Common genetic variants on chromosome 9p21 predict perioperative myocardial injury after coronary artery bypass graft surgery

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

Methods: In a prospective observational study of 846 Caucasian patients undergoing primary coronary bypass surgery at 2 US centers, we genotyped 61 linkage-disequilibrium tagging single nucleotide polymorphisms, encompassing 436 kbp of the 9p21 region. A multivariable logistic model was used to adjust for previously identified clinical covariates of perioperative myocardial injury. Perioperative myocardial injury was defined as a postoperative day 1 cardiac troponin I in the top 10th percentile ($>9.13 \mu\text{g/L}$) of the cohort. Multiple testing of single nucleotide polymorphisms was corrected for with family-wise errors.

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

Conclusions: We have now demonstrated that common genetic variants in the same 9p21 locus, previously known to be associated with myocardial infarction in nonsurgical populations, are also associated with perioperative myocardial injury after coronary artery bypass grafting. Further investigation is warranted to elucidate functional mechanisms. (*J Thorac Cardiovasc Surg* 2010;139:483-88)

Supplemental material is available online.

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 nonsurgical populations.²⁻⁴ Although 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 antisense noncoding RNA gene, shown to be expressed in cell types integral to atherosclerosis.⁹⁻¹¹

Perioperative myocardial injury (PMI), which afflicts nearly 1 million people annually during or after noncardiac surgery worldwide,¹² has conventionally been associated with transient myocardial ischemia and reperfusion owing to aortic occlusion, cardiectomy, and an obligatory acute inflammatory response associated with cardiopulmonary bypass (CPB). Although 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 and Texas Heart Institute, recruited patients within a single study structure known as the CABG

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Abbreviations and Acronyms

CABG	= coronary artery bypass graft
CAD	= coronary artery disease
CPB	= cardiopulmonary bypass
cTNI	= cardiac troponin I
PMI	= perioperative myocardial injury
POD	= postoperative day
SNP	= single nucleotide polymorphism

Genomics Program (<http://clinicaltrials.gov/show/NCT00281164>). After August 2001, we prospectively enrolled patients aged 20 to 90 years undergoing nonemergency primary CABG surgery with CPB, without other concurrent surgery. Patients with a preoperative hematocrit value of less than 25% or transfusion of leukocyte-rich blood products within 30 days before the operation were not enrolled. To avoid potential population stratification, we restricted analysis to subjects who self-reported 4 Caucasian grandparental ancestry. Study protocols were approved by the respective institutional review boards, and participants were enrolled after providing 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 before induction of general anesthesia, after administration of post-CPB protamine, and on the mornings of postoperative days (PODs) 1 to 5. Serum and plasma were stored in vapor-phase liquid nitrogen until analysis for cardiac troponin I (cTnI), B-type natriuretic peptide, and creatine kinase MB fraction on a sandwich immunoassay on a Triage platform using monoclonal and polyclonal antibodies (Biosite Inc, San Diego, Calif) at a single core facility. Myocardial injury was defined as POD 1 cTnI in the top 10th percentile ($>9.13 \mu\text{g/L}$).

Genotyping

DNA was extracted from white blood cells by standard procedures. Linkage-disequilibrium tagging single-nucleotide polymorphisms (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 of 5% or greater in the HapMap Caucasian cohort were identified using Tagger.¹⁵ Overall, 61 tagging SNPs that described 70.8% of the variation with a mean r^2 of 0.8 in the 394 HapMap-identified SNPs were genotyped (Supplemental Table 1) using the Golden Gate assay with an Illumina Bead Station 500 G system (Illumina, San Diego, Calif), in accordance with the manufacturer's standard recommendations. The raw data were analyzed 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 less than 95%, with significant deviation from Hardy–Weinberg equilibrium ($P < .001$ in controls) and nonrandom missingness ($P < .05$) between cases and controls, were excluded from subsequent analysis.

