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Genetic Variants in P-Selectin and C-Reactive Protein Influence Susceptibility to Cognitive Decline After Cardiac Surgery

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Objectives We hypothesized that candidate gene polymorphisms in biologic pathways regulating inflammation, cell matrix adhesion/interaction, coagulation-thrombosis, lipid metabolism, and vascular reactivity are associated with postoperative cognitive deficit (POCD).

Background Cognitive decline is a common complication of coronary artery bypass graft (CABG) surgery and is associated with a reduced quality of life.

Methods In a prospective cohort study of 513 patients (86% European American) undergoing CABG surgery with cardiopulmonary bypass, a panel of 37 single-nucleotide polymorphisms (SNPs) was genotyped by mass spectrometry. Association between these SNPs and cognitive deficit at 6 weeks after surgery was tested using multiple logistic regression accounting for age, level of education, baseline cognition, and population structure. Permutation analysis was used to account for multiple testing.

Results We found that minor alleles of the *CRP* 1059G/C SNP (odds ratio [OR] 0.37, 95% confidence interval [CI] 0.16 to 0.78; p = 0.013) and the *SELP* 1087G/A SNP (OR 0.51, 95% CI 0.30 to 0.85; p = 0.011) were associated with a reduction in cognitive deficit in European Americans (n = 443). The absolute risk reduction in the observed incidence of POCD was 20.6% for carriers of the *CRP* 1059C allele and 15.2% for carriers of the *SELP* 1087A allele. Perioperative serum C-reactive protein (CRP) and degree of platelet activation were also significantly lower in patients with a copy of the minor alleles, providing biologic support for the observed allelic association.

Conclusions The results suggest a contribution of P-selectin and CRP genes in modulating susceptibility to cognitive decline after cardiac surgery, with potential implications for identifying populations at risk who might benefit from targeted perioperative antiinflammatory strategies. (J Am Coll Cardiol 2007;49:1934–42) © 2007 by the American College of Cardiology Foundation

Postoperative cognitive dysfunction (POCD), including impairments in attention, memory, language, and processing time, serves as a marker of long-term cognitive decline and is associated with a reduced quality of life despite patients' expectations that recovery of physical status will generally improve their lives (1). Although many adverse events related to cardiac surgery have been minimized, little progress has been made in reducing POCD, occurring in as many as 53% of patients at hospital discharge and 36% at 6 weeks (2). The etiology of perioperative neurologic injury is multifactorial and includes cerebral embolism and hypoperfusion, but preliminary reports of heritability of cognitive decline (3,4) suggest that genetic factors may also modulate response to this type of neurologic injury. We therefore hypothesized that polymorphisms in candidate genes regulating biologic pathways for inflammation, cell matrix adhesion/interaction, coagulation-thrombosis, lipid metabolism, and vascular reactivity are associated with incidence of POCD after CABG surgery.

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Methods

Study population. Patients enrolled in the study were part of the PEGASUS (Perioperative Genetics and Safety Outcomes Study), an ongoing Institutional Review Board-approved prospective longitudinal study at Duke University Medical Center. The present substudy targeted a cohort of patients undergoing isolated coronary artery bypass graft (CABG) surgery using cardiopulmonary bypass (CPB) between April 1994 and May 2002 in whom detailed genotyping and prospective cognitive testing were performed. Patients were excluded if they had a history of symptomatic cerebrovascular disease, psychiatric illness, renal failure, active liver disease, bleeding disorders, or less than a seventh-grade education.

Measurement of cognitive function phenotype. Cognitive function was assessed the day before surgery and at 6 weeks after surgery by investigators experienced in neuropsychologic testing and who were blinded to the genetic data. In accordance with the Consensus Statement on Assessment of Neurobehavioral Outcomes After Cardiac Surgery (5), we used a cognitive test battery comprising the following 5 instruments: Short Story module of the Randt Memory Test (6), Modified Visual Reproduction Test from the Wechsler Memory Scale (7), Digit Span subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) exam (8), Digit Symbol subtest of the WAIS-R (8), and the Trail Making Test (Part B) (9).

