ORIGINAL ARTICLE

A Functional Synonymous Coding Variant in the *IL1RN* Gene Is Associated with Survival in Septic Shock

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Abstract

Rationale: Death from infection is a highly heritable trait, yet there are few genetic variants with known mechanism influencing survival during septic shock.

Objectives: We hypothesized that a synonymous coding variant in the IL-1 receptor antagonist gene (*IL1RN*), rs315952, previously associated with reduced risk for acute respiratory distress syndrome, would be functional and associate with improved survival in septic shock.

Methods: We used a human endotoxin (LPS) model of evoked inflammatory stress to measure plasma IL-1 receptor antagonist (IL1RA) following low-dose Food and Drug Administration–grade LPS injection (1 ng/kg) in 294 human volunteers. RNA sequencing of adipose tissue pre- and post-LPS was used to test for allelic imbalance at rs315952. In the Vasopressin and Septic Shock Trial cohort, we performed a genetic association study for survival, mortality, and organ failure–free days.

Measurements and Main Results: Adipose tissue displayed significant allelic imbalance favoring the rs315952C allele in subjects of European ancestry. Consistent with this, carriers of rs315952C had slightly higher plasma IL1RA at baseline (0.039) and higher evoked IL1RA post-LPS (0.011). In the Vasopressin and Septic Shock Trial cohort, rs315952C associated with improved survival ($P = 0.028$), decreased adjusted 90-day mortality ($P = 0.044$), and faster resolution of shock ($P = 0.029$).

Conclusions: In European ancestry subjects, the *IL1RN* variant rs315952C is preferentially transcribed and associated with increased evoked plasma IL1RA and with improved survival from septic shock. It may be that genetically determined IL1RA levels influence survival from septic shock.

Keywords: septic shock; polymorphism; functional genetic variant; RNA-seq

Septic shock remains a common cause of death in the intensive care unit, with mortality rates as high as 35% (1, 2). Although improved recognition of the syndrome and careful attention to early antibiotic therapy (3) and hemodynamic targets (4) have decreased mortality over time (1), there remains no specific pharmacotherapy for septic shock. Furthermore, among patients meeting criteria for septic shock, there may be unappreciated heterogeneity in molecular pathophysiology (5–7) or genetic predisposition (8, 9) that influences response to treatment or outcome. Several

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At a Glance Commentary

Scientific Knowledge on the Subject: Death from infection is a highly heritable trait, yet there are few replicated genetic variants associated with death from septic shock, and fewer still with known molecular function.

What This Study Adds to the Field: The present study demonstrates that a synonymous *IL1RN* single nucleotide variant is a site for preferential transcription of one allele in adipose tissue and is associated with higher evoked plasma IL-1 receptor antagonist in response to endotoxin and with increased survival in septic shock.

studies have suggested that either high initial or persistent proinflammatory cytokine levels, including IL-1β, in the plasma may correlate with organ dysfunction and death (7, 10, 11).

We previously identified a synonymous coding variant in the IL-1 receptor antagonist gene (*IL1RN*) associated with lower risk of developing the acute respiratory distress syndrome (ARDS) in three critically ill populations (combined odds ratio, 0.81; $P = 4.2 \times 10^{-5}$) (12). The largest population, with more than 2,000 subjects, had sepsis as the primary risk factor for ARDS (12). The *IL1RN* gene encodes for IL-1 receptor antagonist protein (IL1RA), the naturally occurring antagonist for IL-1α and IL-1β. IL1RA competes with IL-1α and IL-1β to bind the IL-1 receptor 1 (IL1R1), yet IL1RA does not trigger IL1R1 signaling, and instead acts as a brake on inflammasome activation (13–15).

Among critically ill subjects, we demonstrated that the ARDS low-risk *IL1RN* single nucleotide polymorphism (SNP) rs315952C associated with higher plasma levels of IL1RA (12), consistent with a hypothesis that genetically determined higher endogenous plasma IL1RA levels might mitigate ARDS risk. Other groups have examined the association between sepsis outcomes and *IL1RN* variation, and found a variable number of tandem repeat polymorphism known as allele 2 (*IL1RN*2*) to associate with increased susceptibility to sepsis (16, 17) or increased risk of death from sepsis (11, 18), although the effects of *IL1RN*2* on secreted IL1RA protein have been inconsistent (11, 19). In addition, a well-described *IL1RN* promoter SNP rs4251961C has been consistently associated with decreased IL1RA levels in response to infection or pathogen-associated molecular patterns (20–23). These three *IL1RN* variants (rs4251961, *IL1RN*2* tagged by rs419598, and rs315952) exhibit little to no linkage disequilibrium between one another in either European or African ancestry (EA, AA) populations (24).

