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Original Research

Critical Care

Leucyl/Cystinyl Aminopeptidase Gene Variants in Septic Shock

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Background

Vasopressin is an essential peptide hormone regulating cardiovascular homeostasis and an adjunctive vasopressor therapy for septic shock.

Methods

We tested for association between single nucleotide polymorphisms (SNPs) in vasopressin pathway genes and altered outcome in derivation (n = 589) and replication (n = 616) cohorts of patients with septic shock. The primary outcome was 28-day mortality and the secondary outcome was vasopressin clearance. In a third cardiac surgical cohort (n = 977), we tested for locus-specific heritability of serum sodium concentrations.

Results

Of 17 tested tag SNPs in five vasopressin pathway genes (arginine vasopressin [AVP], arginine vasopressin receptor 1A

and 1B [AVPR1A, AVPR1B], leucyl/cystinyl aminopeptidase [LNPEP], and oxytocin receptor [OXTR]), rs18059 in LNPEP (also known as vasopressinase) was associated with 28-day mortality in the derivation cohort (P = .037). Therefore, we resequenced the 160-kb haplotype block encompassing the LNPEP gene, including rs18059, and genotyped the 230 identified SNPs in the derivation cohort. The strongest signal was found for LNPEP rs4869317 (adjusted P = .044). The rs4869317 TT genotype was associated with increased 28-day mortality in the derivation cohort (51.0% [TT] vs 34.5% [AA/AT]; adjusted hazard ratio [HR], 1.58; 95% CI, 1.21-2.06; P = .00073) and the replication cohort (38.6% vs 29.6%; HR, 1.36; 95% CI, 1.03-1.80; P = .030). We found that the TT genotype was associated with increased plasma vasopressin clearance (P = .028), and the rs4869317 genotype accounted for 80% of the variance of serum sodium concentrations (locus-specific heritability) in cardiac surgical patients.

Conclusions

The genetic variation in LNPEP (vasopressinase) is associated with 28-day mortality in septic shock and is associated with biologic effects on vasopressin clearance and serum sodium regulation. Further confirmation in additional cohorts is required.

Abbreviations

APACHE

: Acute Physiology and Chronic Health Evaluation

AVP

: arginine vasopressin

AVPR1A

: AVP receptor 1A

AVPR1B

: AVP receptor 1B

HR

: hazard ratio

LNPEP

: leucyl/cystinyl aminopeptidase

OXTR

: oxytocin receptor

SNP

: single nucleotide polymorphism

SPH

: St. Paul's Hospital

UBC

: University of British Columbia

VASST

: Vasopressin and Septic Shock Trial

Low-dose vasopressin is infused as an adjunct vasopressor when patients with septic shock do not respond adequately to fluid and vasopressor therapy. [1] , [2] , [3] , [4] Vasopressin is particularly important during septic shock because a relative deficiency of vasopressin is reported in this condition. [1] , [5] , [6] Vasopressin, a stress hormone, helps to maintain cardiovascular homeostasis in hypotensive states via the V1a receptor, [1] , [4] modulates adrenocorticotropic hormone and cortisol by the V1b receptor, [7] controls water balance (and serum sodium concentration) via the V2 receptor, [8] , [9] and also binds the oxytocin receptor. [1] , [10] The half-life of vasopressin in human plasma is 4 to 24 min [11] , [12] and is primarily determined by leucyl/cystinyl aminopeptidase (LNPEP) (also known as vasopressinase), a physiologically essential enzyme that cleaves peptide bonds of vasopressin sequentially from the amino terminus and thus contributes to regulation of circulating vasopressin levels. [13] , [14]

Due to a crucial role of vasopressin, we postulated that genetic variants of vasopressin pathway genes (arginine vasopressin [*AVP*], arginine vasopressin receptor 1A and 1B [*AVPR1A, AVPR1B*], oxytocin receptor [*OXTR*], and *LNPEP*) might be associated with an altered clinical course in patients with septic shock. We tested this hypothesis using two large cohorts of well characterized patients who had septic shock. Our primary outcome variable was 28-day mortality. To test for evidence of biologic effect, we measured vasopressin clearance in patients with septic shock, and, in a third cardiac surgical cohort, we measured locus-specific heritability of serum sodium concentrations.

Materials and Methods

Study Patient Cohorts

St. Paul's Hospital Cohort

Septic shock was defined by the presence of two or more diagnostic criteria for the systemic inflammatory response syndrome, [15] proven or suspected infection, at least one new organ dysfunction by Brussels criteria, [3] and hypotension [3] despite adequate fluid resuscitation. All patients admitted to the ICU at St. Paul's Hospital (SPH) in Vancouver, British Columbia, Canada, between July 2000 and January 2004 were screened (n = 1,626). Of these, 601 patients had septic shock on admission, were extensively phenotyped, [16] and had DNA available. Twelve patients, who were also enrolled in the Vasopressin and Septic Shock Trial (VASST), [3] were excluded from this cohort. Thus, 589 patients were included in the analysis. The Institutional Review Board at SPH and the University of British Columbia (UBC) approved the study (approval number, H02-50076).