To account for correlation among cognitive test scores, we performed factor analysis on the 10 cognitive test scores from baseline, as previously described (2). We used SAS Proc Factor (SAS/Genetics version 8.02, SAS Institute, Cary, North Carolina) with a principal components method without priors. Factors were rotated by an orthogonal varimax transformation yielding uncorrelated rotated factors and independent scores representing 4 cognitive domains: 1) verbal memory and language comprehension (short-term and delayed); 2) attention, psychomotor processing speed, and concentration; 3) abstraction and visuospatial orientation; and 4) figural memory. The factor analysis was performed on baseline (preoperative) scores from all eligible patients in our ongoing prospective post-CABG cognitive database (n = 867), of which the patients in the present study were a subset. Factor weights were then used to score the patients in the present study, both at baseline and at 6 weeks. Postoperative cognitive deficit was defined as a decline from baseline score of at least 1 standard deviation for 1 or more of the 4 domain scores at 6 weeks after surgery. Baseline cognitive score was defined as the mean of the 4 preoperative factor scores. A complete description of the factor analysis, including a scree plot and a table of factor loadings, is available in the online appendix.

Candidate gene and polymorphism selection. A candidate gene set, representing pathways putatively involved in cognitive dysfunction, was selected a priori based on comprehensive analysis of existing expression studies, linkage data, neuropharmacology, population-based association studies, and expert opinion. Principal among these are inflammation, cell matrix adhesion/interaction, thrombosis, lipid metabolism, and vascular reactivity pathways. Preference was given to genes previously implicated in memory, learning, cognition, or mental retardation in both humans and experimental animal models of cognition (10). A panel of 37 specific polymorphisms in these candidate genes was identified from public databases, with emphasis on variants with demonstrated or high likelihood of functionally significant effects (Table 1).

Abbreviations and Acronyms

- **AA** = African American
- **CABG** = coronary artery bypass graft
- **CD62P** = P-selectin
- **CPB** = cardiopulmonary bypass
- **CRP** = C-reactive protein
- **EA** = European American
- **NA** = Native American
- **POCD** = postoperative cognitive deficit
- ***SELP*** = P-selectin gene
- **SNP** = single-nucleotide polymorphism

Isolation of genomic DNA and genotype analysis. After isolation of genomic DNA from whole blood, genotyping assays were conducted by matrix assisted laser desorption/ionization time-of-flight mass spectrometry on a Sequenom MassArray system (Sequenom, San Diego, California) at Agencourt Bioscience Corporation (Beverly, Massachusetts). Primers used and polymorphism details are available at: http://anesthesia.duhs.duke.edu/pegasus/cognition/1/cognition-webTable1.htm. Genotyping accuracy of the Sequenom MassArray system was estimated at 99.6% (11). Using direct sequencing on an ABI3700 capillary sequencer (Applied Biosystems, Foster City, California), genotyping reproducibility in this study was validated to be >99% by scoring a panel of 6 polymorphisms in 100 randomly selected patients. The *ACE* insertion/deletion polymorphism was genotyped by polymerase chain reaction amplification followed by size-fractionation through electrophoresis (12).

Statistical analysis. Categoric and continuous demographic characteristics were compared between POCD groups with Pearson chi-squared and Wilcoxon rank sum tests, respectively. To test for association between the 37 candidate gene polymorphisms and incidence of POCD, we used a previously described 2-stage analysis approach: marker selection by set association followed by model building (13). This method has been shown to be much more powerful than individual marker analysis when multiple genes are likely involved in a disease phenotype (14).

Allele and genotype frequencies were calculated for each polymorphism, and Hardy-Weinberg equilibrium was evaluated using an exact test. All association analyses were based on 2 genotypic classes, distinguished by the presence or absence of at least 1 copy of the minor allele (homozygote minor and heterozygote versus homozygote major). In the first stage, the set association approach (15) was used to identify a set of markers jointly associated with POCD as follows: Pearson's chi-square statistics were computed for

JACC Vol. 49, No. 19, 2007
May 15, 2007:1934–42

Table 1 Polymorphisms Evaluated for Their Relationship to Cognitive Decline After CABG Surgery