Given these findings, we sought to identify the genetic mechanism by which rs315952C might associate with higher evoked plasma IL1RA and to test prior *IL1RN* candidate SNPs for association with evoked IL1RA. We used intravenous low-dose endotoxin (LPS) as a human experimental model of inflammatory stress to understand the dynamics of IL1RA in response to a standardized insult. In addition, we sought to test whether rs315952C demonstrated any protective associations during septic shock, including improved survival, faster resolution of shock, or reduced time on the ventilator. These complimentary approaches allowed us to investigate the mechanistic effects of rs315952 both in response to a uniform inflammatory stimulus and during clinical septic shock. Our primary hypothesis was that rs315952 would be more strongly associated with innate immunity-evoked IL1RA, and that this would translate into improved survival during septic shock. Some of the results of this study have been previously reported in the form of abstracts (25, 26).

Methods

Study Populations

GENE study. The Genetics of Evoked response to Niacin and Endotoxemia (GENE) study recruited 294 healthy nonobese subjects (27). The protocol was approved by the University of Pennsylvania Institutional Review Board, had regulatory oversight by the US Food and Drug Administration (LPS: IND 5,984), and was monitored by a National Institute of Health–appointed data safety and monitoring board. Subjects in GENE were admitted to the clinical translational research center inpatient unit for administration of intravenous endotoxin (LPS; 1 ng/kg) and were monitored closely as described (27). Serial blood draws were collected immediately before and 1, 2, 4, 6, 12, and 24 hours post-LPS for plasma. Gluteal adipose biopsy using a liposuction catheter under local anesthesia was performed at baseline and 4 hours post-LPS as described for RNA extraction (28, 29).

VASST cohort. The Vasopressin and Septic Shock Trial (VASST) was a multicenter, double-blind, randomized controlled trial evaluating vasopressin versus norepinephrine for septic shock (30). The study enrolled 778 patients with septic shock and requiring at least 5 μg/min norepinephrine infusion; details have been published (30, 31). The research ethics boards of all participating institutions approved the trial, and written informed consent was obtained from all patients or their authorized representatives, including permission to perform downstream mechanistic testing. Of 778 patients in the VASST trial, 632 had available DNA and were included in this study (8, 31, 32). A subgroup of subjects, determined by study personnel availability, also had plasma collected at study enrollment (n = 399). Clinical outcomes included mortality at 28 and 90 days; site of infection; Acute Physiology and Chronic Health Evaluation II (APACHE II) score; duration of vasoactive drug infusion; and days free of moderate, severe, or extreme organ failure as defined by the Brussels criteria (33).

Genotyping and Protein Analysis

Genomic DNA was extracted from whole blood using a QIAmp kit (Qiagen, Missaugua, ON, Canada). The GENE study was genotyped with the Illumina (San Diego, CA) Infinium Exome chip, filtered for rs315952, rs4251961, and rs419598 and SNPs within 1 kb of *IL1RN*. Plasma IL1RA was measured by ELISA (R&D Systems, Minneapolis, MN) in duplicate. The VASST cohort was genotyped using the Human 1M Duo platform (Illumina) and filtered for rs315952 and SNPs within 1 kb of *IL1RN*. Plasma IL1RA and IL1β levels were measured in VASST in a subgroup of patients by human multiplex kits (EMD Millipore, Billerica, MA) using an antibody-linked magnetic bead assay according to the manufacturer's recommendations. For RNA sequencing (RNA-seq), RNA was extracted from adipose using RNeasy Lipid Tissue total RNA mini kit (Qiagen, Valencia, CA), and prepared and sequenced as previously described (29, 34). Results

were filtered for RNA-seq reads flanking rs315952 (chr2:113890304), and the number of reads carrying each allele (C or T) was counted.

