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Toll-like Receptor 1 Polymorphisms and Associated Outcomes in Sepsis Following Traumatic Injury, A Candidate Gene Association Study

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Abstract

Objective—To determine if SNPs in *TLR1* are associated with mortality, specifically sepsis-associated mortality, in a traumatically injured population.

Summary Background Data—Innate immune responses mediated by toll-like receptors (TLRs), induce early inflammatory responses to pathogen and damage-associated molecular patterns. Genetic variation in TLRs has been associated with susceptibility and outcomes in a number of infectious and non-infectious disease states.

Methods—Patients admitted to the trauma intensive care unit at a level one trauma center serving 4 states were enrolled and followed for development of infection, sepsis, and death. Genomic DNA was genotyped and logistic regression analysis performed to determine associations between *TLR1* SNPs and mortality. We further examined for associations between *TLR1* SNPs and mortality in subgroups based on the presence of sepsis and the type of sepsis-associated organism.

Results—We enrolled 1,961 patients. *TLR1*.7202G (*rs5743551*) was associated with increased mortality after traumatic injury and this association was primarily observed in the subset of patients who developed sepsis (adjusted OR 3.16; 95% CI 1.43–6.97, *P*=0.004). This association persisted after further restriction to gram-positive sepsis. *TLR1*742A/G(*Asn248Ser*) (*rs4833095*), a coding SNP in LD with *TLR1*.7202G was also associated with mortality in gram-positive sepsis (adjusted OR 4.16; 95% CI 1.22–14.19, *P*=0.023).

Conclusion—Genetic variation in *TLR1* is associated with increased mortality in patients with sepsis following traumatic injury and may represent a novel marker of risk for death in critically injured patients.

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INTRODUCTION

Genetic variation in the form of single nucleotide polymorphisms [SNPs] has been associated with both the risk for and outcomes from infection in critically ill patients.[1–4] Studies have shown that genetic variation predisposing to increased inflammation may alter outcomes in sepsis both in laboratory models and clinical cohorts.[5, 6] One example of this paradigm involves the gene for toll-like receptor 1 (*TLR1*). Two SNPs in *TLR1* [*TLR1*-7202A/G (rs5743551) and *TLR1*1804G/T (rs5743618)] have previously been shown to confer higher innate immune inflammatory responses to Pam3CysSerLys4 (Pam3CSK4), a synthetic analog of bacterial lipoproteins and specific ligand for TLR1/TLR2 heterodimers. [1] These SNPs have also been associated with higher mortality, worse organ dysfunction, and higher prevalence of gram positive infection in sepsis cases in a cohort of patients admitted to an intensive care unit with primarily non-surgical illness.[1] In patients with traumatic or thermal injury, candidate gene studies have linked other components of the innate immune system with risk of sepsis and mortality including lipopolysaccharide binding protein (*LBP*), toll-like receptor 4 (*TLR4*), and tumor necrosis factor-alpha (*TNF-α*). [3, 7, 8] In addition to their role in host defense through the recognition of pathogen associated molecular pattern (PAMP) responses, TLRs have also been shown to recognize endogenous ligands that are released in response to cellular necrosis or tissue damage. These endogenous activators are termed damage associated molecular patterns (DAMPs) and have been implicated in a wide variety of clinical illnesses (Reviewed in [9, 10]) including hemorrhagic shock in trauma.[11, 12] Thus, variation in genes of innate immunity could influence outcomes in critically injured patients in a variety of ways. In the study reported here, we sought to determine if SNPs in *TLR1* are associated with mortality in patients who have been traumatically injured. Specifically, because TLR1 is thought to be involved in the innate immune response to infection through the recognition of PAMPs[13, 14] we sought to identify associations between SNPs in *TLR1* and sepsis-associated mortality after traumatic injury. We also examined whether variants in *TLR1* might have a differential effect in the presence of gram-positive sepsis given that prior reports have associated *TLR1* variants with increased prevalence of gram-positive infection.[1] The results presented here show that variation in *TLR1* may play a role in modulating risk of death in trauma-related sepsis.