Statistical Analysis

Categorical and continuous demographic characteristics were compared between groups with likelihood ratio χ^2 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 logis-

tic regression model along with clinically relevant variables using stepwise selection. PLINK (version 1.04) and SAS (version 9.1.3; SAS Institute, Inc, Cary, NC) were used for genetic association analysis. Hardy–Weinberg equilibrium was evaluated by 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 of every SNP for PMI were estimated by the aforementioned multivariable logistic regression model. To correct for multiple testing, we report tests of significance as family-wise empirical P values based on permuting case–control status.

RESULTS

Between August 2001 and August 2006, 877 Caucasian patients were genotyped as described in the Methods section. Thirty-one patients had genotyping rates of less than 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 10th percentile) had a POD 1 cTnI level greater than $9.13 \mu\text{g/L}$ and therefore were classified as having PMI. Patients who had a history of myocardial infarction, lower preoperative left ventricular ejection fraction, longer CPB duration or aortic crossclamp time, or were not receiving preoperative angiotensin-converting enzyme inhibitors were more likely to have PMI. Similarly, elevated preoperative cTnI and creatine kinase MB, but not preoperative B-type natriuretic peptide, were 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 had PMI and those who did not. Patients with PMI were hospitalized for 1 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 nonrandom 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 (Table E1). Levels of postoperative cTnI were incrementally increased for each additional copy of the minor allele (ie, the risk allele in our cohort) 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–Bayesian information criterion), revealed very substantial incremental improvement of adding these 9p21 variants into the clinical predictive multivariable model of PMI.

TABLE 1. Demographics and clinical characteristics

	cTnI < 9.13 μg/L (N = 761)	cTnI ≥ 9.13 μg/L (N = 85)	P value
<i>Demographics</i>			
Gender (N, % male)	628 (83)	67 (79)	.374
Institution (N, %)	615 (81)	70 (82)	.884
Age (y)	64 (57–72)	65 (59–74)	.291
BMI (kg/m ²)	28.5 (25.8–31.9)	28.3 (25.0–32.7)	.972
<i>Medical history</i>			
LVEF preop (%)	55 (45–60)	50 (45–60)	.014
Diabetes (insulin-dependent or non-insulin dependent; %)	203 (27)	16 (19)	.150
Pulmonary disease (COPD, asthma; %)	32 (4)	3 (4)	.767
Creatinine (mg/dL)	1 (0.9–1.2)	1.1 (0.9–1.3)	.401
Hematocrit (%)	40 (34–44)	40 (37–43)	.520
Hypertension (%)	570 (75)	60 (71)	.360
Hypercholesterolemia (%)	581 (76)	62 (74)	.591
Coronary stenosis (>50%) regions			
≤2	165 (22)	14 (16)	
3	388 (51)	49 (58)	
≥4	208 (27)	22 (26)	.417
Previous myocardial infarction	309 (41)	46 (54)	.021
Myocardial infarction within prior 2 wk (%)	119 (16)	26 (31)	.001
<i>Medications—preoperative (N, %)</i>			
ACE inhibitor	30 (35)	362 (48)	.039
β-blocker	587 (77)	66 (78)	.915
Ca ⁺⁺ antagonist	103 (14)	8 (9)	.396
Aspirin	568 (75)	60 (71)	.434
HMG CoA reductase inhibitor	583 (77)	60 (71)	.229
<i>Biomarkers—preoperative</i>			
BNP (μg/L)	18 (5–56)	26 (6–72)	.112
CKMB (μg/L)	0.5 (0.2–1.2)	0.9 (0.3–3.0)	<.001
cTnI (μg/L)	0.01 (0–0.03)	0.03 (0–0.9)	<.001
<i>Surgery</i>			
No. of grafts (N, %)			
≤2	119 (16)	13 (15)	
3	356 (47)	41 (48)	
≥4	286 (38)	31 (36)	.968
CPB duration (min)	94 (66–117)	108 (85–138)	<.001
Aortic crossclamp duration (min)	71 (41–90)	82 (61–107)	<.001
<i>Postoperative data</i>			
HLOS (d)	7 (6–9)	8 (6–10)	.001
Mortality (%; N) up to 5 y	52 (7)	10 (12)	.120

cTnI, Cardiac troponin I; BMI, body mass index; LVEF, left ventricular ejection fraction; COPD, chronic obstructive pulmonary disease; ACE, angiotensin-converting enzyme; HMG CoA, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase; BNP, B-type natriuretic peptide; CKMB, creatinine kinase MB fraction; CPB, cardiopulmonary bypass; HLOS, hospital length of stay.