Functional Category	Gene Name	SNP ID*	Major/Minor Allele	Genomic Context	Minor Allele Frequency in EA Patients Without Cognitive Deficit
Inflammation	CRP (C-reactive protein)	rs1205	2147C/T	3′UTR	0.338
		rs1800947	1059G/C	L184L	0.080
	IL1A (interleukin-1 alpha)	rs17561†	10876G/T	A114S	0.315
		rs1800587	−889 C/T	5′UTR	0.317
	IL1RN (interleukin-1 receptor antagonist)	rs315952‡	16857T/C	S112S	0.302
		rs2229235	13760T/C	A39A	0.297
	IL6 (interleukin-6)	rs1800795	−174G/C	5′UTR	0.473
		rs1800796	−572G/C	5′UTR	0.044
		rs1800797	−597G/A	5′UTR	0.469
	TNFA (tumor necrosis factor-alpha)	rs1800610	1078G/A	intron	0.075
		rs1800629	−308G/A	5′UTR	0.153
		rs1800750	−376G/A	5′UTR	0.009
		rs361525	−238G/A	5′UTR	0.070
Cell matrix adhesion/ interaction	MMP3 (matrix metalloproteinase-3)	rs3025058	−1171 indel (5A/6A)	5′UTR	0.500
	MMP9 (matrix metalloproteinase-9)	rs3918242	−1562C/T	5′UTR	0.119
	SELP (P-selectin)	rs1800805	−1969A/G	5′UTR	0.412
		rs6131	1087G/A	S331N	0.183
		rs6127	1902A/G	N603D	0.391
		rs6133	2013G/T	V640L	0.117
		rs6136	2361A/C	T756P	0.117
Coagulation-thrombosis	GP1BA (glycoprotein Ib alpha)	rs2243093	−5 T/C	5′UTR	0.102
		rs6065	2217C/T	T161M	0.075
	GP6 (glycoprotein VI)	rs1613662	13254T/C	S219P	0.186
	ITGA2 (glycoprotein Ialla)	rs1126643§	64448C/T	F253F	0.357
		rs28095	−52C/T	5′UTR	0.346
	ITGB3 (glycoprotein IIIa)	rs5918	1565T/C	L59P	0.167
	PAI1 (plasminogen activator inhibitor 1)	rs1799768	−675 indel (5G/4G)	5′UTR	0.459
		rs2227631	−844A/G	5′UTR	0.428
Lipid metabolism	APOE (apolipoprotein E)	rs405509	−219G/T	5′UTR	0.475
		rs429358	448T/C	C130R	0.125
		rs7412	586C/T	R176C	0.071
Vascular reactivity	ACE (angiotensin-converting enzyme)	rs4291	−240A/T	5′UTR	0.405
		rs4344	2350G/A	intron	0.421
		SNP030‖	(in 16) indel (D/I)	intron	0.416
	NOS3 (endothelial nitric oxide synthase)	rs1799983	894G/T	E298D	0.318
		rs1799985	10(in23)G/T	intron	0.307
		rs2070744	−786T/C	5′UTR	0.356

*From NCBI's dbSNP public database: http://www.ncbi.nlm.nih.gov/SNP/. †Formerly rs1799942. ‡Formerly rs2229234. §Formerly rs1800198. ‖Duke polymorphism ID number.
CABG = coronary artery bypass graft; D = deletion allele; EA = European American; I = insertion allele; SNP = single-nucleotide polymorphism; UTR = untranslated region.

each of the 37 polymorphisms and ordered from largest to smallest ($\chi_1 > \chi_2 > \ldots \chi_{37}$); sums ($s_i$; $i = 1, 2, \ldots, 10$) were formed from the 10 largest single-locus statistics; the empirical significance level p_i associated with each s_i was evaluated based on 3,000 random permutations of the data set; the set of markers associated with the smallest p_i was selected for further analysis. A series of logistic regression models was then developed to test for association between each pair of markers in the selected set (both as main effects and in interaction) and POCD. Model p values were Bonferroni corrected for multiple comparisons.

To account for covariable effects and population substructure, self-reported ethnicity, age, baseline cognitive score, and years of education were subsequently included in multiple logistic regression modeling. Interactions between genotype and these covariates were also tested. Patients representing ethnic groups other than European American (EA), African American (AA), and Native American (NA) were excluded from analyses involving race. Furthermore, the structured association method was used to control for the possibility of cryptic heterogeneity within the EA patients (16,17); multilocus genotypes from a panel of 52 unlinked bi-allelic null markers, evenly distributed across the genome, were analyzed using Structure version 2.1 software (Division of Biological Sciences, University of Chicago, Chicago, Illinois) to identify clusters of genetically similar individuals. Posterior probabilities of subpopulation membership (based on 2 putative subpopulations) were subsequently used in the logistic regression models that included age, baseline cognitive score, and years of education as covariates.

Haplotypes were inferred from unphased genotypic data using Phase version 2.0 software (Department of Statistics, University of Washington, Seattle, Washington) (18); subsequent haplotype analyses were carried out using Haplo.stats software (Division of Biostatistics, Mayo Clinic, Rochester, Minnesota) (19), restricted to haplotypes with an inferred frequency of >1%. All statistical analyses were performed using SAS and SAS/Genetics version 8.02 (SAS Institute).