VASST Cohort

VASST was a multicenter, randomized, double-blind, controlled trial evaluating the efficacy of vasopressin vs norepinephrine. [3] Of 6,229 screened patients, 778 patients who had septic shock (≥ 16 years of age) [3] , [15] and received a minimum of 5 μg/min of norepinephrine were assessed in the trial. Exclusion criteria, blinding, and randomization have been previously reported. [3] Of these, 616 patients had DNA available and were included in the study. The research ethics boards of all participating institutions approved this trial, and written informed consent was obtained from all patients or their authorized representatives. The research ethics board at the coordinating center (UBC) approved the genetic analysis (approval number H02-50076).

Cardiac Surgical Cohort

Patients admitted to the cardiac surgical ICU after surgery at SPH between July 2000 and January 2004 were screened (n = 1,234). Of these, 977 patients having DNA available were included in the analysis. Perioperative serum sodium levels during a 24-h perioperative period (preoperative, immediately postoperative, and 4, 12, and 24 h after surgery) were evaluated. The institutional review board at SPH and UBC approved the study (approval number, H02-50076).

Selection of Tag Single Nucleotide Polymorphisms of Vasopressin Pathway Genes and LNPEP Single Nucleotide Polymorphisms and Genotyping

Tag single nucleotide polymorphisms (SNPs) were identified using a linkage disequilibrium-based tag SNP selection method, [17] using genotyping data in European ancestry from the HapMap database for *AVP, AVPR1A, LNPEP, OXTR* and those from SeattleSNPs for *AVPR1B* (http://pga.gs.washington.edu/) with an r^2 threshold of 0.65 for SNPs with a minor-allele frequency > 5%. An additional condition for tag SNP selection was successful SNP genotyping. For *OXTR* we limited the large number of potential tag SNPs to two. We identified 230 SNPs by sequencing the haplotype block (160 kb) containing the *LNPEP* rs18059 tag SNP in six lymphoblastoid cell lines (e-Fig 1). We subsequently genotyped these 230 *LNPEP* SNPs in the derivation cohort. Of the 230 SNPs including rs18059, the strongest signal was found in *LNPEP* rs4869317 in the derivation analysis, and thus patients in the VASST replication cohort and cardiac surgical cohort were

genotyped for rs4869317. DNA was extracted from the buffy coat of discarded blood samples using a QIAamp DNA maxi kit (Qiagen; Mississauga, Ontario, Canada) and genotyped using the Illumina Golden Gate assay (Illumina; San Diego, California).

Plasma Vasopressin Levels and Clearance of Vasopressin

Plasma concentrations of vasopressin were measured by double antibody immunoassay, as reported,[3] over the initial 72 h (at time of randomization and 6, 24, and 72 h after randomization) in samples of the vasopressin (n = 50) and norepinephrine group (n = 46) in the VASST cohort. This subgroup with plasma vasopressin concentrations measured (n = 96) had similar clinical characteristics to the original VASST cohort, including age, gender, APACHE (Acute Physiology and Chronic Health Evaluation) II, ethnicity, and vasopressin use (subgroup vs original VASST cohort, median age [year], 61 vs 63; men [%], 61.7 vs 59.3; median APACHE II, 27 vs 27; white [%], 77.1 vs 83.9; vasopressin use [%], 52.1 vs 51.1).

Vasopressin clearance was calculated in patients receiving constant IV vasopressin infusion with the use of one-compartment model as reported,[18] using the equation: **(Equation 1)** $C_P = R(1 - e^{-kt})/V_D\kappa$

where C_P = plasma concentration; R = infusion rate; t = time; V_D = volume of distribution; κ = elimination rate constant.

When t = ∞, $C_{SS} = R(1 - e^{-\infty})/V_D\kappa$, where C_{SS} = steady-state drug concentration; $V_D\kappa$ = clearance). Thus, **(Equation 2)** $\text{Vasopressin clearance} = R/C_{ss}$

The C_{SS} was obtained from the plasma level-time curve and record of vasopressin infusion dose (at time of randomization and 6, 24, and 72 h after randomization) for each patient. Urinary clearance was reported as 5% of total body clearance of vasopressin.[18]

Statistical Analysis

The primary outcome variable was 28-day mortality and the secondary outcome variables were vasopressin clearance and serum sodium level. We used a Breslow generalized Wilcoxon test for mortality over 28 days for 17 tag SNPs without correction for multiple testing for the purpose of discovery. We tested for Hardy-Weinberg equilibrium using a χ^2 test primarily as a data quality check. We used an Armitage test for trend[19] in the derivation cohort for screening all 230 candidate SNPs densely genotyped in the LNPEP gene region: This is a common approach in genome-wide association studies.[20] We used the Nyholt procedure to correct for multiple testing of SNPs in linkage disequilibrium with each other.[21]