Statistical Analysis

Plasma levels in the GENE population were analyzed by nonparametric methods between genotypes at specific time points and for the area under the IL1RA curve, determined using the trapezoidal rule. Additive (nonparametric trend) and dominant (Wilcoxon rank sum) genetic models were assessed. We adopted a dominant model because there were only 14 homozygous rs315952CC subjects, limiting power in this stratum. To analyze the data across all time points, accounting for large variation in concentration by endotoxin stimulus and for repeated measures within each individual, we quantile-transformed data at each time point and used a linear mixed effects model with an individual-specific random effect to control for the correlations among repeated measures within individuals (35). Analyses were separate by genetic ancestry given significant differences in *IL1RN* gene structure (36). To test whether our results were influenced by genetic ancestral substructure, we included the first three principal components of genetic ancestry determination along with covariates sex and body mass index in a quantile regression model for peak IL1RA response. We also regressed transformed plasma IL1RA levels on all polymorphic typed SNPs (n = 18) within 1 kb of the *IL1RN* gene to assess whether the determinants of baseline and evoked IL1RA were distinct.

We used RNA-seq to analyze for allelic imbalance (AI) by quantifying transcription from both paternal and maternal haplotypes using individuals that are heterozygous at the test SNP (37). To test for AI, we compared the number of RNA-seq reads with C versus T allele by a one degree of freedom chi-square goodness of fit test, with the null hypothesis being that 50% of the reads would contain each allele at this locus.

In VASST, survival analysis was performed by Cox proportional hazards methods. Association between genotype and mortality was tested by chi-square and logistic regression, and between genotype and continuous outcomes by linear regression, assuming an additive genetic model and adjusting for APACHE II score and the first three principal components of genetic ancestry determination. Analyses were restricted to EA given *IL1RN* gene structure and the low numbers of non-EA subjects. Plasma levels were inspected by genotype and analyzed by additive model using analysis of variance with Bonferroni adjustment, or by recessive model using Student *t* test, on log-transformed values. A recessive model was evaluated given the observed data distribution. For all analyses, a two-sided *P* value less than 0.05 was considered significant. Additional details including genotyping quality assurances, determination of genetic ancestry, ELISA and multiplex assay characteristics, and power considerations are provided in the online supplement.

Results

IL1RN Variation and the Response to Intravenous Endotoxin

The GENE population is described in Table 1. Because our prior associations with rs315952 were present only for EA subjects (12), we initially focused on this population. The SNP displayed Hardy-Weinberg equilibrium ($P = 0.69$) and its observed minor allele frequency was 0.27, comparable with HapMap EA populations (36). At baseline, EA carriers of rs315952C had slightly increased plasma IL1RA, and this effect was magnified post-LPS (Table 2), with a peak additive effect at 4 hours (Figure 1). Peak IL1RA response remained associated with rs315952 when adjusting for the first three principal components of genetic ancestry ($P = 0.008$). At 24 hours post-LPS, IL1RA levels remained significantly associated with rs315952C. In a repeated measures mixed effects model conditioned on the individual (Figure 2), rs315952C was associated with increased IL1RA levels whether analyzed by additive ($P = 0.049$) or dominant ($P = 0.023$) models of genetic risk. In a *cis* quantitative trait locus analysis considering 18 genotyped loci falling in the *IL1RN* region, rs315952 ranked highest for peak response, third for area under the curve, and 18th for baseline IL1RA (*see* Table E1 in the online supplement).

We tested previously reported *IL1RN* variants for association with baseline and evoked IL1RA. The *IL1RN* promoter SNP rs4251961, previously associated with lower IL1RA levels (21, 23), associated with lower baseline IL1RA levels but not with altered peak IL1RA response or area under the IL1RA curve (*see* Table E2). In contrast, rs419598, a tag for *IL1RN*2* with perfect linkage disequilibrium in European populations ($r^2 = 1.0$) (12, 38), showed no association with baseline or evoked IL1RA (*see* Table E3). As shown in Table E1, the local determinants of LPS-evoked plasma IL1RA were highly distinct from those determining baseline plasma IL1RA. Results for AA GENE subjects (n = 94) are presented in the online supplement (*see* Table E4). No association between rs315952C and plasma IL1RA levels was observed in AA subjects.