Mechanistic subset analyses. Based on positive genetic associations, platelet activation was measured in a subset of 93 patients for whom serial arterial blood samples had been collected before induction of anesthesia, before aortic cross-clamp release, 10 min after cross-clamp release, at end of CPB, and at end of surgery. After incubation with saturating concentrations of monoclonal antibodies, blood samples were analyzed on a FACScan flow cytometer (Becton-Dickinson, Mountain View, California). The percentage of platelets expressing P-selectin (CD62P) was determined as a marker of platelet activation. Similarly, C-reactive protein (CRP) levels were serially measured in a subset of 239 patients from whom serum had been collected before induction of anesthesia and at 4.5, 24, and 48 h after cross-clamp removal. Immunoassays were forward immunometric assays performed by Biosite Diagnostics (San Diego, California) in 384-well microtiter plates using a Tecan Genesis RSP 200/8 Workstation (Tecan, Research Triangle Park, North Carolina).

The association between percentage of P-selectin–positive platelets and *SELP* genotype and between serum CRP levels and *CRP* genotype was tested using repeated measures analysis of variance (ANOVA) based on log transformation and using an unstructured covariance model. Because baseline concentrations differed between *SELP* genotypes, concentration levels at subsequent times were expressed as ratio to baseline. Genotype differences at individual event times were tested using Wilcoxon rank sum tests when analysis of variance was significant at $p < 0.05$.

Results

Demographic characteristics of the 677 enrolled patients are presented in Table 2. Of these, 164 (24%) did not complete 6-week testing, leaving 513 patients for the final analyses; nonreturners had a lower educational level and total CPB time, were older, and were more likely to suffer from diabetes and pulmonary and vascular disease. Allele frequencies for the single-nucleotide polymorphisms (SNPs) identified below were not different between those who did and did not return for 6-week testing. The incidence of

Table 2 Demographic Characteristics of the Study Population

	All Patients, n = 677	Cognitive Deficit		No 6 Week Test,* n = 164	CogDef Group p Value†	Return Group p Value‡
		No, n = 330	Yes, n = 183			
Age (yrs)	61.7 (10.5)	60.7 (10.3)	61.1 (10.5)	64.2 (10.3)	0.740	<0.001
Height (cm)	171.5 (10.2)	171.6 (10.5)	172.2 (9.5)	170.5 (10.4)	0.837	0.226
Weight (kg)	86.2 (17.9)	85.9 (17.9)	87.8 (17.8)	85 (18)	0.484	0.188
Ejection fraction (%)	54.1 (12.2)	54.2 (11.7)	55.3 (11.9)	52.4 (13.4)	0.377	0.092
CPB time (min)	109.7 (36.7)	107.9 (33.7)	105.2 (29.6)	118.5 (47.2)	0.528	0.003
Cross-clamp time (min)	58.2 (23.6)	57 (23.4)	55.1 (20.2)	64.2 (26.3)	0.476	<0.001
No. of grafts	3.2 (0.9)	3.1 (0.9)	3.1 (0.9)	3.3 (0.8)	0.731	0.172
Years of education	12.4 (3.2)	12.4 (3.2)	12.8 (3.1)	11.8 (3.2)	0.148	0.006
Baseline cognitive score	−0.003 (0.516)	−0.01 (0.488)	0.179 (0.457)	−0.193 (0.562)	<0.001	<0.001
Female gender (%)	30.4	30.6	26.8	34.1	0.365	0.243
Prior CABG (%)	3.1	3.3	2.2	3.7	0.589	0.796
Prior MI (%)	29.1	28.0	21.9	40.1	0.155	0.001
Prior stroke/TIA (%)	2.1	1.3	1.2	4.7	0.999	0.017
Unstable angina (%)	60.4	57.6	65.9	59.7	0.081	0.924
Diabetes (%)	30.2	26.5	29.1	39.0	0.536	0.007
CHF (%)	11.9	10.4	13.9	12.8	0.300	0.772
Hypertension (%)	62.8	61.8	61.8	65.8	0.999	0.438
COPD (%)	9.4	9.4	4.6	14.8	0.074	0.011
PVD (%)	12.4	12.3	8.1	17.4	0.171	0.034
Race					0.153	0.394
European American (%)	86.0	88.2	83.1	84.8		
African American (%)	9.7	8.5	9.8	12.2		
Native American (%)	3.7	3.0	5.5	3.0		
Other (%)	0.6	0.3	1.6	0.0		

Values are expressed as mean (SD) or as %. *Nonreturn group: deceased (13), incomplete testing (3), poor health (31), unable to travel (17), unwilling to return (43), unable to contact (38), or other (19). †CogDef group p values compare patients with and without cognitive deficit. ‡Return group p values compare patients with and without 6-week cognitive tests.

