

β_2 -Adrenergic Receptor Gene Polymorphism Is Associated with Mortality in Septic Shock

Taka-aki Nakada¹, James A. Russell¹, John H. Boyd¹, Rosalia Aguirre-Hernandez¹, Katherine R. Thain¹, Simone A. Thair¹, Emiri Nakada¹, Melissa McConechy¹, and Keith R. Walley¹

¹University of British Columbia Critical Care Research Laboratories, Heart + Lung Institute, St. Paul's Hospital, Vancouver, British Columbia, Canada

Rationale: The *CysGlyGln* haplotype of the β_2 -adrenergic receptor gene (*ADRB2*) is functional and associated with altered responses to adrenergic agonists in patients with asthma. Whether this functional haplotype alters outcome in patients receiving adrenergic agonists in septic shock is unknown.

Objectives: To determine whether genetic variation of *ADRB2* influences outcome in septic shock.

Methods: Two cohorts of patients with septic shock were studied: a single center (St. Paul's Hospital [SPH]) cohort (n = 589) and the Vasopressin and Septic Shock Trial (VASST) cohort (n = 616). The A allele of the rs1042717 G/A polymorphism is in complete linkage disequilibrium with the *CysGlyGln* haplotype of *ADRB2*; therefore, rs1042717 was genotyped. Modulation by norepinephrine and salbutamol of IL-6 production by stimulated *in vitro* lymphoblastoid cells was measured by genotype.

Measurements and Main Results: Patients who had the AA genotype of rs1042717 displayed increased 28-day mortality in SPH (adjusted hazard ratio, 2.23; 95% confidence interval, 1.33–3.72; *P* = 0.0022), and this result was replicated in VASST (adjusted hazard ratio 2.82; 95% confidence interval, 1.56–5.09; *P* = 0.0006). This genotypic effect was eliminated in patients treated with acute low-dose corticosteroids. In all patients, the AA genotype was associated with more organ dysfunction. Patients with the AA genotype had a higher heart rate (SPH; *P* < 0.05; VASST; *P* < 0.05) and required a higher norepinephrine dose over Days 1 through 3 (VASST; *P* < 0.05). The AA genotype was associated with decreased norepinephrine and salbutamol inhibition of IL-6 production by stimulated lymphoblastoid cells *in vitro* (*P* < 0.05).

Conclusions: The AA genotype of *ADRB2* rs1042717, identifying homozygotes for the *CysGlyGln* haplotype, was associated with increased mortality and more organ dysfunction in septic shock.

Keywords: single nucleotide polymorphisms; septic shock; genetic association study; lymphoblastoid cells

The β_2 -adrenergic receptor gene (*ADRB2*) plays a key role in outcome and response to adrenergic agonists in cardiovascular diseases, including hypertension and heart failure, and in respiratory and inflammatory diseases, including asthma (1–3). The *ADRB2* gene is very small and intronless. Three common single nucleotide polymorphisms (SNPs) in *ADRB2* (*Cys/Arg-19*, *Gly/Arg16*, and *Gln/Glu27*) are functionally important *in vitro* and *in vivo* (4–6). The *Cys/Arg-19* SNP is located within a short open reading frame (called the 5' leader cistron) of *ADRB2* and is associated with altered *ADRB2*

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Adrenergic agonist therapy is a cornerstone of cardiovascular management of septic shock. The β_2 -adrenergic receptor (*ADRB2*) is involved in regulation of the cardiopulmonary system and exerts antiinflammatory effects. Functional effects of several single-nucleotide polymorphisms of the *ADRB2* gene have been demonstrated *in vitro* and *in vivo*.

What This Study Adds to the Field

The AA genotype of *ADRB2* gene polymorphism rs1042717, marking the *CysGlyGln* haplotype of *ADRB2*, was associated with increased mortality, more organ dysfunction, and a higher heart rate and a higher dose of norepinephrine infusion in septic patients with shock. The same genotype demonstrated reduced antiinflammatory effects as measured by norepinephrine or salbutamol inhibition of IL-6 production in *in vitro* stimulated lymphoblastoid cells.

translation *in vitro* (5). *Gly/Arg16* and *Gln/Glu27* are non-synonymous SNPs in the extracellular N-terminus of *ADRB2* and alter the degree of agonist-mediated receptor expression *in vitro* (4) and agonist-mediated desensitization *in vivo* (6). These SNPs lie within a 125-base pair segment of DNA, and there is marked linkage disequilibrium (LD) between these three SNPs and marked LD of this haplotype with other SNPs in *ADRB2* (see Figure E1 in the online supplement). This adds to data from previous studies that also emphasize the importance of taking haplotype structure into account (5–9). Recently, lymphocytes homozygous for the *CysGlyGln* haplotype of these three SNPs were reported to be more susceptible to desensitization of the β -agonist-mediated response in patients with asthma (8).