Significant pairwise linkage disequilibrium was present in the region that precludes identification of a single associated variant (Figure 2). The SNP rs10811661, previously associ-

ated with type 2 diabetes,¹⁶ had no association with PMI in our cohort and is also not in linkage disequilibrium 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 nonsurgical 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, although variants within the 9p21 chromosomal locus have been associated with CAD in nonsurgical patients, the association we observed between 9p21 SNPs and PMI in our cohort was independent of CAD severity assessed by coronary angiogram. Similarly, while the SNPs associated with CAD and MI in nonsurgical patients are the same as for PMI in our cohort with severe CAD, the specific risk alleles in each population are different. Nonetheless, 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 transforming growth factor β-induced growth inhibition, the cyclin-dependent kinases 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 leukocytes¹⁸ 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 noncoding 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 cyclin-dependent kinase.¹⁰

In addition to being a risk factor for CAD and having an association with myocardial infarction, variation in 9p21

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)	.378
Gender (male)	1.25 (0.69–2.28)	.457
Institution	0.66 (0.86–3.2)	.131
Preoperative HMG CoA reductase inhibitor use	0.73 (0.44–1.22)	.232
Myocardial infarction within previous 2 weeks	2.29 (1.37–3.84)	.002
Coronary stenosis (>50%) regions		
≤ 2	1	
3	1.14 (0.60–2.18)	
≥ 4	0.97 (0.47–2.01)	.80
CPB time	1.14 (1.08–1.21)	<.0001
Preoperative creatinine	1.56 (0.16–15.5)	.70

Important clinical and demographic variables from Table 1 selected by stepwise regression and entered into multivariable model. *CI*, Confidence interval; *HMG CoA*, 3-hydroxy-3-methyl-glutaryl coenzyme A; *CPB*, cardiopulmonary bypass, in increments of 10 minutes.

has also been associated with carotid atherosclerosis, progression of atherosclerosis,²⁰ and abdominal aortic aneurysm.^{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. Nonsurgical myocardial infarction is predominantly associated with prolonged stress-induced myocardial ischemia (ie, non-ST-segment elevation myocardial infarction) or coronary plaque disruption (ie, ST-segment elevation myocardial infarction).^{24–26} By contrast, CABG surgery with CPB requires aortic cross-

FIGURE 1. Cardiac troponin I level for 9p21 risk allele. The SNP rs10116277 plotted against cardiac troponin I values on the 7 perioperative time points. The bars on an individual day represent the copies of the risk allele; the first bar represents two copies, the second bar one copy, and the third bar no copy of the risk allele. Each bar represents median with interquartile range and outliers are shown by the dotted lines. * $P < .01$. *cTnI*, Cardiac troponin I; *Pre*, preoperatively; *Post*, immediately after protamine.

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 nonsurgical 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 genome-wide association studies 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

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*			Prior nonsurgical MI studies
			Cases	Controls	Odds ratio (95% CI)	Asymptotic P value†	FWER permuted P value‡	
rs10116277	22,071,397	G/T	0.53	0.40	1.79 (1.29–2.51)	.001	.019	(7)
rs6475606	22,071,850	C/T	0.53	0.40	1.79 (1.28–2.50)	.001	.020	
rs1333040	22,073,404	C/T	0.46	0.33	1.66 (1.20–2.28)	.002	.054	(7)
rs2383206	22,105,026	A/G	0.50	0.38	1.67 (1.20–2.32)	.002	.061	(2)
rs2383207	22,105,959	A/G	0.50	0.37	1.71 (1.23–2.38)	.001	.040	(7)
rs10757278	22,114,477	A/G	0.54	0.42	1.70 (1.22–2.38)	.001	.052	(7)

Six most significant SNPs by asymptotic *P* value selected. For entire SNP list, see Supplement Table 1. *SNP*, Single nucleotide polymorphism; *PMI*, perioperative myocardial injury; *Chr*, chromosome; *CI*, confidence interval; *FWER*, family-wise error rate; *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.