For the primary analysis, we chose a Cox regression to test for differences in hazard of death over 28 days by genotype, which allows for correction of potential confounding factors, including age, gender, surgical vs medical primary diagnosis, ancestry, and with vasopressin vs without vasopressin infusion as covariates in both the SPH and VASST cohorts. We tested for association between 28-day mortality and rs4869317 TT genotype using a logistic regression analysis. The locus-specific heritability of rs4869317 to serum sodium levels was calculated by the Wijsman procedure.[22]

We tested for differences in baseline characteristics and days alive and free of both cardiovascular dysfunction (Brussels criteria)[3] and vasopressor use during 28 days using a Mann-Whitney *U* or a χ^2 test. Differences were considered significant using a two-tailed $P < .05$. Analyses were performed using R, version 2.8.1 (www.R-project.org) and SPSS, version 16 (SPSS, Inc; Chicago, Illinois) statistical software packages.

Results

We successfully genotyped 17 tag SNPs listed by HapMap in five vasopressin pathway genes, *AVP, AVPR1A, AVPR1B, OXTR*, and *LNPEP* in the SPH derivation cohort of 589 patients with septic shock (Table 1). Allele frequencies of the 17 tag SNPs in this study are similar to those in the HapMap data (e-Table 1). Since the *LNPEP* tag SNP rs18059 was significantly associated with mortality over 28 days and was in Hardy-Weinberg equilibrium (Table 1), we resequenced the haplotype block (160 kb) containing the *LNPEP* gene in six lymphoblastoid cell lines (e-Fig 1) and identified 230 SNPs. SNPs in *OXTR* were significantly associated with 28-day mortality but were not in Hardy-Weinberg equilibrium (χ^2 test for Hardy-Weinberg equilibrium for whites, rs11706648 $P = .0048$, rs237887 $P = 7.9 \times 10^{-5}$) and were not investigated further.

Table 1 -- Allele Frequency and Effect on Mortality of 17 Tag SNPs in Five Vasopressin Pathway Genes in the Derivation Cohort of Septic Shock

Gene	SNP	Major/Minor Allele	MAF (n = 589)	HWE *P* Value	Trend Test Slope	Mortality *P* Value
AVP	rs1410713	C/A	0.292	.20	0.015	.94

Gene	SNP	Major/Minor Allele	MAF (n = 589)	HWE *P* Value	Trend Test Slope	Mortality *P* Value
	rs4815566	C/T	0.091	.066	0.018	.77
	rs857240	C/T	0.113	5.1×10^{-8}	-0.049	.37
	rs857242	C/A	0.123	.031	-0.020	.54
AVPR1A	rs10877970	T/C	0.150	.84	-0.013	.91
	rs1495027	C/T	0.432	.49	-0.0013	.56
	rs3803107	C/T	0.174	.97	-0.034	.88
AVPR1B	rs28418396	A/T	0.153	.0050	0.040	.14
	rs28517473	G/A	0.232	.17	0.0043	.87
	rs28588803	C/T	0.120	.0054	-1.2×10^{-5}	.51
	rs28607590	G/C	0.202	.0	0.036	.24
LNPEP	rs10051637	A/G	0.406	.30	0.012	.69
	rs18059	T/C	0.488	.57	-0.040	.037
	rs27711	G/A	0.400	.75	0.023	.96
	rs38041	G/A	0.487	.20	-0.011	.24
OXTR	rs11706648	A/C	0.350	.0015	-0.073	.016
	rs237887	A/G	0.434	1.1×10^{-5}	0.084	.0039

Derivation cohort of septic shock, St. Paul's Hospital cohort, n = 589. *P* values were calculated with the use of χ^2 test for Hardy-Weinberg equilibrium and Breslow generalized Wilcoxon test for mortality over 28 d. *AVP* = arginine vasopressin; *AVPR* = arginine vasopressin receptor; HWE = Hardy-Weinberg equilibrium; *LNPEP* = leucyl/cystinyl aminopeptidase; MAF = minor allele frequency; *OXTR* = oxytocin receptor; SNP = single nucleotide polymorphism.

All 230 *LNPEP* SNPs were successfully genotyped in the SPH derivation cohort (e-Table 2), and, of these, 179 SNPs had a minor allele frequency of > 0.01. We then screened these SNPs for a genetic association with 28-day mortality in the derivation cohort using an Armitage test for trend to choose the SNP with the strongest association for further study (Fig 1). The major [T] allele of *LNPEP* rs4869317 [A/T] SNP was significantly associated with increased 28-day mortality (uncorrected $P = 4.4 \times 10^{-4}$), even after Nyholt correction for multiple testing (corrected $P = .044$).[21] A major allele model (TT vs AT/AA genotype) best fits 28-day mortality (28-day mortality: TT = 51.0% [172/337], AT = 34.0% [67/197], AA = 36.4% [20/55]). We next performed Cox regression analysis including potential confounding factors: age, gender, surgical vs medical primary diagnosis, ancestry, and with vasopressin vs without vasopressin infusion as covariates. The TT genotype patients had an increased hazard ratio (HR) of death compared with the AT/AA patients (TT vs AT/AA, SPH: adjusted HR, 1.54; 95% CI, 1.19–2.01; $P = .0013$) (Fig 2, Table 2).

