blocker (BB), statins, or antiarrhythmics. The aims of our analyses were to replicate and confirm the findings of the association between 4q25 and postoperative AF in patients undergoing CABG, to evaluate whether these associations are modulated by the choice of pre-operative therapy used to decrease the incidence of post-operative AF, and to evaluate whether these polymorphisms could also predict long term development of AF in patients undergoing CABG. As a secondary aim, we evaluated whether polymorphisms in chromosome 4q25 are associated with postoperative stroke, long term stroke incidence, and long term survival in patients undergoing CABG.

METHODS

TexGen is a collaborative, prospective genetic registry which enrolls patients with any personal or family history of cardiovascular disease who seek care at several institutions within the Texas Medical Center including the University of Texas Health Science Center, The University of Texas M.D. Anderson Cancer Center, Baylor College of Medicine and their affiliated hospitals, and St. Luke's Episcopal Hospital at the Texas Heart Institute. The cohort includes patients admitted with acute coronary syndromes, strokes, transient ischemic attacks, those undergoing percutaneous coronary interventions or CABG, as well as those undergoing vascular surgical procedures.

For the current analyses, we restricted the population to TexGen patients undergoing CABG (with or without valve surgery procedures) from September 2001 through September 2008, who self-reported their race as Caucasian to avoid the issue of population stratification.

Pre-operative, intra-operative and postoperative characteristics of patients were obtained through a database maintained at the St. Luke's Episcopal Hospital at The Texas Heart Institute. This research database prospectively collects information on patients enrolled in TexGen. The database has written documentation for each field that is coded and has systems in place to ensure reliability of the data, including range edit limits for every data field, consistency checks between fields, and periodic inter-rater reliability. Data from patient charts were abstracted by 3 trained abstractors, with 95% agreement among abstractors. Monthly electronic quality-control checks were conducted to detect logical errors. When verification coding (recoding the same admission) was performed on a minimum of 10 admissions per month, it found that 95% of the data collected was correct.

The variables used for our analysis included age, sex, history of acute coronary syndromes, hypertension, diabetes mellitus, concomitant valve surgery, prior history of AF, reduced left ventricular ejection fraction (defined as <0.35), New York Heart Association functional

class at the time of surgery, pre-operative BB, anti-arrhythmic medication, or statin use, and aortic cross-clamp and total bypass time.

Prospective follow-up of outcomes included annual follow-up phone calls by the TexGen research nurses as well as annual surveys mailed to each patient. In addition, any hospitalization for patients enrolled in the database was also verified using hospitalization records.

Genotyping of rs2200733 and rs10033464 variants was performed using validated TaqMan assays (Applied Biosystems). These two polymorphisms on chromosome 4q25 were chosen *a priori* as these polymorphisms are the ones extensively studied in the literature thus far.¹⁻⁹ PCR product was amplified utilizing 0.9 μ M each of the forward primer and reverse primers, 0.2 μ M each of the FAM and VIC MGB (minor groove binder) sequence-specific probes, 3 ng DNA, 5.0 mM MgCl₂, and 1X TaqMan Universal PCR Master Mix containing AmpliTaq Gold DNA Polymerase in a 5.5 μ l reaction volume. Both the SNPs had a call rate of greater than 99%. Hardy Weinberg (HW) equilibrium of $P > 0.05$ was calculated using a chi-square goodness-of-fit model. QC concordance for 37 samples was 100%.

Our primary outcomes of interest included the development of postoperative AF after CABG surgery and long term development of AF after discharge. Postoperative AF was defined as the occurrence of any AF during the hospitalization for the index surgery. All patients were on continuous telemetry (either in the intensive care units or the telemetry floors) throughout their hospital stay, and any episode of AF was included in the analysis. Long term development of AF after the index hospitalization for CABG included any new AF which either necessitated hospitalization or was noted on an electrocardiogram for a hospitalization for a reason other than AF. We also performed exploratory analyses to assess whether the associations between these polymorphisms and the risk of postoperative AF could be modulated by age, gender or the type of pre-operative therapy (pre-operative BB, statin, or anti-arrhythmic) used.

Our secondary outcomes of interest included the development of postoperative stroke, development of stroke at long term follow-up, as well as long term survival. Stroke was defined as clinical evidence of a focal neurologic deficit along with a radiologic defect on computed tomogram or magnetic resonance imaging scan of the brain. Vital status on these patients was ascertained using data from the Texas State Bureau of Vital Statistics.

Categorical and continuous variables were compared between the groups using Pearson chi square test or Wilcoxon rank sum test, respectively. Hardy-Weinberg equilibrium expectation of allelic frequencies was tested using a chi-square goodness-of-fit model.