Because rs315952C is a synonymous coding SNP in the terminal exon of *IL1RN* and not previously shown to fall within a transcription factor binding site (39, 40), we hypothesized that AI might be the genetic mechanism responsible for higher plasma IL1RA levels (37). Using RNA obtained from adipose biopsy at baseline and 4 hours post-LPS in nine subjects heterozygous for rs315952C/T (chr2:113890304), we performed RNA sequencing (RNA-seq) at a median read depth of 414 million (range, 298–492 million) reads per sample. Results for EA subjects are shown in Table 3. Of nine

Table 1. GENE Population Clinical Characteristics Stratified by Genotype

	TT (*n = 122*)	CT (*n = 115*)	CC (*n = 35*)	*P* Value
Age	25.4 ± 6.7	26.1 ± 6.7	26.5 ± 7.2	0.33
Female sex	55 (47.8%)	16 (45.7%)	19 (76.0%)	0.33
Body mass index	23.7 ± 2.8	24.1 ± 3.0	23.4 ± 2.8	0.87
African ancestry	28 (23.0%)	47 (40.9%)	21 (60.0%)	<0.001
European ancestry	94 (77.1%)	68 (59.1%)	14 (40.0%)	

Definition of abbreviation: GENE = Genetics of Evoked response to Niacin and Endotoxemia. Values shown are mean ± standard deviation or number (proportion). Groups were compared in an additive fashion by linear regression for continuous variables and logistic regression for categorical ones.

Table 2. rs315952C Is Associated with Higher Baseline and Evoked IL1RA Post-LPS in European Ancestry Subjects

	TT (*n* = 93)	CT (*n* = 68)	CC (*n* = 14)	*P* Value	Adjusted *P* Value*
Additive model					
Baseline IL1RA, pg/ml	101.8 (84.5–146.6)	120.1 (98.0–157.8)	108.07 (87.2–141.9)	0.12	0.073
IL1RA at 4 h, ng/ml†	39.4 (21.5–63.1)	50.9 (28.8–68.3)	60.1 (14.8–75.5)	0.064	0.008
IL1RA at 24 h, pg/ml	232.8 (183.0–286.1)	272.2 (221.7–338.1)	232.2 (184.6–347.9)	0.064	0.016
AUC IL1RA, ng/ml†	51.4 (26.9–86.1)	64.3 (38.2–90.8)	74.5 (21.5–99.3)	0.11	0.013
Dominant model					
Baseline IL1RA, pg/ml	101.8 (84.5–146.6)	117.3 (94.5–157.4)		0.032	0.039
IL1RA at 4 h, ng/ml†	39.4 (21.5–63.1)	52.2 (28.8–68.8)		0.044	0.011
IL1RA at 24 h, pg/ml	232.8 (183.0–286.1)	268.6 (216.8–338.8)		0.009	0.015
AUC IL1RA, ng/ml†	51.4 (26.9–86.1)	66.0 (37.9–92.4)		0.075	0.026

Definition of abbreviation: AUC = area under the IL1RA curve.
Median (interquartile range) values are shown. Peak IL1RA response was at 4 hours post-LPS, and no shift in peak response was observed by genotype. Given the low number of homozygous CC individuals, we collapsed CT and CC for a dominant model and analyzed by rank sum test or quantile regression adjusting for sex and body mass index.
*Additive genetic models were tested by nonparametric trend and adjusted for sex, body mass index, and the first three components of genetic ancestry using quantile regression.
†Change in scale to ng/ml for peak response and area under the IL1RA curve.

heterozygous subjects, five had fewer than 20 reads at this locus and were excluded, leaving two EA subjects and two AA subjects (*see* Table E5) for the AI analysis. As anticipated, *IL1RN* was up-regulated in adipose post-LPS, with log2(fold change) = 1.71, $P = 5.0 \times 10^{-5}$. Furthermore, in both EA subjects, rs315952 demonstrated strong AI favoring the C allele at baseline and this increased post-LPS, with the most dramatic instance being a 80–20% imbalance post-LPS ($P = 2.1 \times 10^{-36}$).

rs315952 in the VASST Septic Shock Cohort

Characteristics of the VASST cohort subjects with available genotyping are shown in Table 4; rs315952 displayed Hardy-Weinberg equilibrium ($P = 0.86$). Nonsurvivors of septic shock were older and had higher APACHE II scores in addition to other organ failures. Baseline clinical characteristics were largely similar across genotype groups with CC homozygotes being slightly younger and displaying lower APACHE II scores, as shown in Table E6.