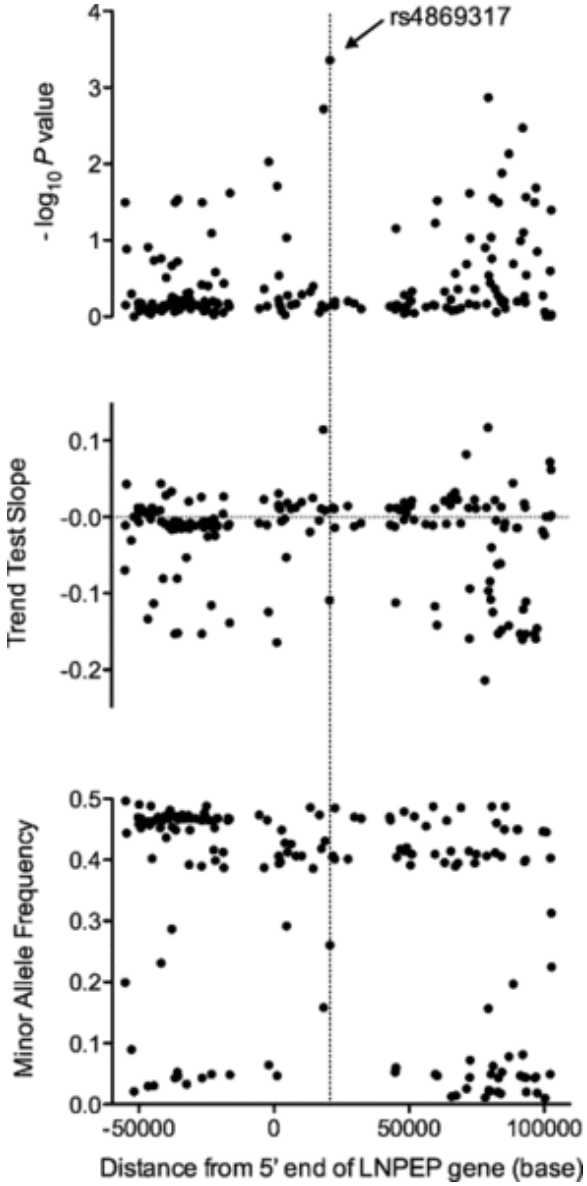

Figure 1 Screening analysis for multiple *LNPEP* single nucleotide polymorphisms (SNPs) in the derivation cohort. The *LNPEP* rs4869317 SNP was most significantly associated with altered 28-day mortality (uncorrected $P = 4.4 \times 10^{-4}$; corrected $P = .044$ [Nyholt correction]). The *LNPEP* rs2303138 SNP was secondly associated with the mortality (uncorrected $P = 1.4 \times 10^{-3}$; corrected $P = .13$ [Nyholt correction]). *P* values of 179 SNPs were calculated with the use of Armitage test for trend. LNPEP = leucyl/cystinyl aminopeptidase.