CABG = coronary artery bypass graft; CHF = congestive heart failure; CogDef = cognitive deficit; COPD = chronic obstructive pulmonary disease; CPB = cardiopulmonary bypass; MI = myocardial infarction; PVD = peripheral vascular disease; TIA = transient ischemic attack.

POCD in the study sample was 35.7% (183 out of 513), and patients with a deficit were similar to those without a deficit with the exception of baseline cognition (Table 2).

Initial demographic comparisons revealed differences in cognitive deficit among racial groups (EA 34.3%, AA 39.1%, NA 50%) after accounting for age, baseline cognition, and years of education in multivariable logistic regression. Therefore, all subsequent analyses were limited to the subset of EA patients (n = 443). Minor allele frequencies for the 37 polymorphisms examined among the EA patients without cognitive deficit are presented in Table 1. Four polymorphisms had deviations from Hardy-Weinberg equilibrium and were excluded from subsequent analyses.

The marker selection process identified 5 SNPs whose sum of chi-square statistics had a minimum p value of 0.121. Genotype frequencies for these 5 SNPs in patients with and without POCD are presented in Table 3. All 10 possible pairs of 5 SNPs were subjected to logistic regression analysis for association with POCD. The smallest Bonferroni-corrected model p value ($p = 0.002$) among models with main effects only occurred for SNPs *CRP* 1059G/C and *SELP* 1087G/A. This pair of SNPs also had the smallest Bonferroni-corrected model p value ($p = 0.005$) among models including both main and interaction effects. The interaction effect, however, was not significant ($p = 0.425$).

Logistic regression in EAs identified age, baseline cognition, and years of education in addition to SNPs *CRP* 1059G/C and *SELP* 1087G/A to be significantly associated with POCD (model $p < 0.0001$) (Table 4). No interaction was found between the SNPs and covariates. Presence of the minor allele at both *CRP* and *SELP* polymorphisms had a protective effect; the incidence of cognitive deficit was 16.7% in carriers of minor alleles at both of these loci compared with 42.9% in patients homozygous for the major allele (Fig. 1). Moreover, the absolute risk reduction in the observed incidence of POCD was 20.6% for carriers of the *CRP* 1059C allele and 15.2% for carriers of the *SELP* 1087A allele. The *CRP* and *SELP* SNPs were not associated with baseline cognition. Detailed analyses of cognitive responses separately by factor and genotype are available in the online appendix and at: http://anesthesia.duhs.duke.edu/pegasus/cognition/1/cognition-webAppendix.htm.

Pairwise estimates of linkage disequilibrium in EAs between 2 *CRP*, among 5 *SELP* SNPs, and between the *CRP* and *SELP* SNPs were low, suggesting no strong correlation between the POCD-associated SNPs and other genotyped SNPs. For the *CRP* gene, containing 2 exons and 1 intron spanning 7 kb, SNPs 1059G/C and 2147C/T exhibited $r^2 = 0.121$ ($p < 0.001$). For the larger *SELP* gene, containing 17 exons and 16 introns and spanning >50 kb, r^2 values between the POCD-associated SNP 1087G/A, and SNPs 1969A/G, 1902A/G, 2013G/T, and 2361A/C were 0.019, 0.011, 0.019, and 0.008, respectively, with the first and third r^2 values significant at $p = 0.01$. Sixteen unique *SELP* haplotypes were inferred from the 5 common *SELP* SNPs, of which 10 exhibited frequencies of >1%.

Table 3 Genotype Frequencies in European Americans (n = 443) for 5 SNPs Selected for Pair-Wise Modeling

SNP ID	Genotype	Frequency In EAs: With Cognitive Deficit	Frequency In EAs: Without Cognitive Deficit
rs361525	A/A	0.000	0.004
TNFA	A/G	0.064	0.132
−238G/A	G/G	0.936	0.864
rs1800797	A/A	0.127	0.188
IL6	A/G	0.530	0.563
−597G/A	G/G	0.343	0.249
rs1800947	C/C	0.007	0.004
CRP	C/G	0.075	0.153
1059G/C	G/G	0.918	0.844
rs6131	A/A	0.035	0.034
SELP	A/G	0.167	0.298
1087G/A	G/G	0.799	0.668
rs1205	C/C	0.518	0.414
CRP	C/T	0.447	0.494
2147C/T	T/T	0.035	0.091

CRP = C-reactive protein; *IL* = interleukin; *SELP* = P-selectin; *TNFA* = tumor necrosis factor-alpha; other abbreviations as in Table 1.