FIGURE 2. Linkage-disequilibrium (LD) plot of 9p21 region. The *upper panel* shows negative $\log_{10} P$ values for the association of the individual single nucleotide polymorphism (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 gray (moderate LD) or black (strong LD). Predicted haplotype blocks are framed by *triangles*. The physical location of the genes *CDKN2A*, *CDKN2B*, and *ANRIL* are shown in the HapMap info track above the SNPs. The type 2 diabetes locus is seen on the far right side of the LD plot, with a nonsignificant association with PMI. *CDKN*, Cyclin-dependent kinase; *ANRIL*, antisense noncoding RNA gene; *T2D*, type 2 diabetes.

with the nonsurgical setting, the incidence of myocardial injury in our population is extremely high.

Although genetic variation in the 9p21 locus is associated with incident cardiovascular disease 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 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 4 generations of Caucasian grandparental 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 for these reasons: (1) personnel and patients were blinded to genotype and perioperative cTnI measurements performed as part of the study; (2) within this cohort, we did not observe any association between genotype and risk factors for PMI; and (3) 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.

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References

1. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. 2004;364:937-52.
2. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. *Science*. 2007;316:1488-91.
3. Consortium WTCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007;447:661-78.
4. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genome-wide association analysis of coronary artery disease. *N Engl J Med*. 2007;357:443-53.
5. Larson MG, Atwood LD, Benjamin EJ, Cupples LA, RB D'Agostino Sr, Fox CS, et al. Framingham Heart Study 100 K project: genome-wide associations for cardiovascular disease outcomes. *BMC Med Genet*. 2007;8(Suppl 1):S5.
6. Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, et al. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. *Circulation*. 2008;117:1675-84.
7. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science*. 2007;316:1491-3.
8. Abdullah KG, Li L, Shen GQ, Hu Y, Yang Y, MacKinlay KG, et al. Four SNPs on chromosome 9p21 confer risk to premature, familial CAD and MI in an American Caucasian population (GeneQuest). *Ann Hum Genet*. 2008;72(Pt 5):654-7.
9. Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, et al. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9 p. *Hum Mol Genet*. 2008;17:806-14.
10. Pasmant E, Laurendeau I, Heron D, Vidaud M, Vidaud D, Bieche I. Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. *Cancer Res*. 2007;67:3963-9.
11. Kim WY, Sharpless NE. The regulation of INK4/ARF in cancer and aging. *Cell*. 2006;127:265-75.
12. Devereaux PJ, Goldman L, Cook DJ, Gilbert K, Leslie K, Guyatt GH. Perioperative cardiac events in patients undergoing noncardiac surgery: a review of the magnitude of the problem, the pathophysiology of the events and methods to estimate and communicate risk. *CMAJ*. 2005;173:627-34.
13. Podgoreanu MV, White WD, Morris RW, Mathew JP, Stafford-Smith M, Welsby IJ, et al. Inflammatory gene polymorphisms and risk of postoperative myocardial infarction after cardiac surgery. *Circulation*. 2006;114(1 Suppl):I275-81.
14. Collard CD, Shernan SK, Fox AA, Bernig T, Chanock SJ, Vaughn WK, et al. The MBL2 "LYQA secretor" haplotype is an independent predictor of postoperative myocardial infarction in whites undergoing coronary artery bypass graft surgery. *Circulation*. 2007;116(11 Suppl):I106-12.
15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21:263-5.
16. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science*. 2007;316:1331-6.
17. Kalinina N, Agrotis A, Antropova Y, Ilyinskaya O, Smirnov V, Tararak E, et al. Smad expression in human atherosclerotic lesions: evidence for impaired TGF-beta/Smad signaling in smooth muscle cells of fibrofatty lesions. *Arterioscler Thromb Vasc Biol*. 2004;24:1391-6.
18. Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, Mohlke KL, et al. INK4/ARF transcript expression is associated with chromosome 9p21 variants linked to atherosclerosis. *PLoS ONE*. 2009;4:e5027.
19. Raffetto JD, Leverkus M, Park HY, Menzoian JO. Synopsis on cellular senescence and apoptosis. *J Vasc Surg*. 2001;34:173-7.
20. Ye S, Willeit J, Kronenberg F, Xu Q, Kiechl S. Association of genetic variation on chromosome 9p21 with susceptibility and progression of atherosclerosis: a population-based, prospective study. *J Am Coll Cardiol*. 2008;52:378-84.
21. Thompson AR, Golledge J, Cooper JA, Hafez H, Norman PE, Humphries SE. Sequence variant on 9p21 is associated with the presence of abdominal aortic aneurysm disease but does not have an impact on aneurysmal expansion. *Eur J Hum Genet*. 2009;17:391-4. Epub 2008 Oct 15.
22. Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. *Nat Genet*. 2008;40:217-24.
23. Samani NJ, Raitakari OT, Sipila K, Tobin MD, Schunkert H, Juonala M, et al. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2008;28:1679-83.
24. Priebe HJ. Perioperative myocardial infarction—etiology and prevention. *Br J Anaesth*. 2005;95:3-19.
25. Landesberg G, Luria MH, Cotev S, Eidelman LA, Anner H, Mosseri M, et al. Importance of long-duration postoperative ST-segment depression in cardiac morbidity after vascular surgery. *Lancet*. 1993;341:715-9.
26. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, et al. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). *J Am Coll Cardiol*. 2000;36:970-1062.
27. Paynter NP, Chasman DI, Buring JE, Shiffman D, Cook NR, Ridker PM. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3. *Ann Intern Med*. 2009;150:65-72.