Whether this functional *CysGlyGln* haplotype alters outcome and response to adrenergic agents in septic shock is unknown. Septic shock is the most common cause of death in Intensive Care Units, and the incidence is increasing (10). Together with adequate and early fluid resuscitation, vasopressors and inotropes (including adrenergic agonists) form the cornerstone of cardiovascular management of septic shock and are an initial therapy of choice in the Surviving Sepsis Campaign: International Guidelines 2008 (11). Polymorphisms of a variety of genes, including cytokines and crucial molecules in the innate immune and coagulation systems (12–14), are associated with altered outcomes in sepsis and septic shock. Despite the known functional polymorphisms within *ADRB2* in patients with asthma (8), the relationship between the *CysGlyGln* haplotype and outcome

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Correspondence and requests for reprints should be addressed to Keith R. Walley, M.D., Critical Care Research Laboratories, 1081 Burrard Street, Vancouver, BC, Canada V6Z 1Y6. E-mail: keith.walley@hli.ubc.ca

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TABLE 1. BASELINE CHARACTERISTICS, BY *ADRB2* rs1042717 GENOTYPE, OF PATIENTS IN TWO SEPTIC SHOCK COHORTS*

	SPH Cohort				<i>P</i> Value*	VASST Cohort				<i>P</i> Value*
	AA (<i>n</i> = 43)	AG (<i>n</i> = 196)	GG (<i>n</i> = 350)	All (<i>n</i> = 589)		AA (<i>n</i> = 29)	AG (<i>n</i> = 205)	GG (<i>n</i> = 382)	All (<i>n</i> = 616)	
Age, years	64 (52.5–76)	64 (48–73)	60 (47–71)	62 (47–72)	0.19	55 (45–70)	64 (48–72)	63 (51–73)	63 (50–73)	0.18
Gender, % male	67.4	64.2	61.7	63.0	0.82	48.3	62.9	58.1	59.3	0.25
APACHE II	27 (21–31.5)	27 (20–33)	25 (20–31)	26 (20–32)	0.20	32 (24–35)	26 (22–31)	26 (22–32)	26.5 (22–32)	0.031
European ancestry, n [†]	22	146	285	453	0.56	18	158	341	517	0.95
Surgical, %	30.2	28.6	30.6	29.9	0.24	17.2	19.5	21.7	20.8	0.73
Preexisting conditions, %										
Chronic heart failure	7.0	6.1	6.3	6.3	0.98	3.4	6.3	8.9	7.6	0.28
Chronic pulmonary disease	14.0	19.4	16.0	17.0	0.52	10.3	14.6	19.6	17.5	0.18
Chronic liver disease	11.6	11.2	8.9	9.8	0.55	10.3	12.7	10.2	11.0	0.65
Chronic renal failure	7.0	8.2	5.7	6.6	0.60	20.7	13.1	9.1	11.0	0.079
Chronic corticosteroid use	2.3	5.1	7.8	6.5	0.26	31.0	21.0	20.2	20.9	0.38
Laboratory variables										
White blood cell count, 10 ³ /mm ³	17.1 (9.8–22.8)	14.5 (9.6–19.8)	14.8 (10–20.1)	14.6 (9.7–20.3)	0.57	10.1 (4.6–19.3)	14.0 (7.5–20.3)	13.4 (7.7–20.8)	13.4 (7.4–20.8)	0.60
Platelet count, 10 ³ /mm ³	140 (92.5–258)	167.5 (97.5–238)	166.5 (90–252)	164 (91–245.5)	0.94	108 (56–166)	147 (85–255)	161 (76.5–247)	150.5 (76.3–248.5)	0.047
PaO ₂ /FiO ₂ , mm Hg	174 (101–246)	151 (98–223)	140 (88–209)	145 (91–217)	0.24	155 (105–291)	183 (132–244)	195 (142–263)	190 (136–258)	0.20
Blood creatinine, μmol/L	138 (76–203)	163 (91–303)	145 (88–261)	147 (88–277.5)	0.25	185 (101–301)	179 (100–273)	141 (89–239)	152 (91–254)	0.016

Definition of abbreviations: *ADRB2* = β₂-adrenergic receptor; APACHE, Acute Physiology and Chronic Health Evaluation; SPH = St. Paul's Hospital; VASST = Vasopressin and Septic Shock Trial.

Data are median (interquartile range) for continuous variables.

* *P* values were calculated with the use of chi-square test and Kruskal-Wallis test.

[†] Chi-square test for Hardy-Weinberg equilibrium.

in patients receiving adrenergic agents in septic shock remains uncertain (15).

We therefore hypothesized that *ADRB2* gene polymorphisms may affect the clinical outcome of patients with septic shock. To test the hypothesis, we focused on the functional *CysGlyGln* haplotype (7, 8). Using HapMap and SeattleSNPs databases, we found that the *CysGlyGln* haplotype is in complete LD with the A allele of the *ADRB2* rs1042717 G/A SNP (Figure E1). This is not surprising because this SNP lies less than 200 base pairs away from the 125–base pair *CysGlyGln* haplotype segment. For simplicity, we genotyped this SNP in two large cohorts of well-characterized patients with septic shock (14, 16).

METHODS

Patients

St. Paul's Hospital Cohort. All patients admitted to the Intensive Care Unit at St. Paul's Hospital (SPH) in Vancouver, Canada between July 2000 and January 2004 were screened (*n* = 1,626). Of these, 601 patients had septic shock on admission (Figure E2), were extensively phenotyped (14), and had DNA available. Twelve patients, who were also enrolled in the Vasopressin and Septic Shock Trial (VASST) (16), 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 approved the study.