Survival curves for EA subjects stratified by rs315952 genotype are shown in Figure 3. The rs315952 genotype satisfied the proportional-hazards assumption (Schoenfeld residual test, $P = 0.53$) and demonstrated a reduced hazard of death with increasing copies of the C allele (hazard ratio, 0.80; 95% confidence interval, 0.65–0.99; $P = 0.038$). This result was unchanged by adjustment for APACHE II score and for the first three components of the genetic ancestry multidimensional scaling analysis (hazard ratio, 0.79; 95% confidence interval, 0.64–0.98; $P = 0.028$). In addition, rs315952C was associated with decreased 90-day adjusted mortality (hazard ratio, 0.75; 95% confidence interval, 0.51–0.99; $P = 0.044$) and increased days alive and free of cardiovascular system failure ($P = 0.041$) (*see* Table E7). Ventilator-free days were higher ($P = 0.061$) with increasing copies of the rs315952C allele, although this result was not statistically significant (*see* Table E7). Eighty SNPs within 1 kb of *IL1RN* were genotyped on the Illumina 1M platform (24). We performed logistic regression of 90-day mortality with all 80 *IL1RN* SNPs assuming an additive model of genetic risk, and rs315952C was the fifth most significantly associated *P* value, at $P = 0.057$, as displayed in Figure E1.

Initial plasma levels of IL-1β and IL1RA were available for 399 subjects, 51% of the overall cohort (*see* Table E8). Plasma IL-1β ($P = 0.038$) was lower for homozygous carriers of rs315952CC, whereas we were unable to demonstrate significant difference in plasma IL1RA ($P = 0.19$). The pair-wise correlation between plasma IL1RA and IL-1β levels was very strong, with $r^2 = 0.90$.

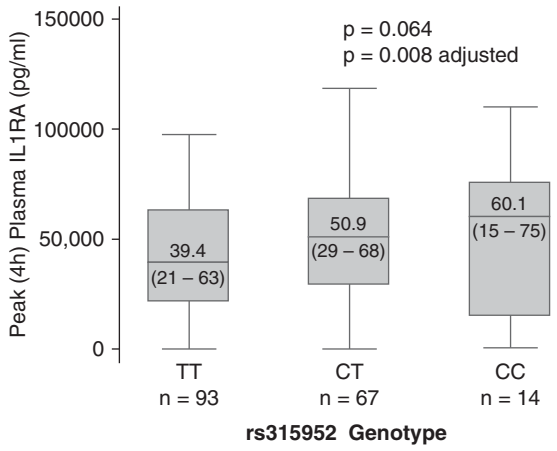


Figure 1. Peak LPS-evoked plasma IL1RA increases with increasing copies of rs315952C. Adjusted for sex and body mass index.

Discussion

Variation of genetic structure across human ancestral populations has been shown to

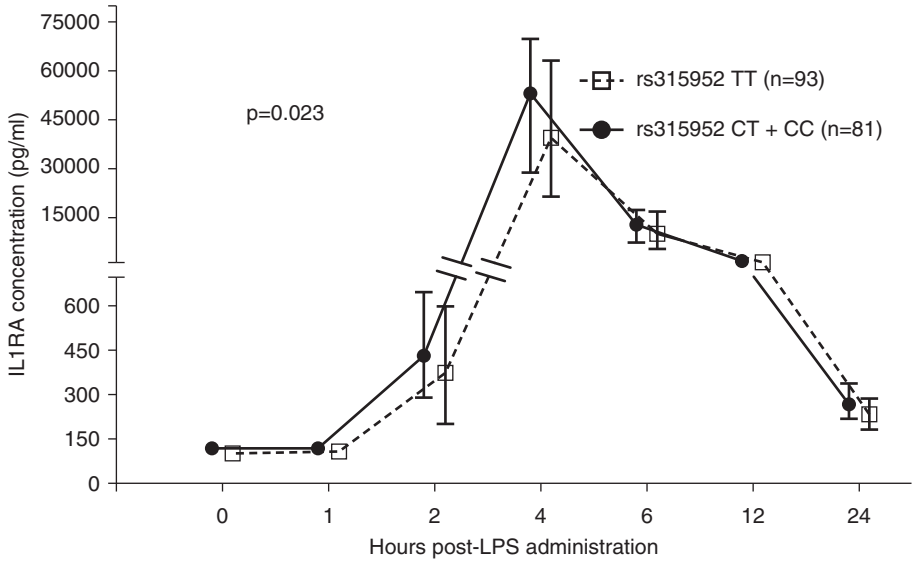


Figure 2. Carriers of rs315952C have increased LPS-evoked plasma IL1RA levels. At each time point, the median (*dot* or *square*) and interquartile range (*whiskers*) are shown, stratified by rs315952C allele carriage. The *y* axis is interrupted to allow discrimination at the earlier time points, when the concentration of plasma IL1RA was 100-fold lower. Repeated measures analysis was performed by linear mixed effects model with an individual-specific random effect and with quantile transformation of the data at each time point.

bear strong marks of selection, where evolutionary pressures have maintained the presence of variant alleles at increased frequency (41–45). Cytokine genes have notably diverse genetic structures across global populations (46), which may be attributable to evolutionary forces including injury or infection shaping human genetic diversity. It may be that by understanding genetic risk factors for death during sepsis, we will uncover sepsis endotypes that may have unique pathophysiology or may respond differentially to specific therapy. We investigated whether rs315952C may identify a genetically determined endotype of septic shock with hypothesized more efficient transcription of *IL1RN*, higher plasma IL1RA, and improved survival.

