Common Variants of TLR1 Associate with Organ Dysfunction and Sustained Pro-Inflammatory Responses during Sepsis

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Abstract

Background: Toll-like receptors (TLRs) are critical components for host pathogen recognition and variants in genes participating in this response influence susceptibility to infections. Recently, TLR1 gene polymorphisms have been found correlated with whole blood hyper-inflammatory responses to pathogen-associated molecules and associated with sepsis-associated multiorgan dysfunction and acute lung injury (ALI). We examined the association of common variants of TLR1 gene with sepsis-derived complications in an independent study and with serum levels for four inflammatory biomarkers among septic patients.

Methodology/Principal Findings: Seven tagging single nucleotide polymorphisms of the TLR1 gene were genotyped in samples from a prospective multicenter case-only study of patients with severe sepsis admitted into a network of intensive care units followed for disease severity. Interleukin (IL)-1β, IL-6, IL-10, and C-reactive protein (CRP) serum levels were measured at study entry, at 48 h and at 7th day. Alleles -7202G and 248Ser, and the 248Ser-602Ile haplotype were associated with circulatory dysfunction among severe septic patients (p = 0.001; #p ≤p ≤0.022), and with reduced IL-10 (#p ≤p ≤0.047) and elevated CRP (#0.011≤p ≤0.036) levels during the first week of sepsis development. Additionally, the -7202GG genotype was found to be associated with hospital mortality (p = 0.017) and ALI (p = 0.030) in a combined analysis with European Americans, suggesting common risk effects among studies.

Conclusions/Significance: These results partially replicate and extend previous findings, supporting that variants of TLR1 gene are determinants of severe complications during sepsis.

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Introduction

Sepsis is a devastating clinical condition characterized by systemic inflammation occurring in the setting of a severe infection. Among intensive care unit (ICU) patients, incidence and mortality has been estimated in ~12% and >40%, respectively, for the most severe forms [1]. Over the past three decades, a plethora of experimental and clinical studies have contributed substantially to our understanding of sepsis development and associated complications, including the acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) [2]. Studies in animal models, and twin and association studies have evidenced that the innate immune responses to pathogens show inter-individual variability that is strongly influenced by genetic factors, which may affect disease susceptibility and severity [3,4,5].

Toll-like receptor (TLR) pathways are critical components of the immune response to pathogens [6], and targeting of TLRs have been demonstrated to protect from lethal sepsis [7,8]. Polymorphisms in genes of the TLR-mediated responses are associated with altered immunity [9,10,11], with susceptibility to...
Infections and with related acute inflammatory syndromes, including sepsis and severe complications such as ALI and ARDS [12,13]. In an elegant study, Wurfl and colleagues screened tagging single nucleotide polymorphisms (SNPs) across 49 TLR-related genes for association with whole blood inflammatory responses to pathogen-associated molecules in normal volunteers [14]. This allowed the identification of various tightly linked SNPs from the TLR1 gene associated with the strongest hyper-inflammatory effects. In a subsequent association study in septic patients focusing on two of the common TLR1 SNPs (-7202A/G and Ser602Ile) revealed associations with organ dysfunction, 28-day hospital mortality, ALI, and the prevalence of gram-positive cultures, constituting the unique association study of TLR1 gene variants with sepsis-associated complications to date.

Given the controversies around the association of genetic factors with sepsis-associated outcomes because of the limited replicability of findings [15,16], here we have examined the association of common variants of TLR1 gene with sepsis outcomes in a cohort of patients with severe sepsis admitted into a network of ICUs from Spain, in order to provide an independent replication of previous results. We additionally explored the relationship of TLR1 polymorphisms and of particular haplotypes with serum levels of four biomarkers of inflammation taken at three stages of sepsis development. We found that TLR1 SNPs and haplotypes were associated with circulatory dysfunction and the source of infection among severe septic patients, and with hospital mortality and ALI in a combined analysis with data from European Americans. Congruently, risk alleles associated with increased nuclear factor \( \kappa \)B (NF-\( \kappa \)B) activation upon TLR1 stimulation in previous studies [17,18], were related with reduced interleukin-10 and elevated C-reactive protein serum levels during the first week of sepsis development.