METHODS

Patient Recruitment, Data Collection, and Definitions

Injured patients admitted to the trauma ICU at Harborview Medical Center in Seattle, Washington were enrolled during the period of August 2003 to December 2005 with approval of the Institutional Review Board. Demographic and clinical data were collected from the electronic medical record and the trauma registry as previously described.[8] A patient was classified as severely injured if their injury severity score (ISS) was greater than or equal to 16. Severe head, thoracic or abdominal traumatic injury was defined as an abbreviated injury scale (AIS) score of greater than or equal to 3. Incident cases of sepsis were identified through chart abstraction according to the guidelines of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Committee.[15] Cultures were drawn at the discretion of the managing physicians for clinical suspicion of infection. Positive cultures from blood, urine, lower respiratory tract, tissue, catheter tips, cerebral spinal fluid and wounds were considered clinically significant if the quantitative count of the culture was >100,000 CFU/ml for urine and >10,000 CFU/ml for lower respiratory, or if a true quantitative count was unavailable, a value of >2+ as reported by our laboratory. For patients with multiple cultures, we used the first significant culture of gram positive and/or non-gram positive organisms occurring after admission to the ICU. Patients were classified as having acute respiratory distress syndrome (ARDS) if they had acute

onset of a PaO₂/FiO₂ < 200, bilateral infiltrates consistent with pulmonary edema on chest x-ray, the need for positive pressure ventilation via endotracheal tube, and no clinical evidence of left atrial hypertension all occurring within the same 24-hour interval. Patients were classified as having acute kidney injury (AKI) if they met stage 2 or 3 AKIN criteria. [16]

Genotyping

We genotyped three SNPs in *TLRI* in a blinded fashion, *TLRI*_{7202A/G} (rs5743551) found in the 5' non-coding region, *TLRI*_{1804G/T} (Ser602Ile) (rs5743618) found adjacent to the predicted transmembrane domain, and *TLRI*_{742A/G} (Asn248Ser) (rs4833095), a non-synonymous SNP within the extracellular domain of *TLRI*. These SNPs were chosen based on existing literature indicating associations with outcomes in critically ill patients. Genotypes were determined by TaqMan-based real-time polymerase chain reaction (RT-PCR) for rs5743551 and rs4833095 as previously described.[17] Genotyping for rs5743618 used a restriction fragment length polymorphism introduced by this SNP. Briefly, a 1334 bp fragment containing this SNP was amplified from genomic DNA, then digested with the restriction enzyme PstI (Thermo Scientific) and the resulting fragments were visualized by gel electrophoresis; "GG" subjects were expected to have two bands at 457bp and 877bp "TT" subjects were expected to have a single band at 1334bp, and "GT" subjects were expected to have all three bands. We included technical replicates on approximately two percent of samples.

Statistical Analysis

Categorical data are presented as counts and percentages. Continuous data are presented as medians and interquartile ranges (IQR). Categorical data were compared by χ^2 analysis and continuous data were analyzed using Student's t-test. For each comparison, the exact *P* values are reported and were considered significant if *P*<0.05. Observed genotype frequencies were compared with expected frequencies to test for deviations from Hardy-Weinberg equilibrium. The primary outcome for this study was in-hospital mortality. The secondary analyses included sepsis-associated mortality, susceptibility to sepsis, sepsis severity, ARDS in sepsis, AKI in sepsis and gram-positive sepsis-associated mortality. We used multiple logistic regression analysis to test for associations between *TLRI* genotypes and mortality. We tested recessive and co-dominant models that were adjusted for identified confounding variables of age, ISS, head AIS, and blood transfusion. Given that we tested two different models per genotype we assigned statistical significance to tests achieving *P*<0.025. We carried out these analyses in the entire cohort as well as subgroups of patients with or without sepsis, and with or without gram-positive sepsis. To minimize the risk of confounding due to racial differences in SNP frequency, we limited our analysis to Caucasian patients. Data were analyzed using Stata 12 Statistical Software (StataCorp LP, College Station, TX). A power analysis was performed using CaTS power calculator from the University of Michigan Center for Statistical Genetics (<http://csg.sph.umich.edu/>).[18]

RESULTS

Demographics and Clinical Outcomes

A total of 1,961 subjects were enrolled and genotyped. Of these, 1,498 (77%) were Caucasian and were included in our analysis. Demographic and outcomes data for these patients are summarized in Table 1. The majority of patients (70%) were male. The most common cause of injury was blunt trauma occurring in 1,341 patients (90%). The majority of patients were severely injured with a median ISS of 22 (IQR, 16–29). Median ICU length of stay was 5 days (2–12) and median hospital length of stay was 12 days (7–22). Sepsis

developed in 691 patients (46%) with 316 patients developing severe sepsis or septic shock (21%).

