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Natriuretic Peptide System Gene Variants Are Associated with Ventricular Dysfunction after Coronary Artery Bypass Grafting

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Background: **Ventricular dysfunction (VnD) after primary coronary artery bypass grafting is associated with increased hospital stay and mortality. Natriuretic peptides have compensatory vasodilatory, natriuretic, and paracrine influences on myocardial failure and ischemia. The authors hypothesized that natriuretic peptide system gene variants independently predict risk of VnD after primary coronary artery bypass grafting.**

Methods: **A total of 1,164 patients undergoing primary coronary artery bypass grafting with cardiopulmonary bypass at two institutions were prospectively enrolled. After prospectively defined exclusions, 697 patients of European descent (76 with VnD) were analyzed. VnD was defined as need for at least 2 new inotropes and/or new mechanical ventricular support after coronary artery bypass grafting. A total of 139 haplotype-tagging single nucleotide polymorphisms (SNPs) within 7 genes (*NPPA, NPPB, NPPC, NPR1, NPR2, NPR3, CORIN*) were genotyped. SNPs univariately associated with VnD were entered into logistic regression models adjusting for clinical covariates predictive of VnD. To control for multiple comparisons, permutation analyses were conducted for all SNP associations.**

Results: **After adjusting for clinical covariates and multiple comparisons within each gene, seven *NPPA/NPPB* SNPs (rs632793, rs6668352, rs549596, rs198388, rs198389, rs6676300, rs1009592) were associated with decreased risk of postoperative VnD (additive model; odds ratios 0.44–0.55; $P = 0.010$–0.036) and four *NPR3* SNPs (rs700923, rs16890196, rs765199, rs700926) were associated with increased risk of postoperative VnD (recessive model; odds ratios 3.89–4.28; $P = 0.007$–0.034).**

Conclusions: **Genetic variation within the *NPPA/NPPB* and *NPR3* genes is associated with risk of VnD after primary coronary artery bypass grafting. Knowledge of such genotypic predictors may result in better understanding of the molecular mechanisms underlying postoperative VnD.**

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VENTRICULAR dysfunction (VnD) has been reported after cardiac surgery in 10–20%[1–3] of patients and has been associated with increased hospital stay and long-term mortality after primary coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass (CPB).[2] B-type natriuretic peptide (BNP) is a member of the natriuretic peptide family, which includes three structurally homologous peptides: A-type (ANP), BNP, and C-type natriuretic peptides.[4] ANP and BNP are primarily released from atrial and ventricular myocytes in response to increased cardiac atrial and ventricular wall stress.[4] C-type natriuretic peptide is released from vascular endothelial cells and other tissues.[4] Natriuretic peptides bind to two guanylyl cyclase-coupled effector receptors (natriuretic peptide receptors A and B) and a clearance receptor (natriuretic peptide receptor C). In addition to compensatory natriuretic, diuretic, and vasorelaxant properties, natriuretic peptides also have mit-

igating paracrine influences on myocardial ischemia–reperfusion injury and cardiovascular remodeling.[4–14]

Circulating plasma BNP levels are used to assess the severity of heart failure and myocardial ischemia and to predict adverse outcomes in patients with acute coronary syndromes or undergoing cardiovascular surgery.[1,2,15–18] Natriuretic peptide system gene variants have been associated with cardiovascular disease states such as hypertension,[19–21] stroke,[22,23] and myocardial infarction.[24] However, to date, the association between natriuretic peptide system gene variants and development of VnD after cardiac surgery with CPB has not been examined. Knowledge of such genotypic predictors may enhance our understanding of the molecular mechanisms underlying postoperative VnD. We thus hypothesized that variants within the natriuretic peptide precursor protein (*NPPA, NPPB, NPPC*), receptor (*NPR1, NPR2, NPR3*), and precursor converting enzyme (*CORIN*) genes are independently associated with the risk of postoperative VnD in patients of European descent undergoing primary CABG surgery with CPB.

Materials and Methods

Study Population

Between August 2001 and June 2005, 1,164 men and women aged 20 to 89 yr scheduled for isolated primary CABG surgery with CPB at Brigham and Women's Hospital, Boston, Massachusetts, and Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas, were prospectively enrolled into an ongoing parent study known as the CABG Genomics Study.## Respective Institutional Review Board approval and subject written informed consent were obtained. The overall objective of the CABG Genomics Study is to identify associations between genotypic variants and adverse perioperative outcomes. CABG Genomics Study exclusion criteria include a preoperative hematocrit less than 25% or transfusion of leukocyte-rich blood products within 30 days before surgery. Enrolled subjects were prospectively excluded from analysis for this study if they underwent emergency surgery, received a preoperative inotrope, intraaortic balloon pump, or ventricular assist device support, had severe preoperative renal dysfunction (preoperative hemodialysis or preoperative serum creatinine greater than 3 mg/dl) or were missing preoperative or peak postoperative plasma BNP or cardiac troponin I (cTnI) measurements. To avoid confounding due to population stratification, subjects were further prospectively excluded from analysis if they reported having a parent or grandparent of non-European descent or if assessment of genomic control single nucleotide polymorphisms (SNPs) indicated non-European ethnicity.[25]

Data and Blood Collection

Data were collected for each enrolled subject during primary hospitalization using a detailed prospectively designed study case report form that includes: (1) preoperative demographic characteristics, comorbidities, and medications; (2) surgical characteristics; and (3) postoperative in-hospital events. Data were subject to automated range and logic checking as well as additional manual audit of a proportion of records.

Plasma samples obtained preoperatively and on postoperative days 1–5 were stored in vapor-phase liquid nitrogen until analysis. Plasma BNP and cTnI were measured at all time points using immunoassays conducted at a single reference laboratory (Biosite, San Diego, CA). DNA was extracted from white blood cells using standard procedures. Genotyping was performed using Sequenom MassARRAY® iPLEX (Sequenom, San Diego, CA) at Helmholtz Zentrum München (Neuherberg, Germany). Raw genotyping signals were analyzed with SpectroTyper 3.4 software (Sequenom) and were manually curated.