VASST Cohort. VASST was a multicenter, randomized, double-blind, and controlled trial evaluating the efficacy of vasopressin versus norepinephrine in 779 patients with septic shock (16). Of these, 616 patients had DNA available. 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 (University of British Columbia) approved the genetic analysis.

Selection of SNP and Genotyping

Using genotyping from HapMap (<http://www.hapmap.org/>) and SeattleSNPs (<http://pga.gs.washington.edu/>), we found that the A allele of the nearby rs1042717 G/A SNP was in complete linkage disequilibrium with the functional *ADRB2* *CysGlyGln* haplotype (*r*² = 1.0). Therefore, we selected rs1042717 for genotyping, which was successful in all patients. DNA was extracted from buffy coat of blood samples using a QIAamp DNA maxi kit (Qiagen, Mississauga, Ontario, Canada) and genotyped using the Tag-It platform (Luminex Molecular Diagnostics, Toronto, ON, Canada).

Inhibition of IL-6 Production by Norepinephrine and Salbutamol

Five rs1042717 AA and five GG homozygous lymphoblastoid cell lines from unrelated individuals of European ancestry genotyped by HapMap were used (17). Cells were incubated with norepinephrine bitartrate (100 μM), salbutamol sulfate (100 μM) (18, 19) (concentrations based on initial dose–response experiments), or control vehicle for 24 hours and then stimulated by the addition of cytomix (20, 21) (2.5 ng/mL each of TNF-α, IL-1β, and IFN-γ [R&D Systems, Minneapolis, MN] and 12.5 μM of CpG [Sigma-Aldrich, Oakville, ON, Canada] based on initial dose–response experiments) and incubated for 24 hours. The concentration of IL-6 protein was measured in supernatants using the ELISA assay (R&D Systems).

Statistical Analysis

Our primary analysis used Cox regression to test for differences in hazard of death over 28 days by rs1042717 genotype. We chose this approach because analysis with respect to *ADRB2* genotype was observational, and correction for potential confounding factors due to baseline imbalances of covariates (age, gender, and primary

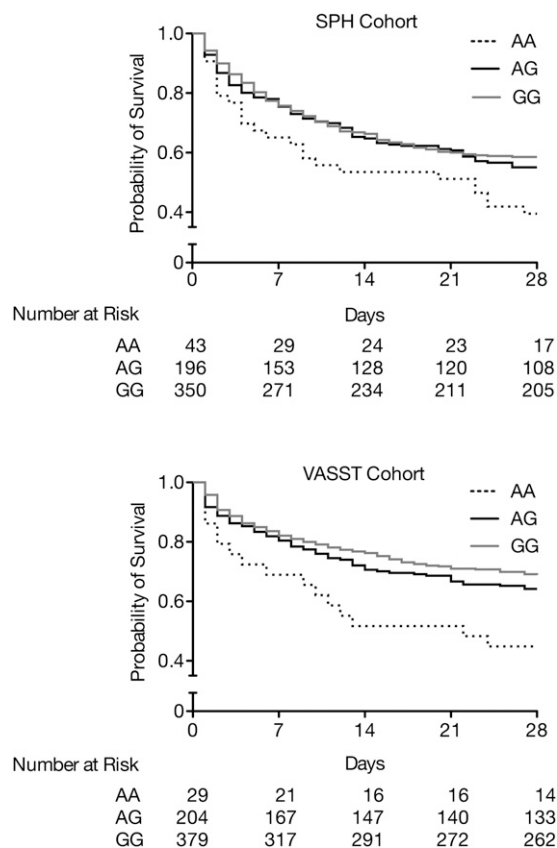


Figure 1. Survival curves for all patients with septic shock in the St. Paul's Hospital (SPH) and Vasopressin and Septic Shock Trial (VASST) cohort by genotype of *ADRB2* rs1042717. Patients who were homozygous for rs1042717 A (AA) (dashed line) had significantly decreased survival in the SPH cohort and the VASST cohort. *P* values were calculated using Cox regression analysis corrected for age, gender, and medical versus surgical diagnosis (SPH: adjusted hazard ratio [HR], 2.23; 95% confidence interval [CI], 1.33–3.72; *P* = 0.0022. VASST: adjusted HR: 2.82; 95% CI, 1.56–5.09; *P* = 0.0006).

diagnosis surgical vs. medical) was potentially important. Based on previous reports (7, 8), we used a minor allele model (AA vs. GA+GG) in the statistical analysis. Additional univariate analysis was performed using chi-square tests for categorical data and Kruskal-Wallis tests or one-way ANOVA for continuous data. We tested for Hardy-Weinberg equilibrium using a chi-square test. Differences were considered significant using a two-tailed *P* < 0.05. Analyses were performed using R (version 2.8.1; www.R-project.org) and SPSS (version 16; SPSS, Chicago, IL) statistical software packages.