A power analysis was performed *post-hoc* and we estimated that using a recessive model our study would have adequate statistical power ($1-\beta > 0.8$) to detect a strong association with sepsis-associated mortality (odds ratio, OR 2.8) with SNPs having a minor allele frequency (MAF) of > 0.2 . Assuming a co-dominant model, we would have adequate power to detect a moderate association (OR 1.5) with SNPs having a MAF of > 0.2 .

Clinical Risk Factors for Mortality

In-hospital mortality was 10% (151 patients). Compared to those who survived, those who died were older, had higher injury severity scores, higher head AIS scores, and had more blood transfusions ($P < 0.001$), these identified covariates were subsequently used in our multiple logistic regression analysis and are summarized in table 2. Sepsis-associated mortality was 5.5% (83 patients). There were no differences in rates of laparotomy, thoracotomy or operative fracture fixation between the patients with sepsis that died compared to those who survived (data not shown).

There were a total of 4,313 positive cultures recorded for the 1,961 subjects; most subjects had more than one positive culture during their hospital stay. Among the subjects who developed sepsis there were 523 with clinically significant cultures; 371 patients had cultures of gram-positive organisms (54% of patients with sepsis) and 342 patients had cultures of gram-negative organisms (49% of patients with sepsis). For the gram-positive cultures, the most common microorganism identified was *Staphylococcus aureus* and the most common source of the culture was the lower respiratory tract. A summary of the clinically significant gram-positive infections by species and source for the patients with sepsis can be found in supplemental table 1. For the gram-negative cultures, the most common microorganism identified was *Escherichia coli* and the most common source was again the lower respiratory tract. A summary of the clinically significant gram-negative culture data for the patients with sepsis can be found in supplemental table 2.

TLR1 SNPs Are Associated with Higher Mortality After Traumatic Injury

*TLR1*_{-7202A/G} was successfully genotyped in 1,426 patients (95%) and *TLR1*_{1804G/T} was genotyped in 1,406 patients (94%). The genotype frequencies of both SNPs were consistent with Hardy-Weinberg equilibrium (Table 3). Table 3 illustrates the SNP genotype frequencies according to survival status. After adjusting for age, ISS, head AIS, and packed red blood cell (PRBC) transfusion, we found an association between *TLR1*_{-7202G} and in-hospital mortality when we assumed a recessive effect of the allele (adjusted odds ratio [OR], 2.41; $P = 0.006$). We did not see a significant association between homozygosity for *TLR1*_{1804T} and in-hospital mortality (OR, 1.68; $P = 0.06$). Using a co-dominant model, we saw an association between *TLR1*_{-7202G} and in-hospital mortality (OR, 1.46; $P = 0.01$) and again we did not see an association between *TLR1*_{1804T} and in-hospital mortality (data not shown). In subsequent analyses we assumed a recessive effect of the variants on the phenotype because that is where we saw the strongest associations, consistent with prior reports.[1]

TLR1 SNPs Are Associated with Higher Mortality with Sepsis After Traumatic Injury

Given the role TLR1 is thought to play in the innate immune response to infection we next investigated the degree to which associations between genetic variation in *TLR1* and trauma-related mortality persisted when the analysis was restricted to patients who developed sepsis. Table 4 illustrates the SNP genotype frequencies in patients with sepsis according to their survival status. We found an association between homozygosity for *TLR1*_{-7202G} and

mortality with sepsis (adjusted odds ratio [OR], 3.16; $P=0.004$). We also saw an association between homozygosity for *TLR1*_{1804T} and mortality with sepsis (OR, 2.48; $P=0.008$). Notably, we observed no significant association between *TLR1* genetic variation and mortality in patients without sepsis (Supplemental table 3). In addition, we did not identify associations between any of the *TLR1* polymorphisms and susceptibility to sepsis (Adjusted risk for sepsis with *TLR1*_{-7202A/G}: 1.34 (95% CI 0.85–2.10; $P=0.207$ and for *TLR1*_{1804G/T}: 1.13 95% CI 0.78–1.64; $P=0.517$) consistent with prior findings [1].