Table 4 Predictors of Cognitive Deficit After Cardiac Surgery in European Americans (n = 443; Area Under the Receiver-Operating Characteristic Curve for This Model Was 0.70)

Variable	Odds Ratio	95% Confidence Interval	p Value
CRP 1059G/C SNP	0.37	0.16–0.78	0.013
SELP 1087G/A SNP	0.51	0.30–0.85	0.011
Years of education	0.90	0.83–0.98	0.021
Age (per yr)	1.03	1.01–1.06	0.013
Baseline cognitive score	5.71	2.98–11.31	<0.001

Abbreviations as in Tables 1 and 3.

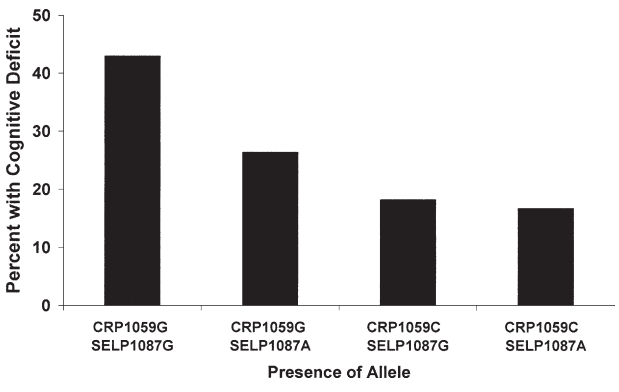

Figure 1 Incidence of Postoperative Cognitive Deficit by *CRP* 1059G/C and *SELP* 1087G/A Genotypes

The incidence of cognitive deficit was 16.7% in carriers of minor alleles at both of these loci compared with 42.9% in patients homozygous for the major allele. CRP = C-reactive protein; SELP = P-selectin. n = 386.

Tests for association between *SELP* haplotypes and POCD were significant for 2 haplotypes, each bearing the A allele of *SELP* 1087G/A. These haplotypes had frequencies below 5% and add little to the observed association of *SELP* 1087G/A to POCD (data not shown).

In separate logistic regression models that included self-reported race in the entire sample, or subpopulation membership probabilities in EAs only, the SNP effects remained significant. However, none of the 2-way interactions between *CRP* and *SELP* SNPs with race or subpopulation membership was significant, suggesting that race and population substructure had no effect on the SNP associations described. In non-EAs, the effects of the *CRP* and *SELP* SNPs trended toward being protective but were not statistically significant.

For serum CRP levels, a significant interaction between time and *CRP* 1059G/C genotype was seen ($p = 0.011$). Serum CRP levels were significantly lower at the 24-hour sampling time in patients homozygous (CC) or heterozygous (CG) for the minor allele compared with patients homozygous (GG) for the major allele ($p < 0.001$) (Fig. 2). Similarly, a significant genotype and time difference was seen when the effect of the *SELP* 1087G/A genotype on the percentage of P-selectin–positive platelets was examined. Platelet activation was significantly lower upon release of the cross-clamp ($p = 0.043$) (Fig. 3) in patients homozygous (A/A) or heterozygous (G/A) for the minor allele compared with patients homozygous (G/G) for the major allele.

Discussion

Cognitive dysfunction remains a frequent complication after cardiac surgery, occurring in approximately 36% of patients at 6 weeks after surgery. In the present study of 513 CABG patients tested with a detailed cognitive test battery before and after surgery, we report a potential genetic basis for this cognitive decline. In addition to the previously described risk factors of age, level of education and baseline cognition, we found that the risk of POCD was significantly lower in patients carrying at least 1 copy of the *CRP* 1059C or *SELP* 1087A alleles, with an additive effect between loci. Furthermore, we provide preliminary evidence that perioperative serum levels of CRP and platelet activation were reduced in patients with these polymorphisms, providing biologic underpinning to the observed allelic association.

Coronary artery bypass graft surgery using CPB is associated with ischemia-reperfusion injury, inducing a complex inflammatory response that impacts not only the heart but also the brain, lungs, kidneys, and gut (20). C-Reactive protein is an acute-phase reactant produced primarily in the liver in response to tissue injury or inflammation, implicated not only as a marker, but also a potential participant in the pathogenesis of inflammatory-mediated processes (21). Mean CRP concentrations have been reported to rise as much as 83-fold from the preoperative period to 72 h after CABG surgery, and variation in the extent of increase appears to be influenced by *CRP* genotype (22). Recently, the relationship of patterns of SNP variation at the *CRP* locus to plasma CRP levels has been extensively characterized in both EA and AA populations (23,24). The synonymous 1059G/C in exon 2 (L184L) has been associated with lower CRP levels in several studies (25–27). Zee and Ridker (26) reported significantly lower CRP levels in G/C heterozygotes than in G/G homozygotes (1.05 vs. 1.38 mg/l); Suk et al. (25) found a 29% lower baseline CRP level in carriers of the minor allele at 1059. Furthermore, 1059G/C is the only SNP that tags a haplotype associated with the lowest plasma CRP levels; in a large population

