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Common Genetic Variants on Chromosome 9p21 Predict Perioperative Myocardial Injury after Coronary Artery Bypass Graft Surgery

Kuang-Yu Liu, PhD[*,1], **Jochen D. Muehlschlegel, MD**[*,1], **Tjörvi E. Perry, MD**[1], **Amanda A. Fox, MD**[1], **Charles D. Collard, MD**[2], **Simon C. Body, MBChB, MPH**[1], and **Stanton K. Shernan, MD**[1]

[1]Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[2]Baylor College of Medicine Division of Cardiovascular Anesthesia at the Texas Heart Institute, Saint Luke's Episcopal Hospital, Houston, TX

Abstract

Objective—Approximately 10% of patients undergoing cardiac surgery suffer perioperative myocardial injury (PMI). A recent genome-wide association study identified an association between myocardial infarction in non-surgical populations and common genetic variants on chromosome 9p21. We hypothesized that these variants are also associated with PMI after isolated primary coronary artery bypass graft (CABG) surgery.

Methods—In a prospective observational study of 846 Caucasian primary CABG surgery patients at 2 US centers, we genotyped 61 linkage disequilibrium tagging single nucleotide polymorphisms (SNPs), encompassing 436 kbp of the 9p21 region. A multivariable logistic model was used to adjust for previously identified clinical covariates of PMI. PMI was defined as a postoperative day 1 cardiac Troponin I (cTnI) in the top 10^{th} percentile (> 9.13 μg/L) of the cohort. Multiple testing of SNPs was corrected for with family-wise (FW) errors.

Results—Prior myocardial infarction and longer cardiopulmonary bypass time were significant independent predictors of PMI. Levels of postoperative cTnI were incrementally increased for each additional copy of the risk alleles of three SNPs: rs10116277, rs6475606 and rs2383207. Adjusted additive odds ratios ranged between 1.64 and 1.79 (asymptotic P value between 3.7×10^{-3} - 6×10^{-4}) and remained significantly associated with PMI even after accounting for clinical covariates including severity of coronary disease, and multiple comparisons.

Correspondence to: Jochen D. Muehlschlegel, MD, Department of Anesthesiology, Perioperative and Pain Medicine, CWN L1, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115, Phone: 617-525-8156, Fax: 617-732-6798, jmuehlschlegel@partners.org.
*both authors contributed equally

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Conclusions—We have now demonstrated that common genetic variants in 9p21 previously known to be associated with myocardial infarction in non-surgical populations, are also associated with PMI after CABG. Further investigation is warranted to elucidate functional mechanisms.

Ultramini-Abstract—We identified an association between common genetic variants in the 9p21 region in primary CABG surgery patients and levels of postoperative cTnI, which were incrementally increased for each additional copy of the risk alleles. The association remained highly significant even after accounting for clinical covariates including severity of coronary disease.

Introduction

Multiple epidemiologic studies have documented a significant heritability for coronary artery disease (CAD) and myocardial infarction (1). Using an unbiased genome-wide approach, recent studies identified an association between cardiovascular disease, CAD or myocardial infarction, with common genetic variants on chromosome 9p21 in non-surgical populations (2-4). Though the responsible molecular genetic determinants remain largely unidentified, these variants are adjacent to genes for the cyclin-dependent kinases *CDKN2A/B*, which play a critical role in regulating cell aging, cell proliferation, and apoptosis (5-8), as well as *ANRIL*, a large anti-sense non-coding RNA gene, shown to be expressed in cell types integral to atherosclerosis (9-11).

Perioperative myocardial injury (PMI), which afflicts nearly one million people annually during or after non cardiac surgery worldwide(12), has conventionally been associated with transient myocardial ischemia and reperfusion due to aortic occlusion, cardiotomy, and an obligatory acute inflammatory response associated with cardiopulmonary bypass (CPB). While a few limited studies have implicated a heritable risk of PMI (13,14), variants in the 9p21 region have not yet been examined for an association with this adverse perioperative outcome. Therefore, we hypothesized that variants in the 9p21 region are also independently associated with PMI after isolated primary coronary artery bypass graft (CABG) surgery with CPB.