Seven candidate genes related to the natriuretic peptide system were selected *a priori*: *NPPA, NPPB, NPPC, NPR1, NPR2, NPR3,* and *CORIN*. SNPs were selected for genotyping to achieve approximately 2,500 base pair spacing across each gene region. SNPs were preferentially selected if they were assessed in previously conducted cardiovascular studies, were nonsynonymous coding SNPs, were within exon regions, were within 50 base pairs of intron-exon boundaries, were in promoter regions, or if they "tagged" haplotype blocks (as per the Caucasian HapMap resource).*** Genotyping was performed for 155 SNPs. SNPs were excluded from analysis for one or more of the following reasons: genotyping rate less than 90% (3 SNPs), observed minor allele frequency less than 1% (13 SNPs), or not being in Hardy Weinberg equilibrium in control subjects ($P \leq 0.001$; 1 SNP). No SNPs required exclusion for differential missingness greater than 10% between case and control groups. A total of 71 additional genomic control SNPs were also genotyped to exclude subjects with non-European ethnicities. To account for potential stratification by Northern *versus* Southern European ancestry, 6 SNPs within the lactase gene (rs182549, rs2322659, rs3754689, rs3769005, rs4954490, rs4988235) were also genotyped.[25,26] Subjects were also excluded if they were missing more than 10% of their genotyping data.

Definitions

Postoperative VnD was defined as a new requirement for two or more inotropes or new placement of an intraaortic balloon pump or ventricular assist device either during the intraoperative period after the patient

Available at: http://clinicaltrials.gov/show/NCT00281164; Accessed: December 10, 2008.

*** Available at: www.hapmap.org; Accessed March 10, 2007.

separated from CPB or postoperatively in the intensive care unit. Inotrope support was defined as continuous infusion of amrinone, milrinone, dobutamine, dopamine ($> 5\ \mu g \cdot kg^{-1} \cdot min^{-1}$), epinephrine, isoproterenol, norepinephrine, or vasopressin. Dyspnea score was derived using the New York Heart Association classification (1 = no dyspnea, 2 = mild impairment of daily functioning, 3 = substantial functional impairment when not at rest, 4 = functional impairment at rest).[27] Urgent CABG surgery was defined as occurring within the same hospitalization as the diagnosis of an acute coronary event or coronary artery disease. Stenosis of more than 50% of the left anterior descending left circumflex or right coronary arteries or their major branches were quantified on the basis of cardiac catheterization data and scored as regions of coronary arterial disease (1, 2, or 3 regions total). Stenosis of more than 50% of the left main coronary artery was counted as two regions of significant disease. Peak postoperative plasma BNP and cTnI was assessed if a subject had at least three postoperative day 1–5 measures or, for peak postoperative cTnI, at least postoperative day 1 and 2 measures.

Statistical Analyses

Categorical and continuous patient and clinical characteristics were compared between case and control groups with chi-square, Fisher exact, or Wilcoxon rank sum test as indicated using SAS (version 9.1.3, SAS Institute, Cary, NC). Continuous plasma BNP and cTnI data were $\log_{10}$ transformed to normalize data distribution before analysis. Multivariable logistic regression models of nongenetic clinical and biomarker predictors of VnD were also created using SAS. One model was created using preoperative and intraoperative covariates, and the other model also included peak postoperative cTnI to adjust in addition for the effects of postoperative myocardial injury. The two multivariable clinical prediction models were created using combined forward and backward regression modeling with covariate entry into the models determined by univariate association with VnD ($P < 0.15$; table 1) and exit from the models determined by P value of 0.05. Preoperative cTnI greather than 0.1 μg/l was kept in the model that includes preoperative and intraoperative characteristics because it has been previously associated with VnD in a larger study group.[2] CPB time greater than 120 min was maintained in the model that includes postoperative peak cTnI concentration, as this covariate independently predicts VnD in the multivariable model that includes only preoperative and intraoperative covariates. Demographic characteristics of age, gender, institution, and body mass index greater than 30 kg/m^2 were locked into both multivariable models regardless of significance. Simultaneous inclusion of preoperative myocardial infarction in the model with peak postoperative cTnI did not improve the predictive ability of the model.

Genetic statistical analyses were conducted with PLINK (version 1.01).[28] Hardy Weinberg equilibrium was evaluated using Fisher exact tests. Potential confounding of associations between natriuretic peptide SNP frequencies and VnD by subjects' Northern *versus* Southern European ancestries was determined by investigating association between lactase gene SNPs and VnD. Individual SNP associations with VnD were estimated using logistic regression for additive (each copy of the minor allele has an equivalent additional additive predictive value, *i.e.*, 0, 1, 2), dominant (1 or 2 copies of the minor allele *vs.* 0 copies of the minor allele), and recessive (2 copies of the minor allele *vs.* 0 or 1 copies of the minor allele) genetic models, and corresponding odds ratios (OR) and 95% confidence intervals (CI) were calculated for each SNP according to the role of the SNP's minor allele in each genetic model. SNPs associated with VnD in single covariate logistic assessment were then individually entered into the multivariable clinical logistic regression models for predicting VnD, and multivariate adjusted ORs and 95% CIs were determined for each SNP with regards to developing VnD.

Empirical P values were determined for all SNP associations based on permuting case/control status (15,000 repetitions) and adjusting for family-wise type 1 error within each gene. Point-wise permutation analyses were conducted for each SNP to adjust for potentially random occurrence of the SNP's minor allele in VnD cases *versus* controls. Permutation analyses adjusting for family-wise type 1 error were conducted to adjust for the probability of making false-positive discoveries when assessing multiple SNPs within a gene region for association with the VnD outcome. *NPPA* and *NPPB* were assessed as one genetic region, as these two genes lie in close proximity on chromosome 1.

Linkage disequilibrium blocks and associated haplotypes for *NPPA/NPPB* (fig. 1) and *NPR3* (fig. 2) gene regions were derived in Haploview (version 4.1; The Broad Institute, Cambridge, MA) using default criteria.[29,30] Omnibus tests were conducted to assess if variance in haplotype frequencies within each linkage disequilibrium block had an overall significant association with the VnD phenotype. Haplotypes within linkage disequilibrium blocks that had omnibus test P values < 0.05 were then assessed with Pearson chi-square tests for association with VnD. These univariate haplotype results were consistent with observed SNP associations; therefore, additional permutation analyses and multivariable adjustments for clinical covariates were not conducted.