RESULTS

Baseline Characteristics

In the SPH cohort (n = 589) and VASST cohort (n = 616), allele frequency and genotype distribution were similar to those previously reported (7) (Table 1). In both cohorts, the majority of patients were of European ancestry (SPH cohort, 76.9%; VASST cohort, 83.9%) and in Hardy-Weinberg equilibrium with respect to rs1042717 (SPH cohort, *P* = 0.56; VASST cohort, *P* = 0.95). Subpopulations of non-European ancestry in both cohorts were also in Hardy-Weinberg equilibrium (Table E1). However, due to mixing different populations, the entire population in the SPH cohort was not in

Hardy-Weinberg equilibrium (Table E1). Therefore, we limited the primary analysis to patients of European ancestry and, in a secondary analysis, included all patients in Cox regression analysis adjusted by including ancestry as a covariate.

In the SPH cohort, there was no difference by genotype in baseline characteristics of the patients (Table 1). In the VASST cohort, patients who had the *ADRB2* rs1042717 AA genotype had a lower platelet count and higher creatinine concentration in parallel with a trend to an increased number of patients with chronic renal failure (Table 1). This difference in renal function and the higher APACHE II score in AA patients in the VASST cohort may be due to random baseline imbalances or may be clinical outcomes related to genotype; therefore, the Cox regression test was conducted without and with the APACHE II score entered as a covariate (Table E2).

Patients Who Had *ADRB2* rs1042717 AA Genotype Had Increased 28-day Mortality

Patients who had *ADRB2* rs1042717 AA genotype had a significant increase in the hazard of death over the 28-day observation period in the SPH European ancestry population (adjusted hazard ratio [HR], 2.23; 95% confidence interval [CI], 1.33–3.72; *P* = 0.0022) (Figure 1 and Table E2). This result replicated in the VASST European ancestry population (adjusted HR, 2.82; 95% CI, 1.56–5.09; *P* = 0.0006) (Figure 1 and Table E2). This result remained highly significant after including the APACHE II score in the statistical model (Table E2) and remained significant in the secondary analysis of all patients where ancestry was included in the Cox regression model.

In a recent review (22), Taylor and Hall suggest that corticosteroid treatment may mask or alter *ADRB2* genotypic effects. To test this hypothesis, we compared patients who did with patients who did not receive acute low-dose corticosteroids (200–300 mg hydrocortisone per day) in the SPH and VASST cohorts. Survival curves (Figure 2) demonstrate that steroid treatment abolished the *ADRB2* rs1042717 AA genotype effect (SPH, *P* = 0.12; VASST, *P* = 0.36). In contrast, patients not receiving steroids had an *ADRB2* genotype effect in SPH (adjusted HR, 2.05; 95% CI, 1.06–4.00; *P* = 0.034) and VASST (adjusted HR, 3.74; 95% CI, 1.84–7.61; *P* = 0.00027) despite the smaller sample sizes in these subpopulations.

Patients Who Had *ADRB2* rs1042717 AA Genotype Had Increased Organ Failure

Patients with the AA genotype had more organ dysfunction, defined by Brussels criteria (16), in the SPH and VASST cohorts (Table 2). Significant associations with *ADRB2* rs1042717 AA genotype in the SPH cohort included more renal dysfunction and in the VASST cohort included more renal dysfunction and more renal replacement therapy as well as more hematological, hepatic, and neurologic dysfunction (Table 2).

Cardiovascular Variables and Adrenergic Agonist Therapy

In the SPH and VASST cohorts, patients with the AA genotype had a higher heart rate on Day 1 (SPH cohort, *P* = 0.013; VASST cohort, *P* = 0.0072) (Table 3) and a higher mean heart rate during Day 2 to Day 5 (SPH cohort, *P* = 0.011; VASST cohort, *P* = 0.0001 [VASST cohort, norepinephrine group, *P* = 0.028; vasopressin group, *P* = 0.033]) (Figure E3). A trend to higher blood lactate was observed in the SPH cohort, and