Methods

Two institutions (Brigham and Women's Hospital [BWH] and Texas Heart Institute [THI]), recruited patients within a single study structure known as the *CABG Genomics* Program (http://clinicaltrials.gov/show/NCT00281164). After August 2001, we prospectively enrolled patients aged 20-90 years undergoing non-emergent primary CABG surgery utilizing CPB, without other concurrent surgery. Patients with a preoperative hematocrit < 25% or transfusion of leukocyte-rich blood products within 30 days before surgery were not enrolled. In order to avoid potential population stratification, analysis was restricted to subjects who self-reported four Caucasian grand-parental ancestry. Study protocols were approved by respective Institutional Review Boards, and participants were enrolled following informed written consent. The funding agencies had no involvement in study design, data interpretation or data analysis.

Data Collection

At each site, we recorded patient demographics, perioperative risk factors, medications, and postoperative outcomes using study-specific case report forms. Blood samples were drawn prior to induction of general anesthesia, after administration of post-CPB protamine, and on the mornings of postoperative days (POD) 1-5. Serum and plasma were stored in vapor-phase liquid nitrogen until analysis for cardiac Troponin I (cTnI), B-type natriuretic peptide (BNP), and creatine kinase MB fraction on a sandwich immunoassay on a Triage® platform using monoclonal and polyclonal antibodies (Biosite Inc., San Diego, CA) at a single core facility.

Myocardial injury was defined as postoperative day (POD) 1 cTnI in the top 10^{th} percentile (> 9.13 μg/L).

Genotyping

DNA was extracted from white blood cells using standard procedures. Linkage-disequilibrium (LD) tagging SNPs between chromosome 9 positions 21,930,588-22,366,970 (NCBI Genome Build 36 assembly) encompassing the originally-identified 9p21 region with minor allele frequencies ≥5% in the HapMap Caucasian cohort were identified using Tagger (15). Overall sixty-one tagging SNPs that described 70.8% of the variation with a mean r2 of 0.8 in the 394 HapMap-identified SNPs were genotyped (Supplemental Table 1) using the Golden Gate assay with an Illumina Bead Station 500G system (Illumina, San Diego, CA), in accordance with the manufacturer's standard recommendations.. Analysis of the raw data was performed with the clustering algorithm of the Illumina BeadStudio software and individual examination of all intensity plots, with manual curation of genotype calls. SNPs with genotyping call rate <95%, with significant deviation from Hardy-Weinberg equilibrium ($P < 0.001$ in controls) and non-random missingness ($P<0.05$) between cases and controls were excluded from subsequent analysis.

Statistical Analysis

Categorical and continuous demographic characteristics were compared between groups with likelihood ratio chi-squared and Wilcoxon rank sum tests, respectively. A multivariable logistic model was used to derive perioperative and demographic variables associated with PMI, including severity of CAD. Factors previously associated with PMI after cardiac surgery in prior studies, including acute coronary syndrome, were forced into the logistic regression model along with clinically relevant variables using stepwise selection. PLINK (version 1.04) and SAS (version 9.1.3, SAS Institute, Cary, NC), were used for genetic association 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 PMI, based on log-additive genetic models. Adjusted odds ratios (OR) of every SNP for PMI were estimated using the above-mentioned multivariable logistic regression model. To correct for multiple testing, tests of significance are reported as family-wise empirical P values based on permuting case/control status.

Results

Between 8/2001 and 8/2006, 877 Caucasian patients were genotyped as described in the methods. 31 patients had genotyping rates <90% or were missing biomarkers and were excluded.

Patient characteristics stratified by PMI status after primary CABG surgery are shown in Table 1. Eighty-five patients (top 10^{th} percentile) had a POD1 cTnI level >9.13mcg/L, and therefore were classified as having PMI. Patients who had a past history of myocardial infarction, lower preoperative left ventricular ejection fraction, longer CPB duration or aortic cross clamp time, or were not receiving preoperative ACE inhibitors, were more likely to develop PMI. Similarly, elevated preoperative cTnI and CKMB, but not preoperative BNP, was associated with a higher risk of PMI. The degree of coronary artery stenosis by angiography or the number of vessels grafted intraoperatively was not significantly different between patients who developed PMI and those who did not. Patients with PMI were hospitalized for one additional day, but did not have significant differences in all-cause mortality. Multivariable logistic model identified recent myocardial infarction and longer duration of CPB time as significant independent predictors of PMI (Table 2).