Methods

Ethics Statement

This study was approved by the Ethics Committees of Hospital Universitario N. S. de Candelaria and Hospital Universitario Rio Hortega (Spain). Written informed consent was obtained from each subject or appropriate surrogates.

Study Subjects

Samples were collected as part of a prospective, observational study of adults admitted during 2003–2005 into a network of Spanish ICUs. The study has been reported elsewhere [19] and included consecutive patients admitted to ICUs older than 18 years old fulfilling the international criteria for severe sepsis (\( n = 218 \)) [20]. We collected basic demographic data, previous health status, severity of illness scores and clinical information until discharge from the ICU, including source of infection, pathogens, and development of organ dysfunction. All patients were followed prospectively for the development of ALI, as defined by the American-European Consensus Conference [21] and for the development of organ dysfunction included in the sequential organ failure assessment (SOFA) scale [22]. For the purpose of this study, patients with ALI and ARDS were analyzed as a single group of ALI patients. Genomic DNA was isolated from peripheral whole blood using commercial kits (GFX kit, GE Healthcare, Little Chalfont, UK) and stored at -20°C until use. A summary description of cases can be found in Table 1.

| Variable | Cases (n = 218) |
|----------|----------------|
| Gender, male (%) | 59.2 |
| Age, median years (P25–P75) | 67 (54–75) |
| Previous health status (%) | |
| Previous surgery | 74.5 |
| Hospitalized &gt;=24 h | 78.8 |
| Diabetes | 16.1 |
| Hypertension | 46.0 |
| Ischemic cardiac disease | 9.3 |
| Smoker | 27.6 |
| Source of infection (%) | |
| Pulmonary | 34.4 |
| Extra-pulmonary | 65.6 |
| Identified pathogen (%) | |
| Gram negative | 23.9 |
| Gram positive | 15.1 |
| Fungi | 2.9 |
| Mixed Gram negative and positive | 7.3 |
| Polymicrobial | 2.0 |
| Negative blood cultures | 48.8 |
| Organ dysfunction (%) | |
| Circulatory | 83.1 |
| Neurologic | 22.1 |
| Coagulation | 22.1 |
| Renal | 33.8 |
| Hepatic | 15.5 |
| APACHE II, median (P25–P75) | 23 (18–27) |
| ALI or ARDS (%) | 86.1 |
| Hospital mortality (%) | 52.7 |

Abbreviations: APACHE II, acute physiology and chronic health evaluation score II; ALI, acute lung injury; ARDS, acute respiratory distress syndrome. doi:10.1371/journal.pone.0013759.001

SNP selection and genotyping

Instead of focusing exclusively on the two previously associated variants [14], we selected tagging SNPs (tSNPs) to efficiently study common TLR1 gene variation in order to test whether additional gene variants associated with the expression of the clinical phenotype. TagIT 3.0.3 software [24] was used to select a set of 7 tSNPs from data available from 23 European-Americans retrieved from the Innate Immunity NHLBI Program for Genomic Applications (PGA) website (http://innateimmunity.net/IIPGA2/index.html, accessed October 2008), forcing the inclusion of previously associated tSNPs by Wurfl and colleagues [14]. A SNP-dropping-with-re-sampling method [24] was used to evaluate the expected properties of the TLR1 7 tSNPs set, indicating an average haplotype \( r^2 &gt; 0.90 \).