Results

Of the 1,164 subjects enrolled into the source cohort during the study period, 407 were prospectively excluded from analysis for one or more of the following

Table 1. Patient Demographics, Medications, and Perioperative Risk Factors in 697 Patients of European Descent with and without VnD after Primary Coronary Artery Bypass Graft Surgery

Preoperative Demographics and Risk Factors	No VnD (n = 621)	VnD (n = 76)	*P* Value
Age, yr	64 ± 10	64 ± 11	0.82
Female gender	116 (18.7%)	12 (15.8%)	0.64
Institution			0.18
Brigham and Women's Hospital	486 (78.3%)	65 (85.5%)	
Texas Heart Institute	135 (21.7%)	11 (14.5%)	
Diabetes mellitus	168 (27.1%)	23 (30.3%)	0.59
Hypertension	466 (75.3%)	59 (77.6%)	0.78
Hypercholesterolemia	469 (75.8%)	59 (78.7%)	0.67
Obesity (BMI > 30 kg/m^2)	240 (38.7%)	27 (35.5%)	0.62
Smoking, > 30 pack year history	167 (27.8%)	29 (42.0%)	0.02
Renal insufficiency (creatinine = 1.6–3.0 mg/dl)	26 (4.2%)	9 (11.8%)	0.009
Myocardial infarction ≤ 2 weeks preoperatively	91 (14.7%)	27 (35.5%)	< 0.0001
LVEF %	54 ± 12	42 ± 15	< 0.0001
Dyspnea score (NYHA classification)			
None or mild	307 (53.7%)	32 (44.4%)	0.06
Moderate	205 (35.8%)	26 (36.1%)	
Severe	60 (10.5%)	14 (19.4%)	
Coronary artery regions with > 50% stenosis			0.07
0–1 region	33 (5.3%)	1 (1.3%)	
2 regions	100 (16.2%)	7 (9.2%)	
3 regions	485 (78.5%)	68 (89.5%)	
Mitral insufficiency (moderate or severe)	12 (2.0%)	5 (6.9%)	0.03
Past arrhythmia	60 (9.7%)	8 (10.5%)	0.84
Median preoperative BNP, pg/ml (10th, 90th percentile)	15.5 (1.6, 102.8)	61.0 (3.4, 369.4)	< 0.0001
Preoperative cTnI > 0.1 μg/l	76 (12.2%)	21 (27.6%)	< 0.0001
Preoperative medications			
ACE inhibitor	290 (46.8%)	48 (63.2%)	0.008
Diuretic	118 (19.0%)	25 (32.9%)	0.007
Statin	468 (75.4%)	55 (72.4%)	0.58
Digoxin	19 (3.1%)	4 (5.3%)	0.30
β-blocker	469 (75.5%)	61 (80.3%)	0.40
Aspirin	462 (74.4%)	54 (71.1%)	0.58
Nonaspirin platelet inhibitor	106 (17.1%)	17 (22.4%)	0.26
Perioperative risk factors			
Urgent surgery	326 (52.6%)	44 (57.9%)	0.40
Cardiopulmonary bypass time, minutes	93.2 ± 36.8	111.4 ± 34.4	< 0.0001
Aortic cross clamp time, minutes	69.5 ± 31.3	79.0 ± 29.3	0.007
Peak postoperative cTnI, μg/l (median, 10th, 90th percentile)	1.57 (0.50, 3.37)	3.14 (0.74, 8.60)	< 0.0001

Data are shown as n (%) for dichotomous variables and mean ± standard deviation or median (10th, 90th percentiles) for continuous variables.

ACE = angiotensin converting enzyme; BMI = body mass index; BNP = B-type natriuretic peptide; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; VnD = ventricular dysfunction.

exclusion criteria: non-European ethnicity (n = 176), no CABG surgery performed (n = 6), history of cardiac surgery (n = 4), concurrent valve surgery performed (n = 51), emergency surgery (n = 4), CPB not used (n = 39), aortic cross-clamp not used (n = 68), preoperative ventricular assist device (n = 1), preoperative intraaortic balloon pump (n = 27), preoperative inotropes (n = 4), no preoperative or peak postoperative BNP or cTnI measurements (n = 72), preoperative hemodialysis (n = 1), preoperative serum creatinine greater than 3 mg/dl (n = 4), or no genotyping performed (n = 83). In addition to these exclusions 60 subjects were excluded for having less than 90% success for their individual genotyping.

Patient Characteristics

Perioperative patient characteristics for 697 subjects included in the study analysis are shown in table 1, stratified by occurrence of postoperative VnD (n = 76). None of the subjects who developed the VnD phenotype underwent ventricular assist device placement. Twelve VnD subjects received an intraaortic balloon pump intraoperatively after separating from CPB. An additional seven VnD subjects underwent intraaortic balloon pump insertion postoperatively in the intensive care unit. Forty-seven VnD subjects (6.7%) required at least 2 inotropes intraoperatively, and 43 subjects (6.2%) required at least 2 inotropes postoperatively.