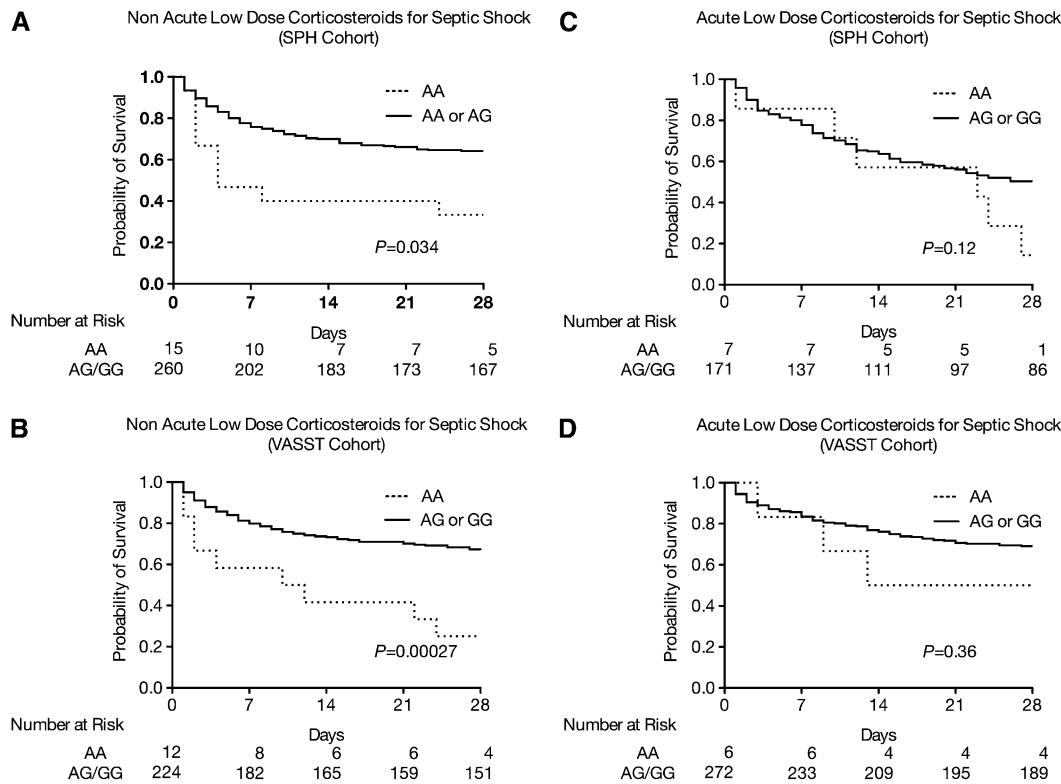


Figure 2. Survival curves for patients with septic shock who received acute low-dose corticosteroids (200–300 mg hydrocortisone per day) or not in the St. Paul’s Hospital (SPH) and Vasopressin and Septic Shock Trial (VASST) European ancestry cohorts. Patients with the *ADRB2* rs1042717 AA genotype who did not receive acute low-dose corticosteroids for septic shock had an increased hazard of 28-day mortality in the SPH European ancestry cohort (A: adjusted HR, 2.05; 95% CI, 1.06–4.00; $P = 0.034$) and in the VASST cohort (B: adjusted HR, 3.74; 95% CI, 1.84–7.61; $P = 0.00027$). In contrast, patients with the *ADRB2* rs1042717 AA genotype who received acute low-dose corticosteroids for septic shock did not have an increased hazard of 28-day mortality in the SPH European ancestry cohort (C, $P = 0.12$) and in the VASST cohort (D, $P = 0.36$). P values were calculated using Cox regression analysis corrected for age, gender, and medical versus surgical diagnosis.

a directionally similar pattern was highly significant in VASST ($P = 0.0026$). A nonsignificant trend toward increased norepinephrine dose was observed at baseline in the SPH and VASST cohorts (Table 3). However, over the Day 1 to Day 3 time period, patients in the VASST cohort who had the AA genotype received a higher mean norepinephrine infusion dose ($P = 0.016$) (Figure E3).

Inhibition of IL-6 Production by Norepinephrine and Salbutamol

Lymphocytes homozygous for the *CysGlyGln* haplotype have been previously reported to be more susceptible to *ADRB2* desensitization (8). We tested norepinephrine- and salbutamol-induced inhibition of IL-6 production under mixed inflammatory stimulation (TNF- α , IL-1 β , IFN- γ , and CpG) in lymphoblastoid cell lines of known *ADRB2* rs1042717 genotype and known *CysGlyGln* haplotype. Cells were treated with norepinephrine or salbutamol or a vehicle control for 24 hours before stimulation. Concentrations of IL-6 in conditioned media after 24-hour incubation were evaluated by genotype. Stimulated cells with the AA genotype displayed decreased inhibition of IL-6 production after treatment with norepinephrine ($P = 0.026$) or salbutamol compared with the GG genotype ($P = 0.021$) (Figure 3A). The mean IL-6 levels of the stimulation alone group with the AA genotype were 3.5-fold higher than those with the GG genotype ($P = 0.0016$) (Figure 3B).

DISCUSSION

We found that the AA genotype of the β_2 -adrenergic receptor gene rs1042717 G/A polymorphism, identifying homo-

zygotes for the known functional *ADRB2* *CysGlyGln* haplotype, was significantly associated with increased mortality and more organ dysfunction in the SPH and the replication VASST cohorts of patients with septic shock. In addition, we found significant associations between *ADRB2* rs1042717 AA genotype and relevant cardiovascular variables in patients with septic shock, including higher heart rates on Day 1 and during Days 2 through 5 in both cohorts and a higher norepinephrine dose during Days 1 through 3 in the VASST cohort. Because lymphocytes express the β_2 -adrenergic receptor (23, 24) and have been used to demonstrate the functional effects of the *CysGlyGln* haplotype (8), we used lymphoblastoid cell lines of the known *ADRB2* *CysGlyGln* haplotype and the corresponding rs1042717 genotype. Our hypothesis, arising from our clinical observations, states that homozygotes for this haplotype have decreased down-regulation of the inflammatory response, which is mediated by adrenergic agents. In line with our clinical observations, we found that patients with the rs1042717 AA genotype (*CysGlyGln* homozygotes) were less responsive to norepinephrine (the most common adrenergic agonist used in the patient cohorts) and salbutamol (a more selective β_2 -adrenergic agonist) in down-regulating IL-6 production in response to a mixed inflammatory stimulus. Thus, it is perhaps not surprising that the known functional *ADRB2* *CysGlyGln* haplotype, marked by the A allele of the rs1042717 SNP, appears to play an important role in patients with septic shock who are treated with adrenergic agonists.