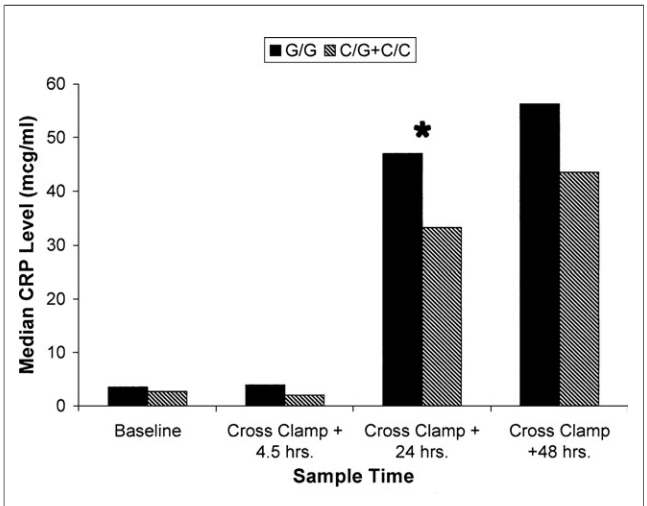

Figure 2 **Median CRP Levels by *CRP* 1059G/C Genotypes**

C-reactive protein (CRP) levels at the 24-h sampling time in patients homozygous (C/C) or heterozygous (C/G) for the minor allele were lower compared with patients homozygous (G/G) for the major allele. n = 225. *$p < 0.05$.

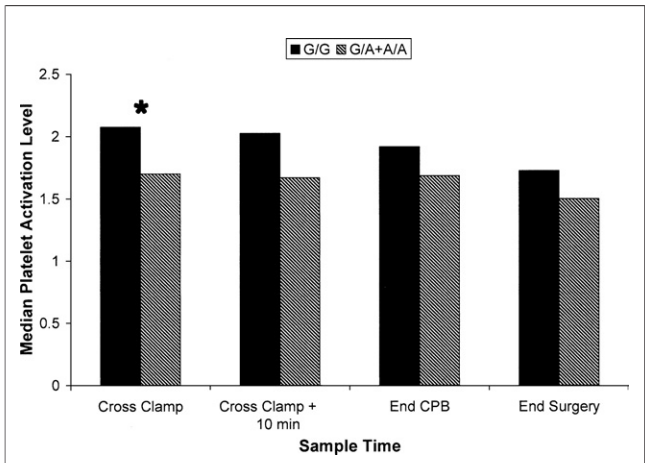

Figure 3 **Median Platelet Activation (Relative to Baseline) by *SELP* 1087G/A Genotypes**

Platelet activation was significantly lower upon release of the cross-clamp in patients with the homozygous minor (A/A) or heterozygous (G/A) genotype compared with patients homozygous (G/G) for the major allele. CPB = cardiopulmonary bypass; SELP = P-selectin. n = 93. *$p < 0.05$.

sample 1059G/C decreased plasma CRP an average of 1.5 mg/l/copy (23). Therefore, it is likely that this polymorphism directly influences CRP levels, although the mechanism for a direct effect remains unclear.

The immune system and the central nervous system form a bidirectional communication network. Host defense against infection and recovery from tissue injury includes not only immune activation, but also an integrated neuroendocrine response coordinated by the central nervous system, and several proinflammatory cytokines have been identified as the signaling molecules for immune-to-brain communication (28). In a double-blind crossover study of 20 healthy male volunteers given an intravenous injection of endotoxin, significant positive correlations were found between cytokine secretion and endotoxin-induced decreased verbal and nonverbal memory functions (29). These findings are consistent with reports that memory impairment is a common adverse effect of cytokine therapy and viral infection (30,31). Inflammatory mechanisms and immune activation have been hypothesized to play a role not only in neurodegenerative conditions such as Alzheimer's disease (32) and vascular dementia (33) but also in age-associated cognitive decline (34–36). Yaffe et al. (36) followed 3,031 well-functioning subjects for 2 years and reported that a high baseline level of serum markers of inflammation, most notably CRP, was associated with poor cognitive performance and greater risk of cognitive decline over the follow-up period. Similar associations between elevated baseline CRP levels and greater cognitive decline have been reported in patients followed for 5 to 6 years (34,35). In the present study, the lower incidence of POCD in patients with the *CRP* 1059G/C polymorphism may be related to a lesser degree of perioperative inflammation as evidenced by the lower levels of serum CRP.