Patients who developed postoperative VnD were significantly more likely to have a more than 30 pack-year history of smoking, renal insufficiency, recent myocardial infarction, lower preoperative left ventricular ejection fraction, higher preoperative plasma BNP concentration, moderate to severe mitral regurgitation, or to be taking an angiotensin-converting enzyme inhibitor or diuretic. Subjects who developed VnD had significantly

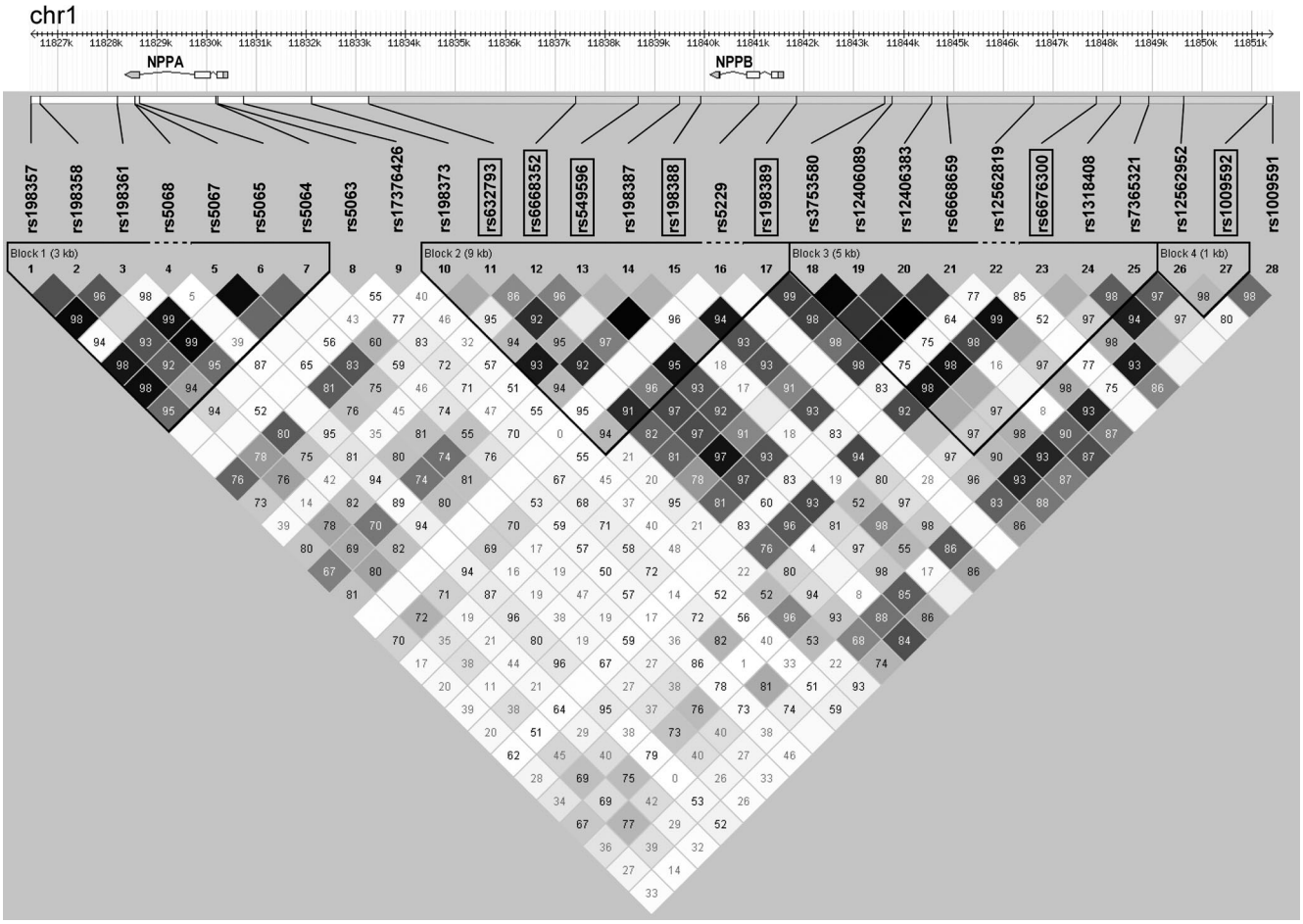

Fig. 1. Block structure of linkage disequilibrium for the 28 successfully genotyped single nucleotide polymorphisms (SNPs) in the natriuretic peptide precursor protein A and B gene regions (*NPPA/NPPB*) on chromosome 1p36.22. SNPs surrounded by *boxes* are the SNPs that were significantly associated with the ventricular dysfunction phenotype after adjustment for clinical covariates and multiple comparisons ($P < 0.05$). Stronger correlations between SNPs are noted by darker color in the intersecting squares linking each pair of SNPs. Stronger correlation indicates a tendency for the minor allele of one SNP to be inherited with the minor allele of the other SNP that it is being linked with in this figure. Correlations and block structures were estimated using Haploview (version 4.1; The Broad Institute, Cambridge, MA).[30]

longer CPB and aortic cross-clamp times and higher peak postoperative cTnI concentrations. Multivariable logistic regression models of patient and clinical predictors of postoperative VnD in the 697 study subjects are shown in table 2 and are consistent with our previous report from a larger, multiethnic cohort, that preoperative left ventricular ejection fraction, BNP, and CPB time greater than 120 min are independent clinical predictors of VnD after CABG.[2]

Natriuretic Peptide System SNP Associations with Ventricular Dysfunction

The 139 SNPs that passed quality control criteria are listed in Supplemental Digital Content along with corresponding *P* values for univariate associations with VnD as assessed with additive, dominant, and recessive genetic models (see appendix, Supplemental Digital Content 1, which shows 139 single nucleotide polymorphisms genotyped within 7 natriuretic peptide system gene regions in 697 patients of European descent with and without ventricular dysfunction after primary coronary artery bypass graft surgery, http://links.lww.com/A819). The genotyping rate for these 139 SNPs was 98.8%. The minor alleles of 13 *NPPA/NPPB* gene region SNPs were univariately associated with a decreased incidence of VnD after CABG surgery, and the minor alleles of seven *NPR3* gene SNPs were univariately associated with an increased incidence of VnD after CABG surgery (asymptotic $P < 0.05$; table 3). SNPs in the *NPPC*, *NPR1,* and *CORIN* gene regions were not associated with VnD. One *NPR3* SNP rs11740580 and one *NPR2* region SNP rs3808864 were univariately associated with *decreased* VnD, but these associations did not survive statistical adjustments for multiple comparisons.