All 61 SNPs passed quality control by genotyping call rate, Hardy-Weinberg equilibrium and non-random missingness. One SNP had an observed minor allele frequency of 1% and was excluded from further analysis leaving a total of 60 remaining SNPs that were included in the analysis (Supplementary Table 1). Levels of postoperative cTnI were incrementally increased for each additional copy of the risk alleles of rs10116277 (Figure 1), rs6475606 and rs2383207. These three SNPs within the coding region of *ANRIL* were also significantly associated with PMI, even after accounting for clinical covariates and multiple comparisons using an additive genetic model (Table 3). Careful inspection of model fit statistics, based on commonly used statistical criteria (Likelihood Ratio, Nagelkerke R^2, Akaike information criterion, and Schwarz's Bayesian information criterion), revealed very substantial incremental improvement of adding these 9p21 variants into the clinical predictive multivariable model of PMI. Significant pair-wise linkage disequilibrium was present in the region that precludes identification of a single associated variant (Figure 2). The SNP rs10811661, previously associated with type 2 diabetes (16), had no association with PMI in our cohort and is also not in LD with SNPs associated with PMI (Figure 2).

Discussion

We have shown that variants on 9p21 previously associated with cardiovascular disease, CAD or myocardial infarction in ambulatory non-surgical populations, are also associated with PMI after CAGB surgery with CPB. Furthermore, we confirmed that this association is independent of type 2 diabetes, and with the SNP rs10811661, previously associated with type 2 diabetes (16). Additionally, though the 9p21 variants have been associated with CAD in non-surgical patients, the association we observed between 9p21 SNPs and PMI in our cohort was independent of CAD severity assessed by coronary angiogram. To our knowledge, this is the first report demonstrating a unique and novel association between 9p21 variants and any adverse perioperative outcome.

Despite considerable efforts by geneticists, the underlying mechanisms of the association of 9p21 with cardiovascular disease, CAD and myocardial infarction have not been elucidated. The 9p21 region encompassing the identified SNPs is flanked by two recombination hotspots and is adjacent to the cyclin-dependent kinases *CDKN2A* and *CDKN2B*, which have important roles in cell cycle regulation (11). Through their role in TGF-β- induced growth inhibition, the CDKNs have been implicated in the pathogenesis of atherosclerosis (17), although their role in acute events such as myocardial infarction has yet to be elucidated. A recent study found that probands with the 9p21 risk allele had decreased expression of the *CDKN2A/2B* locus in peripheral blood leucocytes (18) and that inactivation of *CDKN2B* and/or *CDKN2A* allows cells to escape cell cycle arrest, or become senescent (11). Senescent cells are resistant to programmed cell death, leading to accumulation of these cells in injured tissue with substantial effects on healthy neighboring tissue (19). Hypothetically, ischemic injury may unmask and accelerate the effects of the 9p21 variants with at risk patients more susceptible to senescent cell accumulation. Also of interest, the associated SNPs lie within *ANRIL*, a large antisense non-coding RNA gene, considered to be operating in the transcriptional control repertoire of the cell and expressed in tissues and cell types affected by atherosclerosis (10). Experimental evidence suggests possible coordinated transcriptional regulation of ANRIL mediated by CDKN (10).

In addition to being a risk factor for CAD and having an association with myocardial infarction, variation in 9p21 has also been associated with carotid atherosclerosis, progression of atherosclerosis (20) and abdominal aortic aneurysm (AAA) (21,22). Surprisingly, 9p21 variants are also associated with intracranial aneurysms (22), a disease not mediated by atherosclerosis. However, the role of variants in 9p21 does not appear to be mediated through vascular reactivity.(23) This lack of association with vascular reactivity, along with the relation

of the 9p21 locus to aneurysms, suggests a mechanism more complex than simply promoting atherosclerotic plaque development.