TABLE E1. Sixty 9p21 SNPs considered for association with PMI based on 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‡
rs10965189	21930588	A/C	0.03	0.04	0.76 (0.30–1.93)	.559	1.000
rs10965197	21938666	T/C	0.45	0.42	1.11 (0.79–1.55)	.540	1.000
rs2518722	21942926	T/C	0.27	0.31	0.82 (0.56–1.20)	.310	1.000
rs12335941	21945669	G/A	0.37	0.42	0.82 (0.58–1.16)	.266	1.000
rs2027938	21946078	A/G	0.25	0.28	0.89 (0.61–1.30)	.535	1.000
rs9657608	21946230	T/C	0.09	0.10	0.98 (0.56–1.72)	.952	1.000
rs7041637	21951866	A/C	0.31	0.30	1.02 (0.71–1.47)	.916	1.000
rs3731257	21956221	T/C	0.29	0.26	1.11 (0.77–1.61)	.569	1.000
rs11515	21958199	G/C	0.15	0.17	0.86 (0.55–1.33)	.498	1.000
rs2811708	21963422	T/G	0.26	0.31	0.79 (0.54–1.15)	.213	.996
rs3731239	21964218	C/T	0.35	0.31	1.16 (0.81–1.64)	.416	1.000
rs2811709	21970151	A/G	0.12	0.13	0.95 (0.59–1.54)	.838	1.000
rs4074785	21971583	A/G	0.10	0.09	1.13 (0.66–1.93)	.657	1.000
rs3731222	21973914	G/A	0.13	0.16	0.81 (0.50–1.32)	.400	1.000
rs3731211	21976847	A/T	0.26	0.33	0.72 (0.50–1.06)	.097	.907
rs10757262	21977874	T/A	0.14	0.16	0.83 (0.52–1.30)	.409	1.000
rs3218020	21987872	T/C	0.38	0.41	0.81 (0.58–1.13)	.217	.997
rs3218009	21988757	C/G	0.14	0.08	1.87 (1.15–3.04)	.011	.261
rs3217992	21993223	A/G	0.40	0.45	0.76 (0.54–1.07)	.111	.936
rs1063192	21993367	C/T	0.41	0.34	1.43 (1.01–2.01)	.045	.670
rs2069418	21999698	C/G	0.43	0.36	1.38 (0.98–1.94)	.063	.787
rs495490	22000412	C/T	0.10	0.10	1.08 (0.63–1.86)	.790	1.000
rs573687	22001642	T/C	0.35	0.29	1.38 (0.96–1.96)	.079	.855
rs13298881	22002051	C/T	0.13	0.14	0.87 (0.54–1.41)	.581	1.000
rs545226	22002422	C/T	0.43	0.46	0.86 (0.61–1.19)	.357	1.000
rs643319	22007836	T/G	0.45	0.41	1.25 (0.90–1.76)	.188	.992
rs523096	22009129	C/T	0.43	0.35	1.43 (1.02–2.00)	.038	.604
rs518394	22009673	G/C	0.43	0.36	1.46 (1.03–2.05)	.031	.539
rs564398	22019547	G/A	0.40	0.32	1.52 (1.07–2.15)	.020	.398
rs17694493	22031998	G/C	0.14	0.14	0.94 (0.59–1.51)	.801	1.000
rs1412830	22033612	A/G	0.36	0.29	1.48 (1.05–2.09)	.027	.498
rs1011970	22052134	T/G	0.15	0.18	0.85 (0.55–1.32)	.477	1.000