In secondary analyses we explored whether *TLR1* variants were associated with common forms of organ dysfunction in sepsis, Acute Respiratory Distress Syndrome (ARDS) and Acute Kidney Injury (AKI). The incidence of ARDS in sepsis was 43.9% and the incidence of AKI in sepsis was 46.3%. We did not identify significant associations between the *TLR1* polymorphisms and ARDS (adjusted risk for ARDS in sepsis with *TLR1*_{-7202G}: 1.53 (95% CI 0.82–2.8; $P=0.178$ and for *TLR1*_{1804T}: 1.21 95% CI 0.71–2.1; $P=0.486$) or AKI (adjusted risk for AKI in sepsis with *TLR1*_{-7202G}: 1.33 (95% CI 0.71–2.5; $P=0.371$ and for *TLR1*_{1804T}: 1.2 95% CI 0.7–2.1; $P=0.508$) in the patients with sepsis; though there were trends towards a higher incidence of ARDS and AKI in *TLR1*_{-7202G} and *TLR1*_{1804T} homozygotes (Table 5).

TLR1 forms a heterodimer with *TLR2* at the cell surface and this dimer is thought to specifically play a role in innate immune host responses to gram-positive infection.[13, 19, 20] In light of this biology, we next tested for associations between variation in *TLR1* and mortality in patients with gram-positive sepsis. Three hundred fifty three (24.8%) of the 1,394 patients genotyped for *TLR1*_{-7202A/G} had gram-positive sepsis. We identified a greater than 4-fold increased risk of death in gram-positive sepsis among *TLR1*_{-7202G} homozygotes (Table 6 $P=0.013$). For patients homozygous for *TLR1*_{1804T} we did not identify an association with mortality in gram-positive sepsis. Of note, we found no association between any of the *TLR1* genetic variations and mortality in patients with non-gram positive sepsis (Supplemental table 4).

Nonsynonymous *TLR1* SNP is Associated with Mortality in Gram Positive Sepsis

Given that we did not observe an association between *TLR1*_{1804G/T} a non-synonymous SNP, and mortality in gram-positive sepsis we sought additional coding variations that might explain the association seen with *TLR1*_{-7202A/G}. The non-synonymous SNP, rs4833095 [*TLR1*_{742A/G} (Asn248Ser)], is in significant LD ($R^2=0.93$) with *TLR1*_{-7202A/G} (Table 7). *TLR1*_{742A/G} was genotyped in 1,394 patients (93%, Table 8). Mortality in patients with gram positive sepsis was significantly higher in patients who were homozygous for the rare versus common variant (26.3% vs. 11.3%, $P=0.014$). In the adjusted analysis we observed a significant association of *TLR1*_{742G} with mortality in gram-positive sepsis (OR, 4.13, $P=0.023$).

DISCUSSION

Traumatic injury is the 5th leading cause of death in the United States and the leading cause of death among young adults.[21] The majority of these deaths occur immediately following the injurious event. Patients that survive the early time period after trauma are at risk of developing, and possibly dying from, sepsis and sepsis-associated multiple organ dysfunction and failure. Sepsis following traumatic injury is associated with greater ICU and hospital length of stay, more ventilator days, increased incidence of multiple organ failure and increased in-hospital mortality compared to those who do not develop sepsis. Despite decreases in both the incidence of sepsis and the overall in-hospital mortality of trauma patients, the mortality in trauma patients with sepsis has not changed.[22] The finding of decreased incidence of sepsis and decreased mortality in trauma patients is likely the result of improved management in the early post-trauma period, increased understanding of

clinical risk factors for sepsis in trauma patients and resultant improvements in management of these patients overall. However, the persistent incidence of mortality in trauma-related sepsis remains a troubling problem. Recently, interest has turned to the potential role for genetic variation in altering host susceptibility to sepsis-related outcomes. In this study we have addressed the question of whether genetic differences that affect leukocyte inflammatory response to bacterial products, and, potentially, DAMPs, might be associated with increased sepsis-related mortality in patients who have experienced major trauma.