P-selectin (CD62P) is a membrane glycoprotein that is rapidly mobilized to the surface of activated platelets and endothelial cells where it mediates leukocyte-platelet and leukocyte-vascular endothelial cell adhesion, respectively. Moreover, P-selectin expression on activated platelets appears important for the formation of large stable platelet aggregates and amplification of leukocyte recruitment process (37) and may prime monocytes for tissue factor and cytokine up-regulation (38). In the setting of CPB, activation of platelets, as measured by CD62P expression, peaks 2 to 4 h after CPB and returns to baseline 18 hours after CPB (39). Through increased platelet CD62P expression, CPB results in formation of monocyte-platelet and, to a lesser extent, neutrophil-platelet conjugates (40).

The gene coding for P-selectin (*SELP*) has been reported to be highly polymorphic (41). The *SELP* 1087G/A SNP results in a nonsynonymous amino acid change (S290N) in the consensus repeat (sushi) domain; this extracellular domain has been shown to be important for P-selectin binding to its ligand on leukocytes (42,43). In a study of 582 subjects with myocardial infarction (MI) and 630 age-matched control subjects, Tregouet et al. (44) reported that the risk for MI associated with the *SELP* 1087G/A SNP was reduced but differed according to the haplotype background. In the present study of cardiac surgical patients, we found the 1087G/A variant to be associated with POCD in both genotype and haplotype analyses and provide preliminary evidence for association with lower levels of platelet activation during CPB. Although several studies revealed associations between *SELP* polymorphisms and soluble P-selectin levels (45,46), which appear highly heritable (47), to our knowledge this is the first report of an association between *SELP* genotype and P-selectin expression on circulating platelets. However, based on this preliminary data, we cannot rule out that 1087G/A results in altered stability of membrane-bound P-selectin or differential binding of antibodies used for flow cytometry, and further functional characterization of this SNP is required.

When interpreting positive findings from any genetic association study, several epidemiologic limitations should be considered, including inadequate sample size, poorly matched control groups, subgroup analyses, multiple testing, and population stratification (48). With regard to these concerns, strengths of our study include a relatively large sample of cardiac surgery patients (n = 513) who have undergone intensive cognitive evaluation and a prospective cohort design that reduces the selection bias inherent in case-control designs. Although serum CRP and platelet CD62P data were available only for a subset of patients, we were able to provide preliminary evidence of a genotypic effect on both CRP level and platelet activation. However, it is possible that the *CRP* 1059G/C and *SELP* 1087G/A SNPs are in linkage disequilibrium with other regulatory (causal) variants not within regions previously scanned for polymorphisms and therefore not included in this study. This is particularly true for the *CRP* SNP which is silent at the amino acid level. Further, our structured association analysis revealed no evidence of population stratification in these data. Although we did find differences in cognitive deficit among racial groups, we could not detect a genetic effect that differed between races. It is possible that a larger sample of patients with varying ethnicity may have demonstrated such an effect. The definition of "significant" cognitive change, although commonly used, also remains controversial. Our primary aim was to investigate the role of genetic variability in overall postoperative cognitive function rather than specific brain functions and independent constructs. Post hoc analyses using individual factor scores as well as the raw test scores found no significant associations between the *CRP* and *SELP* polymorphisms and individual cognitive domains, which may only mean that genetic variations in perioperative inflammatory responses have a broad impact on cortical and subcortical function. Evaluation of other SNPs specifically involved in memory and learning may, however, yield a domain-specific effect.

In summary, using a prospective cohort study design, we found 2 candidate gene polymorphisms with additive effects associated with a reduction in the incidence of cognitive

decline after cardiac surgery. Functionally, these *CRP* and *SELP* polymorphisms were associated with reductions in serum CRP and platelet activation, respectively, raising the possibility that therapies aimed at reducing the perioperative inflammatory state may be beneficial. Moreover, using cardiac surgery as a model of neurologic injury, these results provide insight into the biologic factors modulating cognitive performance in humans and further evidence for a genetic basis of cognitive deterioration, which should translate into more precise identification of patients at risk.

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APPENDIX

For a list of the members of the PEGASUS Investigative Team, and a complete description of the factor analysis, including a scree plot and a table of factor loadings, please see the online version of this article.