For the development of postoperative AF, we initially performed univariate analyses to study the associations between each SNP, rs2200733 and rs10033464, and the risk for postoperative AF development. Logistic regression analyses were subsequently performed using a limited adjustment model that included age and gender, followed by a more extensive adjustment model that included age, gender, prior history of AF, hypertension, pre-operative beta-blocker, statin or anti-arrhythmic use, and concomitant valve surgery as covariates to ascertain whether the polymorphisms of interest were independent predictors of postoperative AF in the fully adjusted models.

For long term risk of AF, we initially carried out log rank test to study the association between rs2200733, rs10033464 and time to development of AF. Cox proportional-hazards models (adjusting for age, gender, and hypertension) were subsequently used to determine whether polymorphisms in rs2200733 and rs10033464 were independently associated with long term development of AF. Similarly, logistic and Cox regressions analyses were used to

determine whether rs2200733 and rs10033464 were associated with development of postoperative stroke or long term development of stroke, respectively. The associations between rs2200733, rs10033464 and survival were analyzed using log rank test.

For all the above mentioned analyses, a dominant model was used to study the associations between polymorphisms in rs2200733 and rs10033464 and outcomes of interest. Statistical analyses were performed using SAS statistical software version 9.1 (SAS Institute, Inc.; Cary, NC). All analyses were performed using two-tailed tests for significance. Because we were evaluating these polymorphisms on an *a priori* basis, a p value <0.05 was considered statistically significant and adjustments were not made for multiple comparisons.

RESULTS

Among patients undergoing CABG enrolled in the TexGen database (n=1,568), there were 1,166 patients who identified their race as Caucasian. This group represented the final population included in the analyses. Baseline characteristics of these patients are described in Table 1. The mean age of the cohort was 65 years, and there was a higher proportion of males. There was a high prevalence of hypertension and patients with New York Heart Association functional class III/IV symptoms, whereas 10% of the patients reported a prior history of AF.

Table 2 describes the frequencies for rs2200733 and rs10033464 in the TexGen sample. Overall, 22% of the patients carried at least one risk allele (T) for rs2200733, whereas roughly 16% of the patients carried at least one risk allele (T) for rs10033464.

Overall, the incidence of in-hospital AF after CABG procedures in our cohort was 36.45%. Table 3 describes the odds ratios for risk of the development of postoperative AF after univariate analyses as well as after adjustment for co-variables of interest using the dominant model. Carriers of the risk allele (TT or CT) for rs2200733 had a 41% higher risk for developing postoperative AF in the fully adjusted model. On the other hand, the presence of the minor allele for rs10033464 (TT or GT) was associated with a 47% increased risk for the development of postoperative AF. Similar results were obtained after excluding 117 patients with prior history of AF (Supplemental Table 1).

To assess whether the increased risk of postoperative AF risk associated with these polymorphisms could be modulated by age, gender, pre-operative therapies such as use of beta blockers, statins or antiarrhythmics, we performed stratified analyses in these subgroups of interest (Supplemental Tables 2a and 2b). Although these analyses were limited given the small number of patients in some subgroups, some interesting observations could be made. The presence of the minor allele for either rs2200733 (Supplemental Table 2a) or rs10033464 (Supplemental Table 2b) was associated with a significantly increased risk of postoperative AF in those who received pre-operative beta blockers, whereas these polymorphisms were not significantly associated with postoperative AF in those who did not receive pre-operative beta blockers. For rs2200733, the association with postoperative AF was attenuated in patients receiving pre-operative statins, whereas, for rs10033464, the association with postoperative AF was attenuated in patients using antiarrhythmics (including amiodarone), although the use of pre-operative antiarrhythmics was low in our sample (n=50).

Median follow-up of these patients (after initial hospitalization for CABG) was 1,142 days (mean=1,206 days, standard deviation=850.30 days). Table 4 shows the association of rs2200733 and rs10033464 with long term risk for the development of AF. The presence of the minor allele for rs2200733 was associated with a 32% increased risk of long term development of AF after CABG surgery in a model adjusted for age, gender, and

hypertension. The association between rs10033464 and long term development of AF was of borderline significance (HR=1.28, 95% CI; 1.00–1.66).

Overall, there was a very low incidence of postoperative stroke in this sample (1.1%, n=13). Both rs2200733 and rs10033464 were not associated with either postoperative stroke (OR=0.30, 95% CI; 0.04–2.31 for rs2200733, and OR=0.43, 95% CI; 0.06–3.36 for rs10033464), or long term development of stroke (HR=1.38, 95% CI; 0.65–2.97 for rs2200733, and HR=1.08, 95% CI; 0.45–2.61 for rs10033464).