rs10811650	22057593	A/G	0.55	0.49	1.37 (0.98–1.90)	.064	.792
rs10757270	22062719	A/G	0.55	0.48	1.36 (0.98–1.89)	.063	.787
rs1412832	22067543	C/T	0.31	0.22	1.58 (1.10–2.28)	.013	.290
rs10116277	22071397	G/T	0.53	0.40	1.79 (1.29–2.51)	.001	.019
rs6475606	22071850	C/T	0.53	0.40	1.79 (1.28–2.50)	.001	.020
rs1547705	22072375	C/A	0.13	0.16	0.77 (0.48–1.23)	.276	1.000
rs1333040	22073404	C/T	0.46	0.33	1.66 (1.20–2.28)	.002	.054
rs10738607	22078094	A/G	0.53	0.41	1.65 (1.19–2.30)	.003	.077
rs10757272	22078260	C/T	0.52	0.41	1.59 (1.14–2.21)	.006	.157
rs4977574	22088574	A/G	0.52	0.40	1.63 (1.17–2.27)	.004	.103
rs10125231	22092128	A/G	0.02	0.04	0.53 (0.16–1.75)	.297	1.000
rs1333042	22093813	A/G	0.50	0.38	1.65 (1.19–2.30)	.003	.079
rs2383206	22105026	A/G	0.50	0.38	1.67 (1.20–2.32)	.002	.061
rs2383207	22105959	A/G	0.50	0.37	1.71 (1.23–2.38)	.002	.040
rs1333045	22109195	T/C	0.50	0.39	1.63 (1.17–2.28)	.004	.113
rs10757278	22114477	A/G	0.54	0.42	1.70 (1.22–2.38)	.002	.052
rs10757279	22114630	A/G	0.54	0.42	1.65 (1.18–2.32)	.004	.098
rs1333048	22115347	A/C	0.51	0.40	1.64 (1.18–2.30)	.004	.098
rs1333049	22115503	C/G	0.54	0.42	1.67 (1.20–2.33)	.003	.066

TABLE E1. Continued

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‡
rs2891169	22121825	G/A	0.39	0.40	0.92 (0.66–1.28)	.626	1.000
rs2383208	22122076	G/A	0.14	0.19	0.66 (0.41–1.06)	.084	.870
rs10811661	22124094	C/T	0.13	0.18	0.68 (0.42–1.10)	.117	.946
rs10757283	22124172	T/C	0.43	0.44	0.92 (0.66–1.28)	.603	1.000
rs6475610	22131894	T/C	0.49	0.49	0.99 (0.72–1.36)	.947	1.000
rs10757289	22150454	C/G	0.46	0.41	1.23 (0.89–1.71)	.214	.997
rs1679013	22196987	T/C	0.51	0.50	1.08 (0.78–1.50)	.659	1.000
rs10113901	22364032	C/T	0.49	0.49	0.97 (0.70–1.34)	.837	1.000
rs7046709	22366970	G/A	0.34	0.34	1.00 (0.71–1.41)	.987	1.000

Six most significant SNPs by asymptotic *P* value selected for Table 1 are shaded in gray. *SNP*, Single nucleotide polymorphism; *PMI*, perioperative myocardial injury; *Chr*, chromosome; *CI*, confidence interval; *FWER*, family-wise error rate. *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.