Genotyping was conducted using the iPLEX\textsuperscript{TM} Gold assay on MassARRAY system (Sequenom, San Diego, CA) by the Spanish National Genotyping Center, Santiago de Compostela Node (CeGen, http://www.cegen.org). Briefly, iPLEX\textsuperscript{TM} assays were scanned by MALDI-TOF mass spectrometry and individual SNP genotypic calls were automatically generated using Sequenom
Measurements were done by duplicate using commercial kits: IL-6, IL-1 commonly used biomarkers of acute inflammation, interleukin performed. These samples have been previously measured for four Diagnostic, Basilea, Switzerland). CRP was determined in a Hitachi 917 analyzer (Roche (Siemens Medical Solutions Diagnostics, Caernarfon, UK), and

Serum measurements
Serum was available only from a subset of 121 patients as dry-ice transportation to the coordinating center was not warranted for all participating centers. These samples were collected within 24 hours of meeting severe sepsis criteria, at 48 hours and 7 days after study entry, only if the patient remained hospitalized into the ICU. Given this, serum was available for all 121 patients at inclusion, for 96 of these patients at 48 hours, and for 60 of these patients at the 7th day. Serum was obtained by centrifuging peripheral blood samples for 10 min at 3200 rpm within 35 min after sampling and was kept at −80°C until measurements were performed. These samples have been previously measured for four commonly used biomarkers of acute inflammation, interleukin (IL-6, IL-1β, IL-10, and C-reactive protein (CRP) [25]. Measurements were done by duplicate using commercial kits: IL-6, IL-1β, and IL-10 were assessed in an Immulite analyzer (Siemens Medical Solutions Diagnostics, Caernarfon, UK), and CRP was determined in a Hitachi 917 analyzer (Roche Diagnostic, Baselca, Switzerland).

Statistical analysis
Departures from Hardy-Weinberg equilibrium (HWE) were only tested in controls by means an efficient exact test [26]. Associations of SNPs with sepsis-derived complications were assessed using logistic regression models by means of SNPassoc [27] assuming additive effects for all SNPs. To replicate previous association findings [14], recessive models were also applied for -7202A/G and Ser602Ile SNPs as necessary. Logistic regressions were also used to obtain SNP effects as odds ratios (ORs) with 95% CI adjusting for age, gender, and the acute physiology and chronic health evaluation score (APACHE) II. Genotype data available from 20 unlinked polymorphisms from different parts of the genome [13] were utilized to adjust individual SNP associations for population stratification by means of a modified Cochran-Armitage trend test [28]. A Mantel-Haenszel test over genotype counts was used to perform the joint stratified analysis of the two SNPs overlapping across our study and the previous one [14], under the principle that if SNPs had consistent effects (i.e. allele and effect-wise) among different studies, the joint analysis would improve the results obtained from the two studies by separate. Data were analyzed under recessive models with -7202GG and 602Ile/Ile genotypes as risk factors to emulate previous association findings [14]. For this analysis, we did not consider non-septic control sample genotype counts from Wurtele et al. [14], and used data from the sepsis cohort to test associations with hospital mortality and the CELEG cohort to test associations with ALI development (Table S1). THESIAS [29] was used to assess the association of particular TLR1 haplotypes with sepsis-derived outcomes. A two-tailed \( p \leq 0.05 \) was considered significant. However, in order to estimate the chance that SNP associations were true positives, we calculated the false-discovery rate (FDR) overall comparisons using QVALUE [30]. The patterns of linkage disequilibrium (LD), in terms of \( r^2 \) values, were explored using Haploviev 3.32 [31].

Differences for serum biomarker levels between genotypes were explored using general linear models (GLM) for repeated measures in a longitudinal analysis over the three time points by means of SPSS 15.0 (SPSS Inc., Chicago, IL). Although the four biomarkers were measured for all 121 serum samples at inclusion, 96 serum samples at 48 h and 60 serum samples at the 7th day, the GLM analysis tested for biomarker level differences between genotypes over time. Thus, it only considered the 60 samples for which measures were available for the three time points. Despite dropping the sample size, GLM allowed to explore sustained SNP relationships with biomarkers, under the rationale that these were more likely to identify genuine associations. A post hoc analysis of differences in biomarker levels between genotypes at individual time points was also performed using all available serum measures from each day by means of linear regression. These analyses were performed under recessive models in order to emulate previous functional findings in vitro [17,32,33]. Values for IL-6, IL-1β, and IL-10 were logistically transformed for the analysis.