At a median follow-up of 1,142 days, there were 150 deaths (12.75% of the cohort). Although rs2200733 was associated with an increase in long term mortality (HR=1.57, 95% CI; 1.10–2.24), no such association was seen with rs10033464 (HR=1.04, 95% CI; 0.68–1.60).

DISCUSSION

In this prospective analysis from the TexGen cohort, we found that variants in chromosome 4q25 are associated with postoperative AF, long term development of AF, and long term mortality. We also found that the associations between these polymorphisms and postoperative AF could possibly be increased by the use of pre-operative beta blockers.

Our study supports the findings from one earlier study that polymorphisms in chromosome 4q25 are associated with the development of postoperative AF.⁹ The incidence of postoperative AF (30%) in the study by Body et al was comparable to our study (36%). The risk associated with rs2200733 was somewhat higher in that study (OR=2.14, 95% CI; 1.55–2.96) compared with our study (OR=1.41, 95% CI; 1.04–1.91), although differences in the covariates used in the adjustment model as well as differences in the genetic model used (additive model vs. dominant model of inheritance) could account for the differences in the risk estimates.

Although the presence of these SNPs does identify a group at higher risk for postoperative AF, the treatment approach to circumvent such increased risk is not clear. Our data show that the use of beta blockers is associated with an increased risk of postoperative AF in carriers of these SNPs. This later observation is important as beta blockers are the primary agents used for the prevention of postoperative AF.¹⁰ Though the exact reason for this observation is not known, it has recently been shown that a transcription factor (PITX2) is necessary for the development of the pulmonary myocardial sleeve, a major source of atrial fibrillation.¹¹ The SNPs in chromosome 4q25 are in close proximity to PITX2 and could potentially alter its function. It is possible that the pulmonary myocardial sleeves behave differently to beta blockers compared to other nodal (pacemaker) tissues with different embryologic origins such as the sinoatrial node, myocardium of the systemic veins (sinus venosus) or the atrial myocardium.

Our exploratory post-hoc analyses show that the association between rs2200733 could possibly be attenuated by pre-operative statin use, whereas the association between rs10033464 and postoperative AF could be attenuated by the use of anti-arrhythmic drugs. Antiarrhythmics as well as statins (in small studies) have been shown to diminish the risk of postoperative AF.^{12,13} Therefore, the presence of these SNPs not only identifies a group at higher risk of postoperative AF, but also could potentially alter therapies used to circumvent this increased risk. However, it is important to note that our above-stated stratified analyses were exploratory in nature and hypothesis-generating at best. Therefore, these observations will need to be validated in an independent cohort of patients undergoing CABG, and if confirmed, then a prospective randomized controlled trial could be performed to evaluate the

impact of different pre-operative therapies on the incidence of postoperative AF in the carriers of these polymorphisms.

Our results also extend the earlier findings to show that the risk alleles of rs2200733 and rs10033464 are also associated with long term AF development in patients undergoing CABG; and therefore, the use of these polymorphisms predicts not only short term risk of AF after CABG surgery, but also long term AF risk as well.

The associations between polymorphisms in 4q25 and ischemic stroke have been inconsistent, and the presence of these polymorphisms have been shown to be associated with an increased risk of ischemic, especially cardio-embolic strokes in some⁷ but not all studies.⁶ It would be expected that these polymorphisms, by virtue of being associated with AF, will be associated with an increased risk of strokes of cardio-embolic origin, but we did not find this. The most likely explanation is a lack of statistical power due to the small number of ischemic strokes in our cohort. Similarly, most of these patients were followed vigilantly given their established atherosclerotic vascular disease, and are likely better treated for AF (including the use of anticoagulants), which could diminish any such association in our cohort.

Our finding of association between rs2200733 and an increase in long term mortality after CABG surgery is interesting, and to our knowledge, has not been evaluated in prior studies. One possible explanation would be that rs2200733 is associated with an increase in the risk of fatal strokes, likely decreasing the overall survival in carriers of rs2200733. Although this is possible, our data do not support this. On the other hand, postoperative AF has been shown to be independently predictive of long term mortality in patients undergoing CABG.^{14,15} It is possible that rs2200733 is associated with an increase risk of long term mortality primarily via its direct association with AF, because AF is a marker for the presence of structural heart disease.