To study the genetic contribution to evoked plasma IL1RA more precisely, we turned to an experimental model of inflammatory stress with intravenous LPS, ensuring that each subject received an identical stimulus. Low-dose LPS reliably induced a 100-fold increase in evoked plasma IL1RA. In this system, we replicated the association between rs315952C and increased plasma IL1RA levels (47), and this effect was most pronounced at the peak inflammatory response compared with baseline. Interestingly, the previously studied genetic variants affecting IL1RA response, rs4251961 and the VNTR tagged by rs419598, did not influence peak evoked IL1RA, and only rs4251961 associated with resting plasma IL1RA levels. We interpret these results as implicating rs315952 as an important locus for regulating evoked inflammation, such as might occur during septic shock.

Our RNA sequencing analyses implicate adipose tissue AI favoring transcription of the C allele in EA individuals and suggest that rs315952 is a functional SNP. Adipose tissue strongly expresses *IL1RN* and is a significant source of plasma IL1RA (48). Body mass index explains a significant proportion of the observed variance in baseline plasma IL1RA (27, 47). In addition, adipose explants secrete IL1RA in response to LPS (48), making adipose a relevant tissue to investigate. However, our group has previously shown dramatic tissue specificity to basal and LPS-evoked gene expression (34), whereas others have shown tissue specificity to AI responses within the same individual (49, 50). The AI that we detected in two EA individuals was statistically impressive, yet the sample size is small. In the future, it will be important to test for similar imbalance in transcription in monocytes and neutrophils, cell types we believe to substantially contribute to plasma IL1RA during sepsis, and to confirm these findings in a larger population. Indeed, optimally such work would test for AI in leukocytes harvested directly from patients with septic shock, and would confirm more efficient *IL1RN* transcription and higher plasma IL1RA levels in comparably timed blood samples.

To confirm the significance of rs315952C as a functional variant, we tested the SNP's association with outcomes following septic shock. In the VASST cohort, we demonstrate an association between carriage of the C allele and improved survival, and faster resolution of shock, a direction of effect consistent with our prior results (12). We also report for

Table 3. RNA-Seq Analysis at rs315952 (chr2:113890304) in Adipose Tissue for Heterozygous European Ancestry Subjects Indicates Strong Allelic Imbalance Favoring the C Allele

	Pre-LPS				Post-LPS			
	C Allele Counts	T Allele Counts	Proportion C Reads	*P* Value	C Allele Counts	T Allele Counts	Proportion C Reads	*P* Value
A	112	36	**0.7568**	4.18×10^{-10}	358	91	**0.7973**	2.10×10^{-36}
B	163	98	**0.6245**	5.74×10^{-5}	289	198	**0.5934**	3.73×10^{-5}

Of four European Ancestry subjects with adipose RNA and heterozygous (C/T) at rs315952, only subjects "A" and "B" met our filtering criteria of greater than or equal to 20 reads at this locus. The expected proportion of reads containing the C allele was 0.50, or 50%. Both subjects demonstrated strong allelic imbalance favoring the C allele (bold) both at rest and post-LPS.

Table 4. Characteristics of the VASST Population with Available DNA for Genotyping