Results and Discussion
Among the 218 severe septic patients, the median APACHE II score was 23 (interquartile range: 18–27), 55.0% percent developed ARDS and 31.1% developed ALI during hospitalization (Table 1). Peritonitis was the leading cause of severe sepsis followed by pneumonia, and most pathogens were characterized as Gram-negative bacteria. In agreement with previous studies [34], no pathogens were identified in blood cultures as the causative microorganism for sepsis albeit having an identified site of infection for 48.8% of patients. Hospital mortality was 52.7%. Table 2 shows that completion rates were \( >97\% \) for all 7 tSNPs and that all followed HWE expectations in control samples. None of the tSNPs was significantly associated with hospital mortality or with sepsis-induced ALI (Table 3). Borderline associations were observed for the SNP Arg80Thr (rs5743611) with hospital mortality (\( p = 0.080 \)), and for SNPs -7202A/G (rs5743551) and Asn248Ser (rs4833095) with sepsis-induced ALI (\( p = 0.100 \) and \( p = 0.090 \), respectively). The association of -7202A/G with hospital mortality or with ALI did not improve if a recessive model was

| rs#          | tSNP position | Location | CR (%) | HWE \( p \)-value in controls |
|--------------|---------------|----------|--------|------------------------------|
| rs5743551    | -7202A/G      | 5‘ flanking | 98.9   | 0.566                        |
| rs574365     | -5531A/G      | Intron 1  | 99.1   | 0.294                        |
| rs5743594    | -2299C/T      | Intron 2  | 99.3   | 0.376                        |
| rs5743596    | -2076G/C      | Exon 3   | 99.2   | 0.469                        |
| rs5743611    | 238C/G (Arg80Thr) | Exon 4 | 98.8   | 1.000                        |
| rs4833095    | 742A/G (Asn248Ser) | Exon 4 | 99.3   | 1.000                        |
| rs5743618    | 1804G/T (Ser602Ile) | Exon 4 | 97.3   | 0.513                        |

Abbreviations: CR, completion rate; HWE, Hardy-Weinberg equilibrium.

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used. However, analyses combining samples from this and the previous study by Wurfel et al. [14], enabled the detection of a weak risk of the -7202G/G genotype for hospital mortality and for ALI consistent across the two studies. The significance of the association of -7202G/G with hospital mortality improved to $p = 0.017$ (OR = 0.028 in the previous study [14] and $p = 0.566$ in this study). Similarly, significance of the association of -7202G/G with ALI among septic patients improved to $p = 0.050$, while separate findings were non-significant ($p = 0.171$ in the previous study [14] and $p = 0.108$ in this study). For ile602ser (rs5743618), analyses combining samples from this and the previous study by Wurfel et al. [14], did not enable the detection of consistent significant effects (results not shown). Contrary to previous findings [14], none of the tSNPs showed a significant association with prevalence of Gram-positive infections versus other types of pathogens, but the SNPs -2299C/T (rs5743594) and -7202G/G showed borderline associations ($p = 0.067$ and $p = 0.110$, respectively). Nevertheless, in our study, three tSNPs had nominal significance in association with pulmonary infection among septic patients, which is frequently caused by Gram-positive bacteria [1,35]: -5531A/G (rs5743565) with $p = 0.004$, -2076C/T (rs5743596) with $p = 0.004$, and Asn248Ser with $p = 0.037$. However, when adjusted for covariates using regression models, results remained significant for -5531A/G (OR for -5531A: 1.84, 95% CI: 1.10–3.10, $p = 0.023$) and for -2076C/T (OR for -2076C: 1.90, 95% CI: 1.07–3.39, $p = 0.029$), but not for Asn248Ser (OR for 248Ser: 1.44, 95% CI: 0.92–2.26, $p = 0.112$). Similar findings were obtained after applying a conservative correction for population stratification, as only the association of -5531A/G with pulmonary infection remained significant ($p = 0.047$, $p = 0.057$ and $p = 0.127$ for -5531A/G, -2076C/T and Asn248Ser, respectively).