After adjusting for clinical predictors of VnD using either multivariable model shown in table 2, the minor alleles of eleven *NPPA/NPPB* gene SNPs and the minor alleles of six *NPR3* gene SNPs remained associated with the occurrence of postoperative VnD (asymptotic $P <$ 0.05). After additional point-wise permutation adjust-

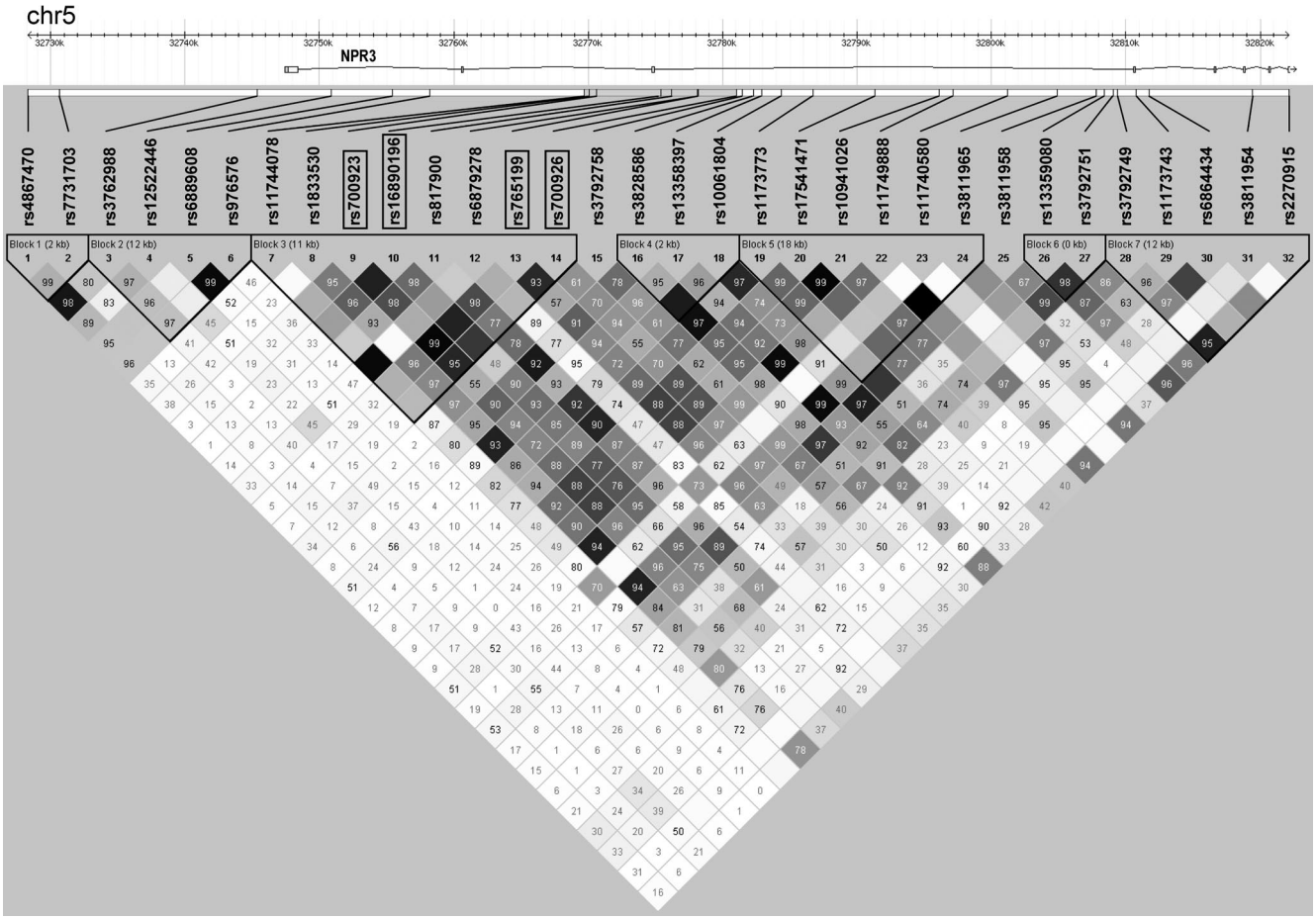

Fig. 2. Block structure of linkage disequilibrium for the 32 successfully genotyped single nucleotide polymorphisms (SNPs) in the natriuretic peptide receptor C gene region (*NPR3*) on chromosome 5p13.3. SNPs surrounded by *boxes* are the SNPs that were significantly associated with the ventricular dysfunction phenotype after adjustment for clinical covariates and multiple comparisons ($P < 0.05$). Stronger correlations between SNPs are noted by darker color in the intersecting squares linking each pair of SNPs. Stronger correlation indicates a tendency for the minor allele of one SNP to be inherited with the minor allele of the other SNP that it is being linked within this figure. Correlations and block structures were estimated using Haploview (version 4.1; The Broad Institute, Cambridge, MA).[30]

ment for subject case-control status and family-wise permutation adjustment for total number of SNPs assessed in the gene region, the minor alleles of seven *NPPA/NPPB* SNPs and four *NPR3* SNPs remained associated with occurrence of postoperative VnD using either multivariable model shown in table 2 (family-wise empirical $P < 0.05$). Table 4 presents the gene association results adjusted for the clinical model containing preoperative

Table 2. Logistic Regression Models of Clinical Predictors of Ventricular Dysfunction after Primary Coronary Artery Bypass Graft Surgery with Cardiopulmonary Bypass

	Model Including Preoperative cTnI > 0.1 μg/l (n = 672*; AIC = 416.30)			Model Including Log_{10} Peak Postoperative cTnI (n = 672*; AIC = 403.08)		
Predictor	Odds Ratio	95% CI	*P* Value	Odds Ratio	95% CI	*P* Value
Age (10-yr increment)	0.91	(0.70, 1.18)	0.46	0.91	(0.70, 1.18)	0.47
Gender (female)	0.81	(0.40, 1.64)	0.55	0.79	(0.38, 1.61)	0.51
Institution	0.75	(0.35, 1.60)	0.45	0.73	(0.33, 1.58)	0.42
BMI > 30 kg/m^2	0.95	(0.55, 1.65)	0.85	0.91	(0.52, 1.60)	0.74
Preoperative LVEF (1% increment)	0.94	(0.92, 0.96)	< 0.0001	0.94	(0.92, 0.96)	< 0.0001
Log_{10} preoperative BNP	1.56	(1.04, 2.36)	0.03	1.61	(1.07, 2.42)	0.02
Preoperative cTnI > 0.1 μg/l	1.76	(0.94, 3.30)	0.08	—	—	—
Log_{10} peak postoperative cTnI	—	—	—	2.80	(1.70, 4.60)	< 0.0001
CPB time > 120 min	2.06	(1.14, 3.70)	0.02	1.55	(0.85, 2.86)	0.16

* Twenty-five subjects were missing one or more of the models' predictor variables and are not included in this analysis.