The etiology of PMI occurring during cardiac surgery is markedly different from myocardial infarction occurring in ambulatory populations. Non-surgical myocardial infarction is predominantly associated with prolonged stress-induced myocardial ischemia (i.e. non-ST-segment elevation myocardial infarction) or coronary plaque disruption (i.e. ST-segment elevation myocardial infarction) (24-26). By contrast, CABG surgery with CPB requires aortic cross-clamping and the use of cardioplegic solutions with obligate ischemia and profound coagulation and inflammatory responses. Thus it is perhaps surprising that the genetic evidence of common 9p21 association with both myocardial infarction in non-surgical populations and PMI, supports common molecular mechanisms in both phenotypes.

Similar to the previously published genome-wide association studies in the ambulatory population, the most likely mode of inheritance in our population is an additive model. In contrast, the odds ratios per copy of the risk allele between 1.66 and 1.79 that we observed were significantly higher than the average of 1.3 in associations with cardiovascular disease in the GWAS in Caucasians(6). The variability in risk may be attributed to interaction with environmental factors and the examination of a provoked phenotype; all patients, cases and controls, had CAD severe enough to warrant CABG surgery. Compared to the non-surgical setting, the incidence of myocardial injury in our population is extremely high.

While genetic variation in the 9p21 locus is associated with incident CVD across many different populations and has a high frequency of the risk allele with a substantial increase in the probability of developing disease with each allele, the utility of screening for this polymorphism to improve risk prediction remains unclear. We observed very substantial incremental improvement of adding 9p21 variants into clinical predictive models of PMI. Similarly, in the Caucasian population of the Atherosclerosis Risk in Communities (ARIC) study (n=10,004), adding 9p21 variation to traditional risk factors improved CAD risk prediction and reclassification, particularly in higher risk categories. Conversely, in the Women's Genome Health Study (n=22,129), genetic variation in 9p21 did not improve discrimination or classification of predicted risk (27). Therefore, further investigation is warranted before the genetic variation in 9p21 can be routinely incorporated into perioperative risk prediction models.

Limitations

We have not yet replicated our findings in a separate cardiac surgical cohort. However, the 9p21 region is the most replicated locus for CAD and myocardial infarction to date across multiple populations. Furthermore, despite examining the entire region, we have observed consistent and strong effect sizes at the same SNPs previously described.

Population stratification, a limitation to all genetic association studies, was addressed by including patients in the analysis who self reported four generations of Caucasian grand-parental ancestry. In addition, we tested genomic controls and tested for differences between Northern and Southern Europeans. Accordingly, we believe that this association is not driven by cryptic heterogeneity.

In a study of this magnitude across two centers and many practitioners, differences in treatment will exist. Institutional differences are unlikely to confound the observed association between 9p21 variants and PMI as (i) personnel and patient were blinded to genotype and perioperative cTnI measurements performed as part of the study; (ii) within this cohort, we did not observe any association between genotype and risk factors for PMI; (iii) within this cohort, it is highly

unlikely that concomitant therapies were associated with genotype with the possible exception of gender which was included in all models.

Our analysis was limited to Caucasians and therefore these results are not generalizable to other ethnic groups with variable allele frequencies.