The *TLR1*_*-7202A/G* SNP is located in the 5' untranslated region of the *TLR1* gene on chromosome 4 at the -7202 position relative to the start codon. *TLR1*_*1804G/T* and *TLR1*_*742A/G* are both located in coding regions of the *TLR1* gene. *TLR1*_*1804G/T* causes an amino acid change from serine to isoleucine in the region of the *TLR1* protein that has been predicted to be in the transmembrane domain.[23] *TLR1*_*742A/G* causes an amino acid change from asparagine to serine in a region predicted to be in the extracellular domain. Both the *TLR1*_*1804T* and *TLR1*_*742G* variants are predicted to be benign by PolyPhen-2[24] with scores of 0 and 0.001, respectively and to be tolerated by SIFT[25] analysis with scores of 0.40 and 0.50, respectively.

*TLR1*_*-7202G* has been associated with a hypermorphic effects on innate immune inflammatory responses *ex vivo* and has been associated with higher mortality, organ dysfunction and susceptibility to gram-positive infection in patients with severe sepsis.[1] *TLR1*_*1804T* is in high LD with *TLR1*_*-7202G* and has been shown to cause hyper-responsiveness to Pam₃CSK₄ in transient transfection assays. Healthy carriers of the G allele have increased cell surface expression of *TLR1* on peripheral blood monocytes. This polymorphism has also been associated with increased mortality in sepsis,[1] protective association against leprosy, [26] and protection from pyelonephritis.[27] *TLR1*_*742A/G* has been associated circulatory dysfunction in sepsis as well as with pulmonary infection among septic patients.[28] This same study also found that *TLR1*_*-7202A/G* and *TLR1*_*1804G/T* were associated with circulatory dysfunction in sepsis. Additionally, *TLR1*_*742A/G* has been associated with increased susceptibility to malaria in pregnancy,[29] and increased risk of IgA nephropathy in Korean children.[30] Taken together, these findings strongly suggest an important role for *TLR1* in modulating host responses to infection.

Our study provides an important extension of previous associations between *TLR1* SNPs and sepsis-related outcomes. In contrast to previous studies that used populations of patients primarily from medical ICUs for whom time of onset of sepsis is ill defined, this study used a traumatically injured cohort who suffered an inciting event at a known time and of a quantifiable severity. Thus, the interpretation of the observed associations is less likely to be influenced by unmeasured confounders. Furthermore, the initial inflammatory stimulus for most of these patients was non-infectious and, thus, we were able to test to what extent *TLR1* polymorphisms may influence outcomes in the presence or absence of severe infection/sepsis. We were able to show that the positive association seen between *TLR1*_*-7202A/G* and mortality in critically ill patients who sustained traumatic injury was driven by the subset of patients that had gram-positive sepsis as we did not observe a significant association in patients without sepsis or those with non-gram-positive sepsis. This study is the first to suggest that *TLR1* variants affect outcomes from primarily gram-positive sepsis.

We have an incomplete understanding of how *TLR1* variants affect risk for death in patients with sepsis. In patients with sepsis from a medical intensive care unit *TLR1*_*-7202G* has been associated with less days free of organ dysfunction[28] and susceptibility to acute lung injury.[1] While we did not identify an association between the *TLR1* polymorphisms and development of ARDS or AKI in trauma-related sepsis there was a trend towards a higher

incidence of both forms of organ dysfunction among subjects homozygous for the rare variant (*TLR1*-7202G). The failure to detect a statistically significant association may be the result of the inadequate power to detect such a relationship. Larger studies in well-phenotyped populations with sepsis will be needed to better elucidate the antecedent events leading to death in patients homozygous for the *TLR1*-7202G variant.