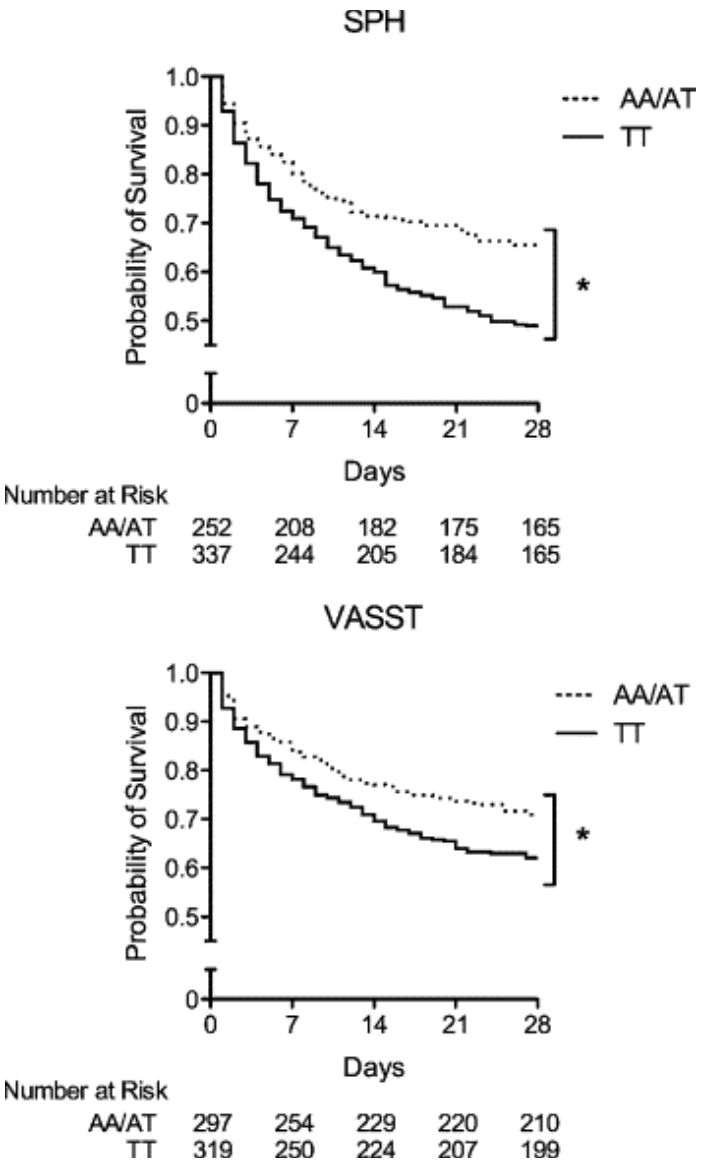

Figure 2 Survival curves of patients with septic shock in two cohorts according to genotype of *LNPEP* rs4869317. Patients with the TT genotype of the *LNPEP* rs4869317 SNP had significantly decreased 28-day survival in the SPH and VASST cohort compared with patients with the AT/AA genotype (TT vs AT/AA genotype, SPH: adjusted hazard ratio [HR], 1.58; 95% CI, 1.21-2.06; *P* = .00073; VASST: adjusted HR, 1.36; 95% CI, 1.03-1.80; *P* = .030). *P* values were calculated using Cox regression analysis corrected for age, gender, surgical vs medical diagnosis, ancestry, and with vasopressin vs without vasopressin infusion. SPH = St. Paul's Hospital; VASST = Vasopressin and Septic Shock Trial. See the Figure 1 legend for expansion of the other abbreviations.

Table 2 -- Hazard of Death Over 28 Days in rs4869317 TT Genotype Patients

	SPH		VASST	
Characteristic	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value**
Age ≥ 60 y	1.68 (1.29–2.18)	9.9×10^{-5}	1.34 (1.01–1.78)	.040
Female	0.94 (0.72–1.21)	.62	0.94 (0.71–1.24)	.67
Surgical	0.83 (0.63–1.09)	.18	0.83 (0.59–1.18)	.31
European ancestry	1.00 (0.75–1.34)	.99	0.89 (0.62–1.27)	.51
Vasopressin infusion	1.89 (1.43–2.52)	1.0×10^{-5}	0.90 (0.68–1.18)	.43
LNPEP rs4869317 TT genotype	1.58 (1.21–2.06)	7.3×10^{-4}	1.36 (1.03–1.80)	.030

Hazard of death over 28 d is calculated using a Cox regression including potential confounding factors: age (≥ 60 vs < 60 y), gender (female vs male), surgical vs medical primary diagnosis, European ancestry vs others, with vasopressin vs without

vasopressin infusion, and *LNPEP* rs4869317 TT vs AT/AA genotype as covariates. HR = hazard ratio; SPH = St. Paul's Hospital; VASST = Vasopressin and Septic Shock Trial. See Table 1 legend for expansion of the other abbreviation.

We next tested for replication of our primary finding in the 616 successfully genotyped patients with septic shock in the VASST cohort.[3] The genotype frequency of *LNPEP* rs4869317 in VASST was similar to HapMap data and to the SPH derivation cohort and was in Hardy-Weinberg equilibrium (e-Tables 1, 3). In the VASST cohort, there was no difference by genotype in baseline characteristics of the patients ([Table 3] , [Table 4] , [Table 5]). In accordance with the primary finding in the SPH cohort, *LNPEP* rs4869317 TT genotype patients in the VASST cohort had significantly increased 28-day mortality with or without adjustment for baseline characteristics compared with the AT/AA patients (TT vs AT/AA: adjusted HR, 1.37; 95% CI, 1.04–1.81; P = .026) (Fig 2, Table 2). In addition, repeating this analysis after limiting both the cohorts to the largest ancestral group, whites, yielded the same conclusions. Logistic regression analysis for 28-day mortality yielded the same conclusions (SPH, TT vs AT/AA: adjusted OR, 2.00; 95% CI, 1.40–2.86; $P = 1.5 \times 10^{-4}$; VASST, TT vs AT/AA: adjusted OR, 1.45; 95% CI, 1.03–2.05; P = .033).