Mechanistically, the *CysGlyGln* homozygous genotype (rs1042717 AA genotype) results in a more rapid and complete desensitization to adrenergic agonists (8). The clinical outcome

TABLE 2. DAYS ALIVE AND FREE OF ORGAN DYSFUNCTION AND ARTIFICIAL SUPPORT IN PATIENTS WITH SEPTIC SHOCK WITH THE *ADRB2* rs1042717 GENOTYPE*

	SPH Cohort					VASST Cohort				
	AA (n = 43)	AG (n = 196)	GG (n = 350)	All (n = 589)	P Value†	AA (n = 29)	AG (n = 205)	GG (n = 382)	All (n = 616)	P Value
Organ dysfunction										
Cardiovascular	5 (0–22)	9 (1–23.3)	14 (0–24)	12 (0–24)	0.16	4 (0–23)	19 (0–24)	19 (0–24)	19 (0–24)	0.19
Respiratory	2 (0–20)	8 (0–23.3)	9 (0–23)	8 (0–23)	0.48	0 (0–12)	3 (0–17)	3 (0–15)	3 (0–15)	0.48
Renal	6 (0–24)	13 (1–28)	15 (2–28)	13 (1–28)	0.042	10 (1–20)	22 (3–28)	23 (4.3–28)	22 (4–28)	0.013
Hematologic	13 (2–28)	24 (4–28)	22 (5–28)	22 (4–28)	0.46	3 (1–23)	24 (6–28)	25 (6.3–28)	24.5 (5–28)	0.0019
Hepatic	9 (1–28)	22 (3–28)	21.5 (4–28)	21 (3–28)	0.18	9 (1–27)	25 (7–28)	27 (5–28)	25 (5–28)	0.021
Neurologic	14 (2–27)	22 (5.8–27)	24 (5–28)	23 (4–28)	0.12	0 (0–23)	10 (0–24)	17 (1–24)	16 (0–24)	0.037
SIRS (two of four SIRS criteria)	0 (0–5)	1 (0–15)	1 (0–13)	1 (0–13)	0.15	0 (0–14)	7 (0–17)	7 (0–17)	7 (0–17)	0.12
Artificial support										
Vasopressor	7 (0–25)	18 (1–26)	21 (2–26)	19 (2–26)	0.28	4 (0–23)	19 (0–24)	19 (0–24)	19 (0–24)	0.18
Ventilator	1 (0–19.5)	5 (0–22)	7 (0–22)	5 (0–22)	0.67	0 (0–21)	8 (0–20)	10 (0–20)	9 (0–20)	0.24
Renal replacement therapy	8 (1.5–28)	15 (2–28)	18 (2–28)	16 (2–28)	0.16	9 (1–27)	24 (6–28)	26 (6.3–28)	25 (6–28)	0.015
28-d mortality, n (%)										
All patients	26 (60.5)	88 (44.9)	145 (41.4)	259 (44.0)	0.059	16 (55.2)	74 (36.1)	121 (31.7)	211 (34.3)	0.029
European ancestry	16 (72.7)	64 (43.8)	114 (40.0)	194 (42.8)	0.011	12 (66.7)	56 (35.4)	105 (30.8)	173 (33.5)	0.0058

Definition of abbreviations: *ADRB2* = β_2 -adrenergic receptor; SIRS = systemic inflammatory response syndrome; SPH = St. Paul’s Hospital; VASST = Vasopressin and Septic Shock Trial.

Data are median (interquartile range) for continuous variables.

* *ADRB2* organ dysfunction was recorded if the patient met the Brussels organ dysfunction criteria (moderate, severe, or extreme).

† P values were calculated with chi-square and Kruskal-Wallis tests.

effects that we observed may be due to altered adrenergic agonist-mediated responses of the heart and vasculature (6) or to altered regulation of the inflammatory response (8, 18, 24–26). In the current study, a higher dose of norepinephrine infusion and a higher heart rate in patients who had the AA genotype were observed. One possible interpretation of this association is cardiovascular dysregulation by *ADRB2* genetic variations. The major effect of norepinephrine infusion for hemodynamic management in patients with septic shock may be as a vasopressor, which is known to function predominantly via the α -adrenergic receptor. However, norepinephrine also binds *ADRB2* to activate the intracellular signaling pathway (24), which in human heart mediates positive inotropic and chronotropic effects (27). Associations between cardiovascular

disorders and altered *ADRB2* signaling via endogenous adrenergic hormones or agonists or antagonists have been substantially demonstrated (28, 29). Together, these points indicate that *ADRB2* signaling influences hemodynamic disorder in patients with septic shock. In patients with hypertension, previous reports demonstrated associations between lower heart rate and the Arg allele of *Arg/Gly16* in *ADRB2* (30). Because Arg is coded for by the opposite allele compared with the *CysGlyGln* haplotype, this result corresponds to our observation. Alternatively or additionally, *ADRB2* signaling activated by adrenergic agonists, which includes norepinephrine and salbutamol, leads to antiinflammatory effects in several cell types when stimulated with inflammatory mediators (18, 24–26, 31), for example via the cAMP/protein kinase A pathway in airway smooth muscle