From a cellular and molecular standpoint, the large hypermorphic effect on innate immune inflammatory responses in human whole blood *ex vivo* that have been seen with *TLR1*-7202G 1 provides a potential explanation for the associations we have observed. We hypothesize that over-activation of the innate immune system via TLR1-specific responses leads to an increase in systemic inflammation predisposing to death. Notably, *ex vivo* studies have shown that the effect of *TLR1*-7202A/G is co-dominant while the clinical association with mortality in this study as well as in our prior report was recessive. In our association study, we observed a trend towards lower sepsis-associated mortality in the heterozygous state (table 4). These findings raise the possibility that the inflammation induced in the heterozygous state may be at least marginally beneficial and that a state of balancing selection might explain why this highly functional allele persists at such a high frequency in the population.

This study has some potential limitations. Although we have associated coding variants within *TLR1* with trauma-related sepsis mortality we have not definitively shown which variants within the LD block are necessary for the effect. Given that there exists extended LD within the *TLR1* locus[31] as well as a gene cluster involving *TLR6-TLR1-TLR10* on chromosome 4,[32] future studies in non-Caucasian or admixed populations will be necessary to more finely map the causative SNPs. In addition, the sub-group analyses testing for association between *TLR1* variant and sepsis-related mortality by infection type were statistically underpowered. Furthermore, only the first clinically-relevant positive culture of a gram-positive and/or gram-negative organism(s) was taken into account for this study but most patients had more than one positive culture during their hospital course. Therefore, it is possible that the first culture did not represent the causal microorganism for the development of sepsis. In spite of these limitations, our findings are congruent with prior findings linking *TLR1*-7202G to a higher prevalence of gram-positive infections in patients with sepsis and predominantly medical illnesses.[1] Larger prospective studies will be needed to accurately determine effect sizes in gram-positive sepsis and exclude significant associations in gram-negative sepsis.

In summary, we have identified an association between genetic variation in *TLR1* and mortality in sepsis after severe traumatic injury. This study extends previous associations with *TLR1* in patients with sepsis in predominantly non-surgical patients. We have also found evidence that *TLR1* variation may have effects on mortality that are specific to gram-positive infection. Future research will determine the mechanisms linking *TLR1* to increased mortality and whether early testing for these genetic variants might drive changes in practice such as more frequent surveillance cultures, timing and choice of prophylactic or empiric antibiotics, or use of novel therapeutic interventions modulating TLR1 function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic and Outcome Data (n=1498)

Variable	Median/Count
<u>Demographics</u>	
Age, yrs	40 (22–54)
Gender	
Male	1050 (70)
Female	448 (30)
Injury Mechanism	
Blunt	1341 (90)
Penetrating	91 (6)
Thermal	66 (4)
Injury Severity Score	22 (16–29)
Severe traumatic brain injury	739 (50)
Severe thoracic injury	620 (42)
Severe abdominal injury	310 (21)
Any PRBC transfusion	1130 (58)
Severity of sepsis	
No sepsis	807 (54)
Sepsis	375 (25)
Severe Sepsis	245 (16)
Septic Shock	71 (5)
<u>Outcomes</u>	
ICU length of stay, days	5 (2–12)
Hospital length of stay, days	12 (7–22)
Died	151 (10)

PRBC=packed red blood cells

Severe traumatic brain, thoracic or abdominal injury defined as abbreviated injury scale score ≥ 3. Continuous variables are presented as median (IQR). Categorical variables are presented as number (%).

Table 2

Demographic and Outcome Data According to Survival

	Died (n=151)	Survived (n=1347)	P-value
Age	54	39	<0.001
Male	109 (72)	941 (70)	0.55
ISS	28	23	<0.001
Severe traumatic brain injury	95 (64)	644 (48)	<0.001
Severe thoracic injury	60 (40)	560 (42)	0.68
Severe abdominal injury	33 (22)	277 (21)	0.7
Any PRBC transfusion	121 (80)	752 (56)	<0.001
Severity of sepsis			<0.001
No sepsis	68 (45)	739 (55)	
Sepsis	27 (18)	348 (26)	
Severe Sepsis	18 (12)	227 (17)	
Septic Shock	38 (25)	33 (2)	

ISS=injury severity score, PRBC=packed red blood cells

Severe traumatic brain, thoracic or abdominal injury defined as abbreviated injury scale score ≥ 3 . Continuous variables are presented as median. Categorical variables are presented as number (%). Exact *P* values are reported and considered significant if $P < 0.05$.