Table 3 -- Baseline Characteristics of Three Cohorts

Baseline Characteristics	SPH (n = 589)	VASST (n = 616)	Cardiac Surgical ICU (n = 977)
Age, y	62 (48–72)	63 (50–73)	66 (58–73)
Gender, % male	63.0	59.3	76.3
Ethnicity, No. (%)			
European ancestry	453 (76.9)	517 (83.9)	855 (87.5)
Asian	105 (17.8)	57 (9.3)	92 (9.4)
NA aboriginal	21 (3.6)	15 (2.4)	3 (0.3)
African	3 (0.5)	14 (2.3)	3 (0.3)
Hispanic	5 (0.8)	6 (1.0)	6 (0.6)
Others	2 (0.3)	7 (1.1)	18 (1.8)

NA = North American. See Table 2 for expansion of other abbreviations.

Table 4 -- Baseline Characteristic of Patients in Two Cohorts of Septic Shock by LNPEP rs4869317 Genotype

	SPH			VASST		
Characteristic	AA/AT (n = 252)	TT (n = 337)	*P* Value	AA/AT (n = 297)	TT (n = 319)	*P* Value
Age, y	62 (45–72)	63 (49–73)	.28	63 (52–73)	63 (49–72)	.57
Gender, % male	61.9	63.8	.64	59.6	58.9	.87
APACHE II	25 (19–30)	27 (21–33)	.014	26 (22–31)	27 (22–32)	.073
Surgical, %	31.3	28.8	.5	22.6	19.1	.29
Vasopressin use, %	17.1	18.1	.74	52.2	50.2	.61
Physiologic dose steroid use, %	48.4	45.1	.43	74.1	76.5	.49
Preexisting conditions, No. (%)						
Chronic heart failure	18 (7.1)	19 (5.6)	.46	25 (8.4)	22 (6.9)	.48
Chronic pulmonary disease	42 (16.7)	58 (17.2)	.86	48 (16.2)	60 (18.8)	.39
Chronic liver disease	26 (10.3)	32 (9.5)	.74	29 (9.8)	39 (12.2)	.33
Chronic renal failure	13 (5.2)	26 (7.7)	.22	38 (12.8)	30 (9.4)	.18
Chronic corticosteroid use	19 (7.5)	19 (5.6)	.35	61 (20.5)	68 (21.3)	.81
Laboratory variables, day 1						

	SPH			VASST		
Characteristic	**AA/AT (n = 252)**	**TT (n = 337)**	***P* Value**	**AA/AT (n = 297)**	**TT (n = 319)**	***P* Value**
WBC count, 10^3/μL	14.6 (10.0–19.8)	14.8 (9.6–21.1)	.68	13.5 (7.7–21.2)	13.2 (7.1–19.6)	.38
Platelet count, 10^3/μL	164 (96–251)	167 (90–243)	.57	161 (81–256)	138 (76–241)	.28
PaO_2/FIO_2, mm Hg	135 (83–195)	152 (98–227)	.0045	189 (133–255)	189 (135–258)	.76
Blood creatinine, μmol/L	133 (87–237)	160 (91–308)	.036	150 (91–242)	155 (91–272)	.84
Blood lactate, mmol/L	2.3 (1.4–4.2)	2.4 (1.4–5.3)	.29	2.4 (1.4–4.7)	2.2 (1.4–4.0)	.36

Data are median (interquartile range) for continuous variables. *P* values were calculated with the use of Mann-Whitney *U* test and χ^2 test. APACHE = Acute Physiology and Chronic Health Evaluation. See [Table 1] , [Table 2] for expansion of other abbreviations.

Table 5 -- Characteristics of Cardiac Surgical ICU Patients by LNPEP rs4869317 Genotype

Characteristic	**AA/AT (n = 454)**	**TT (n = 523)**	***P* Value**
Age, y	66 (58–73)	67 (58–73)	.84
Gender, % male	76.9	75.7	.71
Preexisting conditions, No. (%)			
Hypertension	294 (64.8)	332 (63.5)	.68
Diabetes	117 (25.8)	136 (26.0)	.94
Smoker	128 (28.2)	133 (25.4)	.33
Surgical procedure, No. (%)			
CABG	305 (67.2)	325 (62.1)	.37
Valve repair	78 (17.2)	101 (19.3)	
CABG + valve repair	55 (12.1)	79 (15.1)	
Other	16 (3.5)	18 (3.4)	
ICU survival, %	98.2	97.3	.34
Hospital survival, %	96.3	94.3	.15

Data are median (interquartile range) for continuous variables. *P* values were calculated with the use of Mann-Whitney *U* test and χ^2 test. CABG = coronary artery bypass graft surgery. See Table 1 for expansion of other abbreviation.