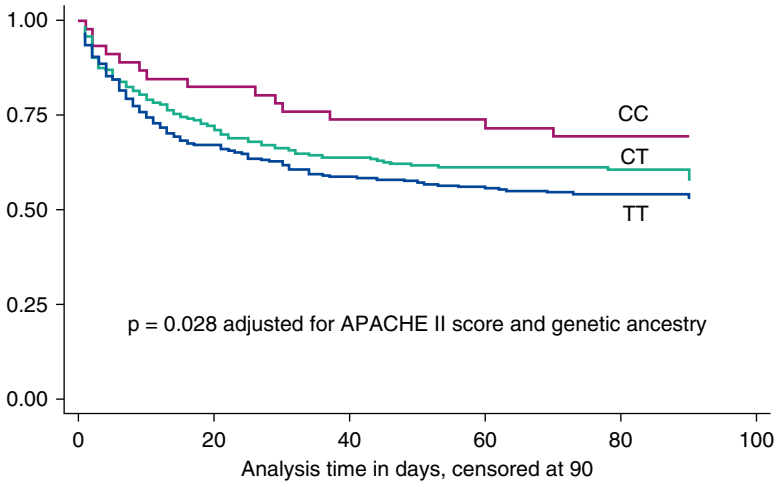
	90-d Nonsurvivors (*n* = *287*)	90-d Survivors (*n* = *345*)	*P* Value
Age	63.5 ± 16	57.7 ± 17	<0.001
Female	118 (41.1%)	144 (41.7%)	0.72
Ancestry			
European	232 (80.8%)	298 (86.4%)	0.065
Asian	24 (8.4%)	17 (4.9%)	0.10
African	9 (3.1%)	5 (1.5%)	0.18
Site of infection			
Lung	129 (45.0%)	147 (42.6%)	0.56
Abdomen	73 (25.4%)	97 (28.1%)	0.39
Other	85 (29.6%)	101 (29.3%)	
Infectious pathogen			
Gram-positive bacteria	79 (27.5%)	110 (31.9%)	0.32
Gram-negative bacteria	55 (19.2%)	83 (24.1%)	0.19
Fungal or viral	38 (13.2%)	41 (11.9%)	0.40
Not identified	131 (46.8%)	143 (41.5%)	0.18
APACHE II	28.0 ± 9	25.3 ± 7	<0.001
Randomized to vasopressin	141 (49.1%)	185 (53.6%)	0.26
Plasma available	164 (57%)	235 (68%)	0.004
Acute organ failure			
Lung	286 (99.7%)	334 (96.8%)	0.032
Kidney	246 (85.7%)	161 (46.7%)	<0.001
Liver	236 (82.2%)	121 (35.1%)	<0.001
Coagulation	269 (96.4%)	289 (87.3%)	<0.001
Central nervous system	269 (93.7%)	289 (83.8%)	<0.001
Days alive and free of vasopressors, 28 d	0 (0–9)	23 (19–25)	<0.001
Days alive and free of ventilator, 28 d	0 (0–4)	18 (10–23)	<0.001

Definition of abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation; VASST = Vasopressin and Septic Shock Trial.
Variables are displayed as mean ± standard deviation, median (25–75 percentile range), or as number (percentage). Site of infection and infectious pathogen were not exclusive and thus the total may exceed 100%. Acute organ failures and organ failure–free days were defined by Brussels criteria within the first 28 days. Comparisons were made by *t* test, Wilcoxon rank sum test, chi-square, or Fisher exact test as appropriate. PF ratio = ratio of Pa_{O_2} to $F_{I_{O_2}}$.

the first time that homozygous carriers of rs315952C (CC) demonstrate lower plasma IL-1β level, consistent with the hypothesis that reduced IL-1β–driven signaling may improve outcomes during sepsis.

We did not identify a consistent relationship between rs315952C and plasma IL1RA in VASST, in contrast to the findings in GENE and in our and other's prior publications (12, 47). Although this was surprising, it may be that the timing of plasma draw in VASST, with a median of 12-hour duration of septic shock (30), a late manifestation of a severe systemic inflammatory response, influenced results. Our previous report used plasma primarily drawn in the emergency department with either severe sepsis or severe trauma (12). If the C allele of rs315952 is more efficiently transcribed, as our RNA-seq data suggest, it may be that earlier up-regulation of IL1RA acts as a brake on subsequent IL-1β–driven self-induction (51), and might explain a lower IL-1β and IL1RA level observed at a later period because IL-1β also regulates IL1RA (14, 52). Previous human and primate studies report that exogenous IL1RA therapy lowers plasma IL-1β and even IL-6 levels, demonstrating the potential for IL1RA to have a broad-based modulation of the cytokine inflammatory response (53, 54). Although this is an attractive theory, it does not explain the observed differences between homozygous and heterozygous carriers of the C allele, because one would predict that each C allele would lower IL-1β in an additive fashion. Several other differences in the populations studied may account for the observed results, including differences between a clinical trial population and observational cohorts, differences between trauma- and sepsis-induced inflammatory responses, or between sepsis and septic shock. Ideally, we would use a prospective cohort of patients with sepsis with uniform repeat blood and leukocyte mRNA sampling to replicate the associations observed in GENE.