AIC = Akaike information criterion; BMI = body mass index; BNP = B-type natriuretic peptide; CI = confidence interval; CPB = cardiopulmonary bypass; cTnI = cardiac troponin I; LVEF = left ventricular ejection fraction.

Table 3. SNPs Univariately Associated with VnD after Primary Coronary Artery Bypass Graft Surgery in 697 Patients of European Descent

SNP	Chromosomal Position NCBI hg36	Gene Region and SNP Role	Minor/ Major Allele	No VnD (n = 621) (aa/aA/AA)	VnD (n = 76) (aa/aA/AA)	Genetic Model*	Odds Ratio	95% CI	Asymptotic *P* Value†	Point-wise Empirical *P* Value‡	Family-wise Empirical *P* Value§
rs198358	chr1:11826663	NPPA; Downstream	C/T	46/235/340	2/24/49	Additive	0.647	0.422, 0.992	0.046	0.039	0.382
rs632793	chr1:11833264	NPPA; Promoter NPPB; Downstream	C/T	104/302/212	7/32/37	Additive	0.616	0.427, 0.889	0.010	0.009	0.108
rs6668352	chr1:11837416	NPPA; Promoter NPPB; Downstream	T/C	43/234/332	1/21/53	Additive	0.509	0.320, 0.809	0.004	0.004	0.0503
rs549596	**chr1:11838682**	**NPPA; Promoter NPPB; Downstream**	**C/T**	**124/306/191**	**9/30/37**	**Additive**	**0.577**	**0.402, 0.827**	**0.003**	**0.002**	**0.037**
rs198388	chr1:11839927	NPPA; Promoter NPPB; Downstream	T/C	118/291/186	8/27/33	Additive	0.586	0.402, 0.856	0.006	0.004	0.060
rs198389	chr1:11841858	NPPB; Promoter	C/T	118/304/195	9/32/35	Additive	0.631	0.441, 0.902	0.012	0.011	0.121
rs3753580	chr1:11843635	NPPB; Promoter	C/T	74/277/265	4/28/43	Additive	0.600	0.405, 0.890	0.011	0.009	0.118
rs12406089	chr1:11843768	NPPB; Promoter	G/C	75/281/258	4/28/42	Additive	0.593	0.399, 0.881	0.010	0.008	0.108
rs12406383	chr1:11844580	NPPB; Promoter	T/G	48/241/330	1/25/49	Additive	0.588	0.381, 0.909	0.017	0.014	0.187
rs6668659	chr1:11844885	NPPB; Promoter	G/T	75/284/256	4/26/41	Additive	0.574	0.383, 0.862	0.007	0.006	0.084
rs6676300	**chr1:11847887**	**NPPB; Promoter**	**C/T**	**78/281/236**	**4/26/41**	**Additive**	**0.538**	**0.358, 0.809**	**0.003**	**0.002**	**0.038**
rs12562952	chr1:11849643	NPPB; Promoter	C/T	9/122/450	0/8/64	Additive	0.431	0.207, 0.899	0.025	0.020	0.254
rs1009592	chr1:11851301	NPPB; Promoter	C/G	91/282/248	5/29/42	Additive	0.587	0.402, 0.857	0.006	0.005	0.061
rs700923	**chr5:32770625**	**NPR3; Intron**	**G/A**	**34/226/352**	**13/19/40**	**Recessive**	**3.746**	**1.873, 7.490**	**0.0002**	**< 0.0001**	**0.004**
rs16890196	**chr5:32775418**	**NPR3; Intron**	**G/A**	**25/205/384**	**10/18/47**	**Recessive**	**3.630**	**1.667, 7.882**	**0.001**	**0.001**	**0.020**
rs765199	**chr5:32778222**	**NPR3; Intron**	**A/G**	**32/235/351**	**13/23/39**	**Recessive**	**3.840**	**1.915, 7.699**	**0.0002**	**< 0.0001**	**0.003**
rs700926	**chr5:32781040**	**NPR3; Intron**	**C/A**	**37/257/322**	**13/24/39**	**Recessive**	**3.229**	**1.630, 6.395**	**0.0008**	**0.001**	**0.015**
rs17541471	chr5:32791346	NPR3; Intron	G/A	29/207/380	9/21/45	Recessive	2.760	1.253, 6.082	0.012	0.007	0.131
rs10941026	chr5:32796132	NPR3	G/A	30/207/383	9/20/47	Recessive	2.642	1.203, 5.801	0.015	0.014	0.183
rs11749888	chr5:32797186	NPR3; Intron	A/G	6/132/475	3/19/54	Recessive	4.156	1.018, 16.98	0.047	0.062	0.464

Bold type signifies single nucleotide polymorphisms (SNP) that remain significantly associated with the ventricular dysfunction (VnD) phenotype after family-wise permutation adjustment for multiple comparisons within a gene region.

* Genetic model with most significant association shown, although other models may have resulted in asymptotic $P \leq 0.05$. † *P* value before permutation adjustments. ‡ *P* value after permutation adjustment for cohort case-control status. § *P* value after permutation adjustment for testing multiple SNP hypotheses within a gene region.

A = adenine; aa/aA/AA = subjects with two minor alleles/subjects with one minor allele and one major allele/ subjects with 2 major alleles; C = cytosine; CI = confidence interval; G = Guanine; NPPA = natriuretic peptide precursor A; NPPB = natriuretic peptide precursor B; NPR3 = natriuretic peptide receptor 3; T = Thymine.

cTnI greater than 0.1 μg/l, but the asymptotic, point-wise, and family-wise significant associations ($P < 0.05$) were unchanged when adjustments were made for the second clinical model.