To test for evidence of biologic plausibility, we next measured the rate of vasopressin clearance by *LNPEP* rs4869317 genotype (vasopressin treatment group: n = 50, TT [n = 28], AT/AA [n = 22]; non-vasopressin treatment group: n = 46, TT [n = 23], AT/AA [n = 23]). We used the constant vasopressin infusion in the vasopressin patients in the VASST cohort (0.03 units/min) and measured vasopressin levels to determine the first-order clearance kinetics of vasopressin (normalized to body mass). The TT genotype patients (n = 28) in the vasopressin treatment group had increased vasopressin clearance (mean ± SD, 28.7 ± 17.7 mL/kg/min) compared with the AT/AA patients (n = 22, 19.7 ± 6.5 mL/kg/min, *P* = .028, Student *t* test with Welch correction). Given this increased TT genotype vasopressin clearance rate, the vasopressin concentration in nontreated TT patients (n = 23) should be 68.6% of the vasopressin concentration in nontreated AT/AA genotype patients (n = 23) (equation 2 in the “Materials and Methods” section). This value closely matches the observed value of 67.4% (Css TT genotype/Css AA/AT genotype = 3.17 ± 2.10 pmol/L/4.70 ± 4.67 pmol/L), although this ratio is not significantly different from 1 (*P* = .16). This concordance suggests that clearance of vasopressin in patients not receiving exogenous vasopressin infusions was similarly increased in the *LNPEP* rs4869317 TT genotype patients compared with the AT/AA patients.

We next tested for this genotypic effect on cardiovascular function. In line with the observation of vasopressin clearance, the TT genotype patient had increased cardiovascular dysfunction and increased vasopressor use in the SPH cohort (median days alive and free [25th–75th percentile], cardiovascular dysfunction: TT = 8 [0–23] vs AT/AA = 17 [3–24], *P* = .012; vasopressor use: TT = 15 [1–26] vs AT/AA = 23 [6–26], *P* = .0010). A nonsignificant trend in the same direction was present in the VASST cohort (cardiovascular dysfunction: TT = 17 [0–24] vs AT/AA = 20 [0–24], *P* = .14; vasopressor use: TT = 17

[0–24] vs AT/AA = 20 [1–24], *P* = .10).

We sought to confirm these findings using an independent population (cardiac surgery patients) and different strategy (V2 receptor effect). Accordingly, we measured perioperative serum sodium concentrations in 977 cardiac surgical ICU patients (Tables 3-5). We calculated locus-specific heritability [22] to determine the heritable fraction of this trait due to *LNPEP* rs4869317. The total variance (σ^2) in serum sodium concentration for 977 patients was 33.2, whereas the variance within each genotype was 6.6 and the variance calculated between the means of each genotype was 26.6. Thus, 80% of the variance (26.6/33.2) in the normally tightly regulated serum sodium concentration was accounted for by *LNPEP* rs4869317 genotype, indicating that this trait is highly heritable and 80% of the heritability is due to *LNPEP* rs4869317 genotype.

Discussion

Patients with septic shock who had the TT genotype of the *LNPEP* rs4869317 SNP had increased 28-day mortality compared with patients who had the AT/AA genotype. The *LNPEP* genotype was also associated with evidence of concordant biologic effects. That is, the TT genotype of *LNPEP* (vasopressinase) rs4869317 was associated with increased vasopressin clearance sufficient to account for decreased plasma vasopressin concentrations and, independently, serum sodium concentrations are heritable, and 80% of this heritability is accounted for by *LNPEP* rs4869317 genotype in a third large cardiac surgical cohort.

Vasopressin is synthesized in the hypothalamus, stored in the posterior pituitary, and released into the systemic circulation, where it is degraded rapidly, having a very short plasma half-life. [11] , [12] Thus, plasma vasopressin concentration is determined by modification of the production (or secretion) and degradation. [14] , [23] Relative deficiency of vasopressin is proposed as a rationale to treat patients with septic shock with vasopressin infusion. [1] , [6] The *LNPEP* rs4869317 TT genotype was associated with an increased mortality in patients with septic shock, perhaps because the TT genotype patients had increased vasopressinase activity as indicated by a greater vasopressin clearance rate. We surmised that increased mortality in the patients with septic shock with the TT genotype may therefore be due to increased vasopressinase (LNPEP) activity, which results in an increased vasopressin clearance, and a decreased plasma vasopressin concentration (an even more pronounced vasopressin deficiency), which leads to inadequate cardiovascular compensation, likely via the V1 receptor.

Vasopressin, also called antidiuretic hormone, binds to the V2 receptor (expressed in renal collecting ducts) to increase water reabsorption by increasing movement of aquaporin-2 water channels from cytoplasm to apical membrane of collecting duct cells. [24] , [25] Thus, vasopressin controls plasma sodium levels. We reasoned that if *LNPEP* genetic variants are associated with altered outcome of septic shock (likely related to V1 receptor responses) due to alterations in vasopressinase activity, then the same genetic variants of *LNPEP* should be associated with altered V2 receptor-mediated response. We discovered that the rs4869317 genotype accounted for 80% of the variance in serum sodium concentrations in cardiac surgical patients, those with the TT genotype having increased serum sodium level compared with those with AT/AA genotype. This discovery is aligned with the associations of the TT genotype with increased vasopressin clearance and decreased vasopressin levels in patients with septic shock.