Our results in both the GENE and VASST populations implicate rs315952 as a variant modifying IL1RA response and septic outcomes in EA populations, with attenuated or no effect in non-EA subgroups. In GENE, AA subjects demonstrated no association between rs315952C and plasma IL1RA levels, and AI at the SNP is either absent or slightly favors the T allele. It may be that we were underpowered to detect a difference in AA populations, but it is also striking how the AI analysis yielded very discordant results for the two ancestries. Although our data suggest this locus is a *cis* regulatory variant or enhancer in EA subjects (49), the region is not an area of known enhancer function by the VISTA Enhancer database (55), nor is it predicted to be a transcription factor binding site by the ChIP-seq experiments performed by the Encyclopedia of DNA Elements project (39). A potential explanation for how ancestral *IL1RN* gene structure might result in different allele-specific results at our locus would be that rs315952 alters binding of the CCCTC-binding factor, a regulator of chromatin and transcription factor binding. Prior work has established that CCCTC-binding factor binding can vary in an allele-specific manner (56–58), and the divergent linkage disequilibrium across chromosome 2q13 in ancestral populations might influence overall conformation in a population-specific



Number at risk

TT:	263	177	155	148	143	0
CT:	217	157	139	133	132	0
CC:	46	38	34	34	32	0

Figure 3. By Cox proportional hazards regression, rs315952C is associated with improved 90-day survival among European ancestry subjects of the Vasopressin and Septic Shock Trial cohort in an additive fashion ($P = 0.038$ unadjusted, $P = 0.028$ adjusted for Acute Physiology and Chronic Health Evaluation [APACHE] II score and genetic ancestry). The *y* axis indicates the proportion alive. *Magenta line* = CC homozygotes; *green line* = CT heterozygotes; *blue line* = TT homozygotes.

manner. Additional studies to understand chromosome conformation at this locus will be important to pursue.

Our investigations had some limitations. We performed multiple tests in the GENE population centered on the hypothesis that rs315952 would associate with increased evoked IL1RA, and our multiple testing may have inflated the overall type I error. The associations we observed between rs315952 and plasma IL1RA levels would not withstand a stringent Bonferroni adjustment for multiple testing; however, they are not truly independent tests. Because the analyses were conducted in a previously completed observational study and clinical trial, our sample size was fixed and may only provide limited power to detect modest genetic effects on complex traits. In GENE, we were powered to detect a difference in half of one standard deviation in means for plasma IL1RA, whereas in VASST, our minimal detectable relative risk for genotype on mortality was 1.49 (59). For both populations, these are moderate to large effect sizes. Our RNA-seq data, while compelling, involved a small number of individuals. Our power limitation was more potent in the AA population in the GENE study, and the VASST cohort lacked sufficient non-EA subjects to make inferences in African or Asian populations. In addition, we acknowledge the inability of a low-dose LPS injection to precisely model the complexity of septic shock, yet we believe the controlled nature of the LPS challenge is ideal for studying evoked response to a uniform stimulus. Furthermore, there are ample data that the inflammatory response to severe infections and to LPS share many features (60–63). We attempted to measure IL-1β in the plasma of all GENE subjects but for many subjects, the level of plasma IL-1β seemed to be at the limits of detection at peak response (4 h), and undetectable at other times. Thus, we were unable to test whether increased peak IL1RA response with rs315952C results in lower IL-1β post-LPS.

Previous clinical trials of recombinant IL1RA, anakinra, for severe sepsis failed to achieve a large reduction in mortality (54, 64, 65). Given the associations of rs315952C with improved survival in the VASST cohort, with potential preferential transcription, and with increased evoked plasma IL1RA, attenuating IL-1β remains an attractive potential treatment paradigm, particularly if it were possible to predict which patients might be more likely to respond to such an intervention. Anakinra may display pharmacogenomic variation in response to treatment for rheumatoid arthritis (66); it is unknown whether response to this drug when used to decrease mortality in sepsis may also have varied by genotype. Beyond genetic variation, it may be that clinical factors result in sepsis endotypes that differ in their degree of inflammasome activation (6). Alternatively, a more successful intervention may need to combine anti–IL-1β therapy with strategies to block IL-18 (67, 68), or to mitigate vascular permeability (69). Despite recent disappointments in the ability of pharmacologic interventions to improve sepsis mortality (70), we remain optimistic that with improved understanding of sepsis endotypes, effective therapies may yet emerge. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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