Conclusion

We have identified common genetic variants in 9p21 within or adjacent to genes CDKN2A/B and ANRIL, involved in cell-cycle regulation and atherosclerosis, which are significantly and independently associated with PMI in patients undergoing CABG with CPB. Further investigation is warranted to elucidate the mechanisms by which the variants exert their effects on the development of PMI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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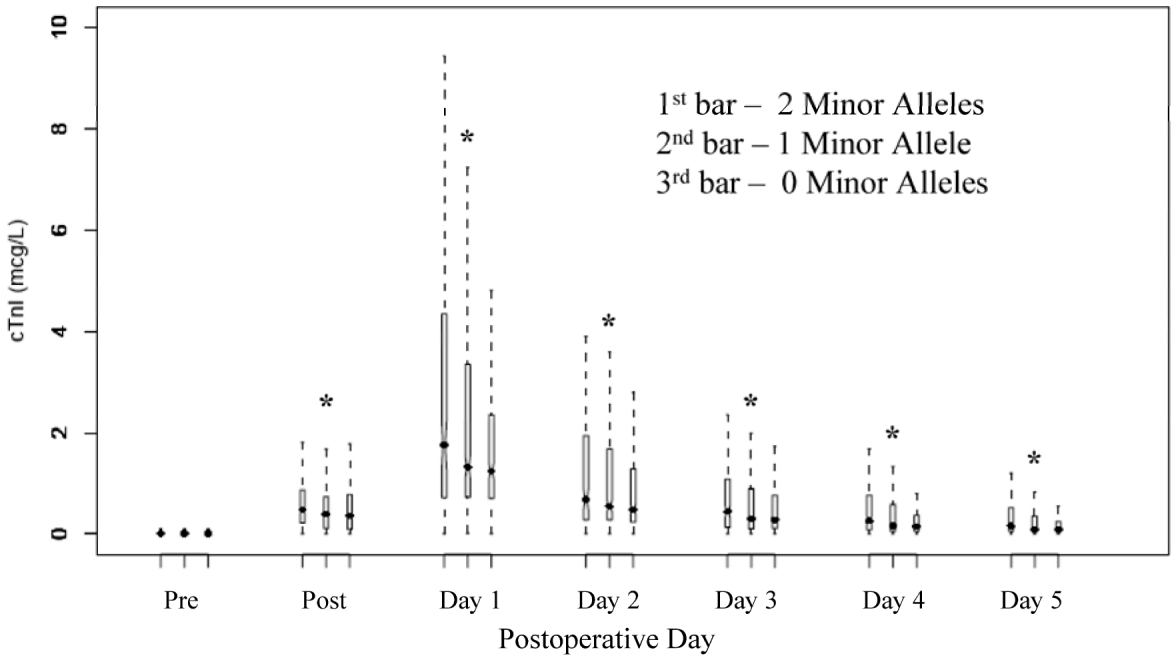

Figure 1. Cardiac Troponin I level for 9p21 risk allele

The SNP rs10116277 plotted against cardiac troponin I values on the seven perioperative time points. The bars on an individual day represent the copies of the risk allele; the first bar represents 2 copies, the second bar one copy, and the third bar no copy of the risk allele. Each bar represents median with inter-quartile range and outliers shown by the dotted lines.