Table 3***TLR1* Genotype Frequencies and Associations with Mortality**

<i>TLR1</i> _{-7202A/G}				
	N	AA	AG	GG
All	1,426	823 (57.7)	517 (36.3)	86 (6.0)
Survived	1,285	750 (91.1)	464 (89.8)	71 (82.6)
Died	141	73 (8.9)	53 (10.2)	15 (17.4)
HWE	0.6885			
	OR for Death	95% CI	P value	
<i>TLR1</i> _{-7202A/G}	2.41	1.28–4.55	0.006	
Age	1.9	1.57–2.29	<0.001	
ISS	1.23	1.01–1.50	0.043	
Head AIS	1.27	1.14–1.42	<0.001	
PRBC Transfusion	3	1.86–4.81	<0.001	
<i>TLR1</i> _{1804G/T(Ser602Ile)}				
	N	GG	GT	TT
All	1,406	706 (50.2)	568 (40.4)	132 (9.3)
Survived	1,265	641 (90.7)	512 (90.1)	112 (84.9)
Died	141	65 (9.2)	56 (9.9)	20 (15.1)
HWE	0.2537			
	OR for Death	95% CI	P value	
<i>TLR1</i> _{1804G/T}	1.68	0.97–2.9	0.06	
Age	1.84	1.52–2.22	<0.001	
ISS	1.22	0.99–1.49	0.056	
Head AIS	1.27	1.14–1.41	<0.001	
PRBC Transfusion	2.96	1.7–4.27	<0.001	

HWE= Hardy-Weinberg Equilibrium, OR=odds ratio, CI=Confidence interval, ISS=injury severity score, AIS=abbreviated injury scale 3, PRBC=packed red blood cell

Odds Ratios and P values were determined by logistic regressions adjusted for age, ISS, head AIS and PRBC transfusion.

Table 4*TLR1* Genotype Frequencies and Associations with Mortality in Sepsis

<i>TLR1</i> _{7202A/G}				
	N	AA	AG	GG
All	662	368 (55.6)	249 (37.6)	45 (6.8)
Survived	583	326 (88.6)	223 (89.6)	34 (75.6)
Died	79	42 (11.4)	26 (10.4)	11 (24.4)
	OR for Death	95% CI	P value	
<i>TLR1</i> _{7202A/G}	3.16	1.43–6.97	0.004	
Age	2.4	1.79–3.23	<0.001	
ISS	1.16	0.87–1.55	0.3	
Head AIS	1.17	1.01–1.35	0.038	
PRBC Transfusion	2.6	1.17–5.76	0.019	
<i>TLR1</i> _{1804G/T(Ser602Ile)}				
	N	GG	GT	TT
All	654	317 (48.5)	272 (41.6)	65 (9.9)
Survived	577	280 (88.3)	247 (90.8)	50 (76.9)
Died	77	37 (11.7)	25 (9.2)	15 (23.1)
	OR for Death	95% CI	P value	
<i>TLR1</i> _{1804G/T}	2.48	1.26–4.88	0.008	
Age	2.28	1.7–3.07	<0.001	
ISS	1.1	0.83–1.47	0.496	
Head AIS	1.19	1.03–1.38	0.018	
PRBC Transfusion	2.15	1.01–4.61	0.048	

OR=odds ratio, CI=Confidence interval, ISS=injury severity score, AIS=abbreviated injury scale 3, PRBC=packed red blood cell
 Odds Ratios and P values were determined by logistic regressions adjusted for age, ISS, head AIS and PRBC transfusion.