The logistic regression model including demographic, clinical, and nongenetic preoperative biomarker variables (table 2) predicted 15.9% of the variability (r^2) in occurrence of postoperative VnD, and the area under the receiver operating characteristic curve related to this model was 0.781. When the SNPs most significantly associated with VnD from the *NPPA/NPPB* region (rs549596; additive model) and the *NPR3* region (rs700923; recessive model) were both added to the clinical model, the proportion of explained variability (r^2) for the model increased to 21.9% ($P < 0.0001$) and the area under the receiver operating characteristic curve for the model increased to 0.828.

Natriuretic Peptide System Haplotype Associations with Ventricular Dysfunction

The seven *NPPA/NPPB* SNPs that remained associated with a decreased risk of VnD after statistical adjustment for clinical predictors and multiple comparisons are contained within the linkage disequilibrium blocks 2, 3, and 4 (chr1:11,832, 122–11, 851, 301) of the *NPPA/NPPB* gene region (fig. 1). Omnibus tests for global

Table 4. SNPs Associated with VnD after Primary Coronary Artery Bypass Graft Surgery in 697 Patients of European Descent after Adjusting for Clinical Predictors of VnD‖

SNP	Gene Region	Genetic Model*	Odds Ratio	95% CI	Asymptotic *P* Value†	Point-wise Empirical *P* Value‡	Family-wise Empirical *P* Value§
rs632793	**NPPA_NPPB**	**Additive**	**0.520**	**0.347, 0.780**	**0.002**	**0.002**	**0.026**
rs6668352	**NPPA_NPPB**	**Additive**	**0.437**	**0.264, 0.722**	**0.001**	**0.002**	**0.022**
rs549596	**NPPA_NPPB**	**Additive**	**0.488**	**0.330, 0.722**	**0.0003**	**0.0004**	**0.010**
rs198388	**NPPA_NPPB**	**Additive**	**0.505**	**0.335, 0.760**	**0.001**	**0.001**	**0.019**
rs198389	**NPPA_NPPB**	**Additive**	**0.545**	**0.370, 0.804**	**0.002**	**0.002**	**0.036**
rs3753580	NPPA_NPPB	Additive	0.525	0.342, 0.807	0.003	0.003	0.053
rs12406089	NPPA_NPPB	Additive	0.527	0.343, 0.812	0.004	0.003	0.057
rs12406383	NPPA_NPPB	Additive	0.506	0.315, 0.814	0.005	0.005	0.073
rs6668659	NPPA_NPPB	Additive	0.521	0.335, 0.810	0.004	0.004	0.058
rs6676300	**NPPA_NPPB**	**Additive**	**0.481**	**0.308, 0.751**	**0.001**	**0.001**	**0.023**
rs1009592	**NPPA_NPPB**	**Additive**	**0.495**	**0.325, 0.752**	**0.001**	**0.0008**	**0.019**
rs700923	**NPR3**	**Recessive**	**4.282**	**1.937, 9.466**	**0.0003**	**0.0006**	**0.007**
rs16890196	**NPR3**	**Recessive**	**4.090**	**1.680, 9.955**	**0.002**	**0.003**	**0.034**
rs765199	**NPR3**	**Recessive**	**4.270**	**1.934, 9.430**	**0.0003**	**0.0008**	**0.007**
rs700926	**NPR3**	**Recessive**	**3.892**	**1.802, 8.409**	**0.0005**	**0.001**	**0.009**
rs17541471	NPR3	Recessive	3.204	1.334, 7.696	0.009	0.007	0.114
rs10941026	NPR3	Recessive	3.140	1.309, 7.530	0.010	0.009	0.129

Bold type signifies single nucleotide polymorphisms (SNP) that remain significantly associated with the ventricular dysfunction (VnD) phenotype after family-wise permutation adjustment for multiple comparisons within a gene region.

* Genetic model with most significant association shown, although other models may also have also resulted in asymptotic $P \leq 0.05$. † *P* value before permutation adjustments. ‡ *P* value after permutation adjustment for cohort case-control status. § *P* value after permutation adjustment for testing multiple SNP hypotheses within a gene region. ‖ SNP associations adjusted for multivariable clinical logistic regression model in Table 2 that includes preoperative cTnI > 0.1 μg/l.

CI = confidence interval; NPPA = natriuretic peptide precursor A; NPPB = natriuretic peptide precursor B; NPR3 = natriuretic peptide receptor 3.

association between haplotype variation within each *NPPA/NPPB* linkage disequilibrium block and the VnD phenotype were significant for blocks 1, 3, and 4 ($P < 0.05$; block 2 $P = 0.055$). Within these blocks, analyses of individual haplotype associations with occurrence of VnD were consistent with the *NPPA/NPPB* region SNP associations.

The four *NPR3* SNPs that remained associated with an increased risk of VnD after statistical adjustment for clinical predictors and multiple comparisons are contained within block 3 of the *NPR3* gene (chr5:32, 769, 725–32, 781,040; fig. 2). Omnibus tests for global association between haplotype variability within *NPR3* linkage disequilibrium blocks and occurrence of the VnD phenotype showed significant associations for blocks 3 and 5 ($P < 0.05$). Within these blocks, analyses of individual haplotype associations were consistent with the associations observed for the *NPR3* SNPs.

Discussion

We have identified novel regions of genetic variation within the *NPPA*, *NPPB*, and *NPR3* genes that are associated with VnD after primary CABG surgery. After adjusting for environmental covariates and multiple comparisons, the minor alleles of the *NPPA/NPPB* SNPs identified in this study associate with a decreased risk of postoperative VnD, and the minor alleles of the *NPR3* SNPs identified in this study associate with an increased risk of postoperative VnD. Furthermore, simultaneous addition of the *NPPA/NPPB* SNP rs549596 and the *NPR3* rs700923 into the clinical multivariable logistic regression model for predicting postoperative VnD improved the predictive ability of the model.