TABLE 3. CARDIOVASCULAR VARIABLES AND ADRENERGIC AGONIST THERAPY ON DAY 1 FOR THE *ADRB2* rs1042717 GENOTYPE

	SPH Cohort					VASST Cohort				
	AA (n = 43)	AG (n = 196)	GG (n = 350)	All (n = 589)	P Value*	AA (n = 29)	AG (n = 205)	GG (n = 382)	All (n = 616)	P Value*
Cardiovascular variables										
Heart rate, bpm	125 (100–140)	115 (100–135)	110 (95–125)	115 (95–130)	0.013	135 (125–151)	125 (110–140)	127.5 (110–140)	126 (110–140)	0.0072
Mean arterial pressure, mm Hg	55 (50–58)	54.5 (50–59)	55 (50–59)	55 (50–59)	0.84	56 (49–61)	54 (49–60)	55 (50–61)	55 (50–61)	0.33
Central venous pressure, mm Hg	10.5 (8–15.5)	12 (10–15)	12 (9–15)	12 (9–15)	0.74	15 (13–20)	14 (11–18)	14 (11–17)	14 (11–18)	0.26
Blood lactate, mmol/L	2.7 (2–4.9)	2.2 (1.3–4.7)	2.3 (1.4–4.6)	2.3 (1.4–4.7)	0.17	3.6 (2.6–5.9)	2.3 (1.4–4.55)	2.1 (1.4–4)	2.3 (1.4–4.4)	0.0026
Adrenergic agonist infusions										
Norepinephrine, μ g/min	18 (10–31.9)	15 (7.6–29.5)	14 (8–23.5)	15 (8–25)	0.27	19 (10.6–35)	15 (9–25)	15 (9–26.6)	15 (9–26.5)	0.22
Dobutamine, μ g/kg/min	6 (5–7.8)	7.3 (5–12)	6 (5–10)	7 (5–10)	0.98	4.4 (3.5–5)	3.8 (2.9–5.3)	5 (2.4–8.6)	4 (2.5–7.1)	0.91
Corticosteroid therapy										
Low dose corticosteroids, %	39.5	50.0	45.4	46.5	0.37	69.0	74.6	76.2	72.4	0.65
Duration of corticosteroids, d	3 (2–8)	4 (2–10.8)	4 (2–12)	4 (2–12)	0.80	9.5 (3.8–14)	7 (4–15)	8 (4–14)	8 (4–14)	0.80

Definition of abbreviations: *ADRB2* = β_2 -adrenergic receptor; SPH = St. Paul’s Hospital; VASST = Vasopressin and Septic Shock Trial.

Data are median (interquartile range) for continuous variables.

* P values were calculated using chi-square and Kruskal-Wallis tests.

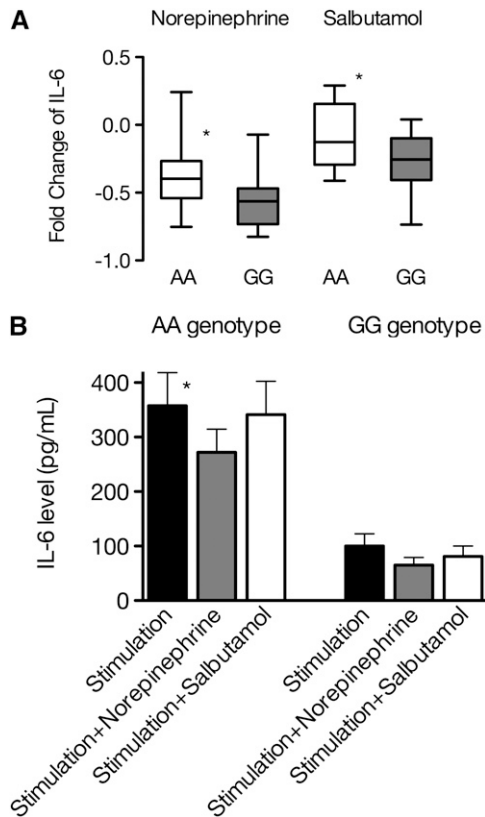


Figure 3. Norepinephrine and salbutamol-induced inhibition of IL-6 production under mixed inflammatory stimulation in genotyped lymphoblastoid cell lines by *ADRB2* rs1042717 genotype. (A) Significant fold change differences in IL-6 concentrations were observed between the AA and GG genotypes when treated with norepinephrine ($P = 0.026$, student's t test) or salbutamol ($P = 0.021$, student's t test). Fold changes were calculated by \log_2 value. (B) Absolute IL-6 concentration values. The mean IL-6 levels of the stimulation alone group with the AA genotype were 3.5-fold higher than those with the GG genotype ($P = 0.0016$, Mann-Whitney U test). There were significant differences in IL-6 levels among the three conditions (stimulation alone, stimulation + norepinephrine, stimulation + salbutamol) in the GG genotype ($P = 0.048$, one-way repeated-measures analysis of variance) but not in the AA genotype ($P = 0.10$, one-way repeated-measures analysis of variance). Error bars indicate SEM.

cells (26). Signaling via *ADRB2* in patients with septic shock is impaired (32), and this impairment has been shown to be associated with endotoxin-induced acute renal failure in rats (33). These findings suggest an additional interpretation of the inflammatory responses being altered via *ADRB2* signaling due to *ADRB2* genetic variations, and this effect altered clinical outcome.