"*"= P<0.01

cTnI, cardiac troponin I; Pre, preoperatively; Post, immediately post protamine

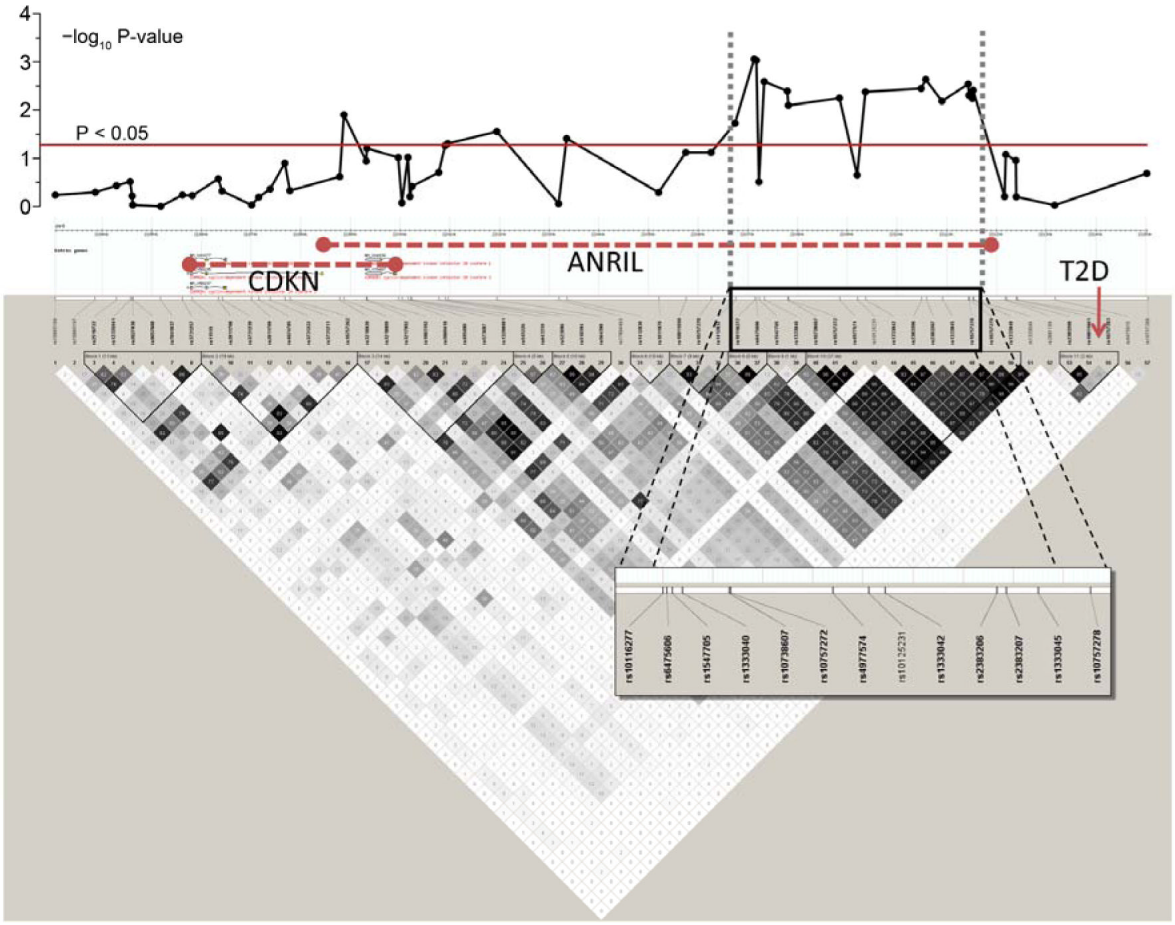

Figure 2. Linkage disequilibrium (LD) plot of 9p21 region

The upper panel shows negative $\log_{10}$ P-values for the association of the individual SNPs at their physical location with perioperative myocardial injury (PMI), after adjustment in multivariable regression model. The lower panel summarizes the LD structure in the HapMap database (CEU European ancestry). Regions of LD are shaded in grey (moderate LD) or black (strong LD). Predicted haplotype blocks are framed by triangles. The physical location of the genes CDKN2A and 2B and ANRIL are shown in the HapMap info tack above the SNPs. The type 2 diabetes (T2D) locus is seen on the far right side of the LD plot, with a non-significant association with PMI.

CDKN= cyclin dependent kinase

ANRIL= anti-sense non-coding RNA gene

T2D= type 2 diabetes

Table 1
Demographics and clinical characteristics

Demographics	cTnI < 9.13mcg/L	cTnI ≥9.13mcg/L	
	(N=761)	(N=85)	P-Value
Gender (N, % male)	628 (83)	67 (79)	0.374
Institution (N, %)	615 (81)	70 (82)	0.884
Age (years)	64 (57-72)	65 (59-74)	0.291
BMI (kg/m^2)	28.5 (25.8-31.9)	28.3 (25.0-32.7)	0.972
Past Medical History			
LVEF preop (%)	55 (45-60)	50 (45-60)	0.014
Diabetes (Insulin or non-insulin dependent; %)	203 (27)	16 (19)	0.150
Pulmonary disease (COPD, Asthma; %)	32 (4)	3(4)	0.767
Creatinine (mg/dL)	1 (0.9-1.2)	1.1 (0.9-1.3)	0.401
Hematocrit (%)	40 (34-44)	40 (37-43)	0.520
Hypertension (%)	570 (75)	60 (71)	0.360
Hypercholesterolemia (%)	581 (76)	62 (74)	0.591
Coronary Stenosis (>50%) regions			
≤2	165 (22)	14 (16)	
3	388 (51)	49 (58)	
≥ 4	208 (27)	22 (26)	0.417
Previous myocardial infarction	309 (41)	46 (54)	0.021
Myocardial infarction within prior 2 weeks (%)	119 (16)	26 (31)	0.001
Medications - preoperative (N, %)			
ACE inhibitor	30 (35)	362 (48)	0.039
β-blocker	587 (77)	66 (78)	0.915
Ca^{++} antagonist	103 (14)	8 (9)	0.396
Aspirin	568 (75)	60 (71)	0.434
HMG CoA reductase inhibitor	583 (77)	60 (71)	0.229
Biomarkers - preoperative			
BNP (μg/L)	18 (5-56)	26 (6-72)	0.112
CKMB (μg/L)	0.5 (0.2-1.2)	0.9 (0.3-3.0)	<0.001
cTnI (μg/L)	0.01 (0-0.03)	0.03 (0-0.9)	<0.001
Surgery			
Number of grafts (N, %)			
≤2	119 (16)	13 (15)	
3	356 (47)	41 (48)	
≥4	286 (38)	31 (36)	0.968
CPB duration (min)	94 (66-117)	108 (85-138)	<0.001
Aortic cross clamp duration (min)	71 (41-90)	82 (61-107)	<0.001
Postoperative Data			