Although *NPPA* and *NPPB* SNPs have not been assessed previously in relation to adverse perioperative cardiovascular outcomes, several *NPPA/NPPB* gene variants have been examined for association with ambulatory cardiovascular diseases.[19,22–24,31–35] In the only study examining *NPPA/NPPB* variants in ambulatory heart failure patients, the *NPPA* nonsynonymous coding polymorphism rs5065 was found to be associated with elevated plasma BNP and amino-terminal BNP (NT-proBNP) levels in patients who were New York Heart Association class III-IV, but not in patients who were New York Heart Association class I–II.[31] rs5065 has also been associated with nonfatal myocardial infarction[24] and coronary artery disease[24] and, along with the rare nonsynonymous coding polymorphism rs5063, has been associated with stroke,[22] hypertension,[19] and left ventricular mass in hypertensive patients.[34] We did not identify any association between rs5065 or rs5063 and VnD after CABG surgery.

In addition to the above studies of *NPPA/NPPB* gene variants in relation to cardiovascular disease, the *NPPA/NPPB* promoter SNPs rs198388 and rs198389 have been associated with elevated circulating NT-proBNP concentrations in ambulatory diabetics with nephropathy,[33] and these same two SNPs along with *NPPA/NPPB* SNPs rs632793, rs6668352, 6676300, and rs198375 have been

associated with elevated plasma BNP levels in a large ambulatory Japanese cohort.[32] The strong overlap between these *NPPA/NPPB* SNPs and the *NPPA/NPPB* SNPs associated with VnD in the current study supports the need for further studies regarding how genetic variation in the region containing these SNPs may interact with the natriuretic peptide system and influence postoperative VnD. The *NPPB* promoter region has multiple *cis* regulatory elements known to be gene regulators, and multiple physiologic stimuli including mechanical stretch, ischemic injury and hypoxia, and inflammatory mediators are known to stimulate regulation of the *NPPB* gene.[36] Furthermore, processing of natriuretic peptide preprohormones to more active fragments is complex and at present incompletely understood.[13] Autocrine and paracrine effects of natriuretic peptides on myocardial ischemia–reperfusion injury and remodeling are also being identified.[13]

In comparison with the *NPPA/NPPB* gene region, less is known about the association between SNPs within the natriuretic peptide receptor C (*NPR3*) gene and adverse cardiovascular outcomes. Although natriuretic peptide receptor C was initially identified as a natriuretic peptide clearance receptor, animal studies indicate that this receptor may locally modulate the physiologic effects of natriuretic peptides and may influence microvascular permeability and cardiac sarcolemmal Na^+-K^+ pump activity.[12,37,38] The major allele of the *NPR3* promoter SNP rs9716700 has been associated with increased family history of hypertension, as well as lower ANP levels and higher systolic blood pressure in obese hypertensive patients,[20,21] and a different study found a univariate association between the major allele of rs9716700 and higher plasma ANP and BNP levels in ambulatory patients without heart failure but not in patients with heart failure.[31] While rs9716700 was not assessed in the current study, we did not find associations between other SNPs in the *NPR3* promoter region and VnD after CABG. Furthermore, the *NPR3* SNPs that we did find to be associated with VnD are within a linkage disequilibrium block that does not include the *NPR3* promoter region and is demarcated from the promoter region by a strong recombination point. Therefore, the NPR3 SNPs associated with VnD in this study are unlikely to be related to rs9716700.

The *NPR3* SNPs (rs700923, rs16890196, rs765199, rs700926) that we identified as significantly associated with decreased postoperative VnD lie within currently assumed noncoding intronic sequences, and there are several potential mechanisms that could explain their association with the study's clinical VnD outcome and warrant further study. These SNPs may be in linkage disequilibrium with promoter SNPs that have not yet been identified or that were not genotyped in this study. Furthermore, these intronic SNPs may have promoter functions that have not yet been identified. Finally, intronic variants may potentially affect receptor function through alternative splicing mechanisms. For example, recent reports have demonstrated associations between intronic SNPs unrelated to the *NPR3* gene and alternatively spliced mRNA transcripts that alter function of ultimately translated protein.[39]

There are several potential limitations to this study. First, the current cardiac surgical literature does not include a standardized outcome definition for ventricular dysfunction after cardiac surgery. Many primary CABG patients at both study institutions do not undergo perioperative monitoring with transesophageal echocardiography or pulmonary artery catheters. We elected to define VnD after CABG surgery as a need for two or more inotropes or new intraaortic balloon pump or ventricular assist device support to best ensure that we were not including patients with normal ventricular function. It is not standard organizational or surgeon-based practice at either study institution to separate from CPB on prophylactic inotropes. Furthermore, we have previously reported that patients with our definition of VnD experience significantly prolonged hospital stays and increased up to 5-yr all-cause mortality after primary CABG surgery.[2] Second, we limited analysis to people of European descent only, as we had an insufficient number of non-European subjects to allow for statistical accommodation of genetic admixture. Thus, further studies are warranted to replicate our findings in non-European cardiac surgical patient populations. Third, our study's sample and effect sizes allowed adjustment for multiple comparisons within each gene, but our findings should be viewed as exploratory until further validation studies are conducted in other European-descent cardiac surgical populations. Fourth, we have identified regions in *NPPA/NPPB* and *NPR3* that contain genetic variants independently associated with VnD after CABG surgery, but there is considerable linkage disequilibrium within these regions. Consequently, we cannot conclude that the SNPs identified in this study are directly related to occurrence of VnD after CABG surgery. Finally, our findings do not identify mechanistic pathways that link identified SNP associations to occurrence of postoperative VnD. The natriuretic peptide system and its regulation are complex.[36] Further investigation is required to determine how the *NPPA/NPPB* and *NPR3* SNPs associations identified in this study relate to development of postoperative VnD.

Conclusions

VnD after CABG surgery with CPB is associated with increased postoperative hospital stay and mortality. Genetic variation within defined regions of the *NPPA/NPPB* and *NPR3* natriuretic peptide system genes is associated with VnD after CABG surgery and improves

ability to predict the VnD outcome beyond what can be predicted using clinical covariates alone. Further investigation of these regions is warranted, as knowledge of such genotypic predictors may enhance our understanding of the molecular mechanisms underlying postoperative VnD and heart failure.

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