It is important to note that these polymorphisms in chromosome 4q25 are in noncoding genomic regions. Although the exact mechanism by which these polymorphisms mediate the increased risk for AF is not known, their proximity to the gene for a transcription factor PITX2 has generated interest. PITX2c has been shown to be important in left-right asymmetry¹⁶ and the development of the left atrium.^{17,18} PITX2c has also been shown to be necessary for the development of pulmonary myocardium that forms the sleeves in the left atrium.¹¹ These sleeves are considered to be the origins of AF,¹⁹ and the currently used pulmonary vein isolation-based AF ablation procedures target these myocardial sleeves.²⁰ In that respect, a recent study showed that carriers of rs2200733 had a higher incidence of AF recurrence after catheter ablation,⁸ supporting a putative role of these polymorphisms with AF development and propagation.

Our study has limitations. We studied the polymorphisms in chromosome 4q25 in Caucasians undergoing CABG; therefore, our findings cannot be generalized to other ethnicities. In addition, we did not validate our findings in another cohort since the association between these polymorphisms and AF has been shown convincingly in prior studies.^{1-6,9} On the other hand, the interaction between these polymorphisms and pre-operative BB, statin, anti-arrhythmic use, as well as their associations with term development of AF and mortality in CABG patients have not been previously described and will need further validation. We used an α level of 0.05. As explained in the Methods section, we used this α level because the polymorphisms were selected on an *a priori* basis, and genome-wide associations were not evaluated. For the long term development of AF, we only captured AF episodes that required hospitalization as well as episodes of AF that occurred during hospitalization for any other reason. Therefore, there is a possibility that we

may have missed some cases of AF especially paroxysmal AF. Our limitations also include the fact these results only show association. As discussed above, these polymorphisms are in a noncoding region of chromosome 4. Although its proximity to PITX2 would suggest that it could possibly modulate the activity of this gene, there has been no direct evidence that supports this assumption. The strengths of our analyses include the strict definition of each variable, as well as regular quality checks performed by the database used for our analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline and intra-operative characteristics of the TexGen patient included in the study cohort (n = 1,166)

Characteristics	
Age mean/SD (years)	64.8/10
Men	930 (79%)
Concomitant valve surgery	188 (16%)
Acute coronary syndrome	555 (47%)
Hypertension (by history)	919 (78%)
Diabetes mellitus	386 (33%)
Rheumatic heart disease	9 (0.77%)
Prior atrial fibrillation	117 (10%)
New York Heart Association functional class III/IV symptoms	794 (68%)
Ejection fraction <35%	156 (13%)
Pre-operative beta blocker use	632 (54%)
Pre-operative anti-arrhythmic use (including amiodarone)	50 (4%)
Pre-operative statin use	650 (55%)
Aortic cross clamp time (min) mean $\pm$ SD	45.6 $\pm$ 32.4
Total bypass time (min) mean $\pm$ SD	75.15 $\pm$ 46.4

SD = standard deviation

Distribution of the genotypes for single nucleotide polymorphisms in rs2200733 and rs10033464 in TexGen coronary artery bypass grafting cohort

Table 2

rs2200733				rs10033464			
TT	CT	CC	HWE*	TT	GT	GG	HWE*
20 (1.7%)	234 (20%)	912 (78.2%)	0.23	8 (0.7%)	180 (15.5%)	979 (84%)	0.65

* p value for Hardy-Weinberg equilibrium (HWE) expectation of allelic frequencies using a chi-square goodness-of-fit model

Table 3

Associations between polymorphisms in rs2200733 and rs10033464, and the risk for development of postoperative atrial fibrillation

	Unadjusted			Model 1 [*]			Model 2 [†]		
	OR	(95% CI)	p	OR	(95% CI)	p	OR	(95% CI)	p
rs2200733 (TT, CT vs.CC)	1.33	(1.002–1.77)	0.048	1.384	(1.04–1.86)	0.028	1.41	(1.04–1.91)	0.025
rs10033464 (TT, GT vs. GG)	1.40	(1.02–1.93)	0.036	1.46	(1.05–2.02)	0.024	1.47	(1.05–2.06)	0.024

* Model 1: Adjusted for age and gender

† Model 2: Adjusted for age, gender, prior history of atrial fibrillation, hypertension, preoperative beta-blocker, statin or anti-arrhythmic use, and concomitant valve surgery

CI = confidence interval, OR = odds ratio

Table 4

Associations between polymorphisms in rs2200733 and rs10033464, and long term risk for the development of atrial fibrillation

	Unadjusted		Adjusted for age, gender, and hypertension	
	HR (95% CI)	p	HR (95% CI)	p
rs2200733 (TT, CT vs. CC)	1.27 (1.01–1.60)	0.038	1.32 (1.05–1.67)	0.017
rs10033464 (TT, GT vs. GG)	1.28 (0.99–1.65)	0.057	1.28 (1.00–1.66)	0.05

CI = confidence interval, HR = hazards ratio