Demographics	cTnI < 9.13mcg/L	cTnI ≥9.13mcg/L	
	(N=761)	(N=85)	P-Value
HLOS (days)	7 (6-9)	8 (6-10)	0.001
Mortality % (N) up to 5 yrs	52 (7)	10 (12)	0.120

BMI, Body mass index; LVEF, left ventricular ejection fraction; ACE, angiotensin converting enzyme; HMG, 3-hydroxy-3-methyl-glutaryl-CoA reductase; BNP, B-type natriuretic peptide; CKMB creatinine kinase MB fraction; cTnI, cardiac troponin I; CPB, cardiopulmonary bypass; HLOS, hospital length of stay.

Table 2
Multivariable clinical predictor model of perioperative myocardial injury

Predictor	Odds Ratio (95% CI)	P-Value
Age (Decile)	1.11 (0.88-1.39)	0.378
Gender (Male)	1.25 (0.69-2.28)	0.457
Institution	0.66 (0.86-3.2)	0.131
Preoperative HMG Co-A reductase inhibitor use	0.73 (0.44-1.22)	0.232
Myocardial infarction within previous 2 weeks	2.29 (1.37-3.84)	0.002
Coronary Stenosis (>50%) regions		
≤2	1	
3	1.14 (0.60-2.18)	
≥4	0.97 (0.47-2.01)	0.80
CPB time	1.14 (1.08-1.21)	<0.0001
Preoperative Creatinine	1.56 (0.16-15.5)	0.70

Important clinical and demographic variables from table 1 selected by stepwise regression and entered into multivariable model.

CPB, cardiopulmonary bypass, in increments of 10-mimutes

Table 3

Selected 9p21 SNPs associated with PMI based logistic regression model

SNP	Chr 9 position	Allele (Minor/Major)	Minor Allele Frequency		Covariate-adjusted additive genetic model*			
			Cases	Controls	Odds Ratio (95% CI)	Asymptotic P-value †	FWER permuted P-value ‡	Prior non-surgical MI studies
rs10116277	22,071,397	G/T	0.53	0.40	1.79 (1.29 - 2.51)	0.001	0.019	(7)
rs6475606	22,071,850	C/T	0.53	0.40	1.79 (1.28 - 2.50)	0.001	0.020	
rs1333040	22,073,404	C/T	0.46	0.33	1.66 (1.20, 2.28)	0.002	0.054	(7)
rs2383206	22,105,026	A/G	0.50	0.38	1.67 (1.20, 2.32)	0.002	0.061	(2)
rs2383207	22,105,959	A/G	0.50	0.37	1.71 (1.23 - 2.38)	0.001	0.040	(7)
rs10757278	22,114,477	A/G	0.54	0.42	1.70 (1.22 - 2.38)	0.001	0.052	(7)

Six most significant SNPs by asymptotic P-value selected. For entire SNP list, see supplement table 1.

SNP, single nucleotide polymorphism; Chr, chromosome; FWER, family-wise error rate; CI, confidence interval; MI, myocardial infarction

*The cohort-specific clinical model consists of predictors from the multivariable model in Table 2.

†Asymptotic P-value covariate adjusted

‡Family-wise empirical P value is used to adjust for multiple comparisons.