In our *in vitro* experiments, lymphoblastoid cells with the rs1042717 AA genotype (*CysGlyGln* homozygotes) had decreased inhibitory effect of stimulation-induced IL-6 production by norepinephrine or salbutamol. Oostendorp and colleagues previously demonstrated that *CysGlyGln* homozygotes, corresponding to the rs1042717 AA genotype in this study, were more susceptible to desensitization of isoproterenol-induced cAMP responses in lymphocytes (8); our findings are consistent with this. This hyporesponsiveness of the rs1042717 AA genotype might account for our observation of a higher dose of norepinephrine infusion in patients with septic shock who had the rs1042717 AA genotype in the VASST cohort. In addition, the mean IL-6 levels of stimulation alone in the AA genotype cell lines were 3.5-fold higher

than those in the GG genotype cell lines. This difference in IL-6 production by genotype, if also present *in vivo*, is consistent with the increased mortality that we observed in patients having the AA genotype.

In a recent review (22), Taylor and Hall suggest that corticosteroid treatment may reduce the effects of *ADRB2* genotype on clinical outcomes in patients with asthma. Corticosteroids exert antiinflammatory effects via the translocation of the glucocorticoid receptor–corticosteroid complex from the cytosol to the nucleus of the cell. Stimulation of *ADRB2* enhances the nuclear translocation of this complex and results in additional antiinflammatory effects (34, 35). In addition, corticosteroids interact with intracellular signaling pathways initiated by the β_2 -adrenergic receptor (36). We therefore tested this as a secondary hypothesis in patients with septic shock. We found that 39.3% of the SPH patients and 54.1% of the VASST patients with European ancestry were treated acutely with low-dose corticosteroids for septic shock, which abolished the differences in mortality by *ADRB2* genotype (Figure 2). In contrast, a greater *ADRB2* genotype survival difference was observed in patients who were not treated with corticosteroids (SPH, $P = 0.034$; VASST, $P = 0.00027$), a strikingly significant result despite the smaller subset sample size. A deeper understanding of the molecular mechanism of this interaction would make this observation more compelling. If the mortality effect is confirmed in additional cohorts, then this result suggests that corticosteroid treatment might be considered in patients with septic shock who carry the AA genotype (*CysGlyGln* homozygotes) to prevent the increased mortality observed in these patients.

This study has several limitations. The analysis of the SPH and VASST cohorts was retrospective. In particular, lack of by-genotype differences in patients treated with corticosteroids must be regarded as hypothesis generating and not conclusive. In addition, these data, demonstrating an association between *ADRB2* genotype and clinical outcome, do not prove a causal link despite many previous reports of mechanistic effect as well as our observation of decreased responsiveness to norepinephrine and salbutamol of stimulated lymphoblastoid cells with the *ADRB2* rs1042717 AA genotype (*CysGlyGln* homozygotes). Another limitation involves the selection of the treatment used for stimulation of the cells. Human sepsis invokes a mixed inflammatory stimulus. The agonists in the well-studied “cyto-mix” (20, 21) represent a number of the key pathways involved. Initially we tried using LPS to invoke the toll-like receptor signaling pathway, but because these cells, which are derived from human B lymphocytes, are generally refractory to TLR4 agonists due to low cell-surface TLR4 expression (37), we had limited success. These cells have well-documented evidence for TLR9 expression; therefore, we chose to add CpG to the cyto-mix (CpG is a known ligand for TLR9) (37). In patients with septic shock, the association of the AA genotype with increased 28-day mortality was very strong in patients of European ancestry and significant in all patients when ancestry was included in the statistical model. Despite this, the current results have inadequate power to verify that a similar finding will be observed in other ancestral groups.

In conclusion, the AA genotype of the *ADRB2* rs1042717 polymorphism, marking homozygotes for the known functional *CysGlyGln* haplotype, was associated with increased 28-day mortality, more organ failure, a higher heart rate during the initial 5 days as well as a higher norepinephrine infusion rate in patients with septic shock. These results are consistent with previous reports and with our observation that the AA genotype is associated with decreased responsiveness to the antiinflammatory effects of adrenergic agonists.

Conflict of Interest Statement: T.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.A.R. received more than \$100,001 from Sirius Genomics, \$5,001–\$10,000 from AstraZeneca, and \$10,001–\$50,000 from Ferring in consultancy fees; \$1,001–\$5,000 from Eli Lilly in lecture fees; and \$10,001–\$50,000 from Eli Lilly and \$10,001–\$50,000 from Novartis (Chiron) in industry-sponsored grants. J.A.R. and J.A.R.'s spouse/life partner each hold more than \$100,001 in stock ownership or options from Sirius Genomics. J.H.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.A.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.R.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.A.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.R.W. is a professor of medicine at the University of British Columbia and a ICU Physician at St. Paul's Hospital and has received more than \$100,001 as a scientific consultant from Sirius Genomics Inc., \$10,001–\$50,000 as a board member of Sirius Genomics Inc., and more than \$100,001 contract research agreement from Sirius Genomics Inc., is a coinventor of a patent with UBC, which holds patent applications regarding genetic predictors of disease outcome, and holds more than \$100,001 in common stock and stock options from Sirius Genomics Inc.

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