The *LNPEP* gene consists of 18 exons (17 introns) on chromosome 5q15 covering 94 kb of genomic DNA and has a wide tissue distribution. [14] , [26] , [27] A huge haplotype block (160 kb) covers the *LNPEP* gene region (e-Fig 1). The LNPEP protein is a type 2 transmembrane protein in the M1 aminopeptidase family and the extracellular domain has aminopeptidase activity. [28] , [29] *LNPEP* has two major transcript variants (NM005575, NM175920). [27] The rs4869317 SNP is located in a regulatory region of the gene in both of these transcript variants: either intron 1 (NM005575) or 5′ upstream (2,151 base upstream from transcription start site, NM175920), and therefore may alter transcription of the *LNPEP* gene. Alternatively, SNPs that are in high linkage disequilibrium with rs4869317 may be functional. The *LNPEP* rs2303138 SNP having the second strongest association in the derivation cohort (Fig 1) was previously reported with autoimmune diseases in a genome-wide association studies using 14,500 nonsynonymous SNPs. [30] *LNPEP* is also called placental leucine aminopeptidase (*P-LAP*), [27] , [31] insulin-regulated aminopeptidase (*IRAP*), [28] , [32] vasopressinase, oxytocinase, and angiotensin IV receptor, [33] and has diverse physiologic roles in addition to its role as the catalytic enzyme for vasopressin. Our study suggests that the *LNPEP* rs4869317 TT genotype was associated with increased mortality of patients with septic shock due to altered clearance of vasopressin. However, we acknowledged that the increased mortality may instead be due to changes in clearance of oxytocin, altered activity of the angiotensin IV receptor, altered glucose transporter type (GLUT)-4 activity, or other actions. [31] , [32] , [33]

There are several limitations of our study. This is a study of mixed races (% largest ancestral group [white]: SPH, 76.9%; VASST, 83.9%; cardiac surgical ICU, 87.5%). Since we did not adjust for the effects of population admixture, this is a major limitation. Replication of further large patient cohorts with correction for population admixture using ancestry informative markers would strengthen these conclusions. We showed associations of the rs4869317 TT genotype with significant increases in both mortality and vasopressin clearance compared with the AT/AA genotype, but these findings do not prove

causation. Furthermore, rs4869317 is located in the haplotype block of 230 SNPs. Therefore, the association of this SNP with the mortality of septic shock may be due to the effect of a single SNP or due to the combined impact of multiple SNPs in the same haplotype block. We did not investigate mechanisms of action, such as changes in clearance of oxytocin, altered activity of the angiotensin-IV receptor, or other actions, according to *LNPEP* rs4869317. We tested 17 tag SNPs of five vasopressin pathway genes in a derivation analysis followed by a screen of 230 LNPEP SNPs whereby we identified rs4869317. An average of three to four tag SNPs per gene in genes of this size is not enough to detect all potential signals and thus is a limitation of this study. Since we tested for Hardy-Weinberg equilibrium primarily as a data quality check in the derivation analysis (Table 1), we did not further investigate OXTR SNPs (rs11706648 and rs237887), despite the significant associations with 28-day mortality. It remains possible that the deviations from Hardy-Weinberg equilibrium in the OXTR SNPs are due to disease (septic shock), and this presents a possible limitation of this study.

In conclusion, the TT genotype of *LNPEP* rs4869317 SNP was associated with increased 28-day mortality and increased vasopressin clearance in patients with septic shock compared with patients who had the AT/AA genotype. Independently, the genotype of *LNPEP* rs4869317 also accounted for 80% of the heritability of serum sodium concentrations in cardiovascular surgery patients. Thus, this *LNPEP* genotype marks a functional genetic variant that results in increased vasopressinase activity, which leads to decreased vasopressin levels associated with decreased survival in patients with septic shock.

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Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following conflicts of interest: Dr Russell holds shares in Sirius Genomics Inc, which has submitted patents owned by UBC and licensed to Sirius Genomics Inc that are related to the genetics of vasopressin. UBC has also submitted a patent related to the use of vasopressin in septic shock. Dr Russell is an inventor on these patents and has received consulting fees from Ferring, which manufactures vasopressin; from AstraZeneca, which manufactures anti-tumor necrosis factor; and from Sirius Genomics Inc; has received grant support from Sirius Genomics, Novartis, Ferring, and Eli Lilly; and has received speaking honoraria from Pfizer and Eli Lilly. Mr Wellman serves as Director of Research and holds shares at Sirius Genomics Inc, which has submitted a patent owned by UBC and licensed to Sirius Genomics Inc that is related to the genetics of vasopressin. Dr Walley is an inventor in a patent application filed by the UBC related to this work. UBC has licensed this to Sirius Genomics Inc. Dr Walley holds shares in Sirius Genomics Inc. Drs T. Nakada, Boyd, E. Nakada, Hirasawa, and Oda, and Mss Thain and Thair have reported that no

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Additional information: The e-Figure and e-Tables can be found in the Online Supplement at http://chestjournal.chestpubs.org/content/139/5/1042/suppl/DC1.

Web Extra Material

Online Supplement

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