Table 5

TLR1 Genotype Frequencies and Associations with ARDS or AKI in Sepsis

TLR1 ^{-7202A/G}						
	N	AA	AG	GG	OR	95% CI P value
ARDS	291	155 (42)	112 (45)	24 (53)	1.53	0.82–2.86 0.178
No ARDS	370	213 (58)	112 (55)	21 (47)		
AKI	303	160 (43)	120 (48)	23 (51)	1.33	0.71–2.54 0.371
No AKI	359	208 (57)	129 (52)	22 (49)		
TLR1 ^{1804G/T}						
	N	GG	GT	TT	OR	95% CI P value
ARDS	287	142 (45)	113 (42)	32 (49)	1.21	0.71–2.1 0.486
No ARDS	366	175 (55)	158 (58)	33 (51)		
AKI	300	139 (44)	129 (47)	32 (49)	1.2	0.7–2.07 0.508
No AKI	354	178 (56)	143 (53)	33 (51)		

ARDS=acute respiratory distress syndrome, AKI=acute kidney injury, OR=odds ratio, CI=Confidence interval
Odds Ratios and P values were determined by logistic regressions adjusted for age, ISS, head AIS and PRBC transfusion.

Table 6*TLR1* Genotype Frequencies and Associations with Mortality in Gram-Positive Sepsis

<i>TLR1</i> _{-7202A/G}				
	N	AA	AG	GG
All	353	196 (49.9)	137 (38.8)	20 (5.7)
Survived	320	176 (89.8)	129 (94.2)	15 (75)
Died	33	20 (10.2)	8 (5.8)	5 (25)
	OR for Death	95% CI	P value	
<i>TLR1</i> _{-7202A/G}	4.88	1.4–17.03	0.013	
Age	2.22	1.43–3.47	<0.001	
ISS	1.33	0.83–2.12	0.237	
Head AIS	1.19	0.96–1.48	0.114	
PRBC Transfusion	9.97	1.27–77.71	0.028	
<i>TLR1</i> _{1804G/T(Ser602Ile)}				
	N	GG	GT	TT
All	346	167 (48.2)	146 (42.2)	33 (9.5)
Survived	313	149 (89.2)	137 (93.8)	27 (81.8)
Died	33	18 (10.8)	9 (6.2)	6 (18.2)
	OR for Death	95% CI	P value	
<i>TLR1</i> _{1804G/T}	1.89	0.66–5.44	0.239	
Age	1.89	1.24–2.88	0.003	
ISS	1.22	0.77–1.92	0.384	
Head AIS	1.23	0.99–1.53	0.058	
PRBC Transfusion	9.43	1.24–71.96	0.03	

OR=Odds Ratio, CI=Confidence interval, ISS=injury severity score, AIS=abbreviated injury scale 3, PRBC=packed red blood cell
 Odds Ratios and P values were determined by logistic regressions adjusted for age, ISS, head AIS and PRBC transfusion.

Table 7 Linkage Disequilibrium between *TLR1* SNPs

	<i>rs5743551</i>	<i>rs5743618</i>	<i>rs4833095</i>
<i>rs5743551</i>	1*		
<i>rs5743618</i>	0.76	1	
<i>rs4833095</i>	0.93	0.76	1

* Values are presented as R^2

Table 8*TLR1*_{742A/G(Asn248Ser)} Genotype Frequencies and Association with Mortality in Gram-Positive Sepsis

	<i>TLR1</i> _{742A/G(Asn248Ser)} ⁺			
	N	AA	AG	GG
All	350	195 (55.7)	136 (38.8)	19 (5.4)
Survived	315	173 (88.7)	128 (94.1)	14 (73.7)
Died	35	22 (11.3)	8 (5.9)	5 (26.3)
	OR for Death	95% CI	P value	
<i>TLR1</i> _{742A/G}	4.16	1.22–14.19	0.023	
Age	2.04	1.34–3.11	0.001	
ISS	1.24	0.79–1.94	0.338	
Head AIS	1.22	0.99–1.5	0.066	
PRBC Transfusion	10.3	1.33–79.2	0.025	

OR=odds ratio, CI=Confidence interval

⁺Hardy Weinberg Equilibrium=0.9209

Odds Ratios and P values were determined by logistic regressions adjusted for age, ISS, head AIS and PRBC transfusion.