The strongest associations of TLR1 tSNPs with sepsis-derived complications were found with circulatory dysfunction (Table 3). Three tSNPs, -7202G/A, Asn248Ser, and Ser602Ile, showed $p$-values ranging from 0.001 to 0.024. The association of -7202G/A remained significant after adjusting for covariates (OR for -7202G: 2.68, 95% CI: 1.40–5.12, $p = 0.003$) and population stratification ($p = 0.031$). The associations for Asn248Ser and Ser602Ile also persisted when adjusting for covariates (OR for 248Ser: 2.13, 95% CI: 1.16–3.90, $p = 0.015$; OR for 602Ile: 1.94, 95% CI: 1.10–3.44, $p = 0.023$) and showed borderline significance after the conservative adjustment for population stratification ($p = 0.061$ for Asn248Ser and $p = 0.064$ for Ser602Ile). In the context of the multiple tests performed, some of the above findings might represent true positives. In particular, FDR was below 5% for the association of -7202G/A with circulatory dysfunction, which partially replicates the study by Wurfel et al. [14], which reported the association of this TLR1 SNP with circulatory, neurologic, coagulation, renal and hepatic dysfunction among septic patients. However, one must take into account the power limitation of this association study. Assuming a minor allele frequency of 30% (as is the case for -7202A/G) and an OR of 2.0, in the range of values reported by Wurfel et al [14], statistical power ranged from 39% to 69% for the outcomes tested, which might be responsible for the lack of replication in the association of TLR1 variants with some of the outcomes.

Many evidences point to the SNP Ser602Ile as the main responsible for the inter-individual variation in TLR1-mediated responses, 602Ser allele being related to a decrease in NF-kB activity [14,17,18,32]. Albeit the SNP Asn248Ser have demonstrated no functional relevance [14,18], previous association studies have indicated that it might be important for disease susceptibility [14,36,37,38]. Thus, we were interested in testing the association of the ancestral haplotype 248Ser-602Ile [18] with sepsis-derived complications, motivated by the observation that this haplotype consistently tends to associate with the highest NF-kB activation upon TLR1 stimulation in independent studies [17]. In particular, we were interested in evaluating whether haplotypes of these two SNPs were more strongly associated with circulatory dysfunction than the two SNPs by separate. However, our results showed that, although 248Ser-602Ile haplotype homozygous subjects were more frequent among patients with circulatory dysfunction (17.5%) than in the rest of patients (5.6%), being a risk factor for circulatory dysfunction (OR: 2.07, 95% CI: 1.12–3.82, $p = 0.028$), statistical significance did not improve considerably compared to those obtained for Asn248Ser and Ser602Ile individually.

A number of studies have explored the functional effects of TLR1 variants in vitro [14,17,32]. However, no study have explored if TLR1 variants are associated with amplified immune responses to pathogens in vivo. Given that serial serum measures for four biomarkers of inflammation were available from these patients during the first week after the onset of severe sepsis (Table S2), we correlated tSNPs genotypes with biomarker serial measures using GLM (Table 4). None of the tSNPs was found to be associated with serially-measured IL-1β and IL-6 serum levels, despite previous studies have demonstrated that TLR1 gene polymorphisms determined IL-6 levels in response to specific gram-

### Table 3. Association $p$-values of TLR1 tSNPs with severe sepsis complications.

| tSNP     | Hospital mortality | Acute lung injury | Gram positive infection | Pulmonary infection | Organ dysfunction |
|----------|--------------------|-------------------|-------------------------|--------------------|-------------------|
|          |                    |                   |                         |                    | Circulatory       |
|          |                    |                   |                         |                    | Neurologic        |
|          |                    |                   |                         |                    | Coagulation       |
|          |                    |                   |                         |                    | Renal             |
|          |                    |                   |                         |                    | Hepatic           |
| -7202A/G | 0.306              | 0.100             | 0.253                   | 0.129              | 0.001             |
|          | (0.566)            | (0.108)           | (0.110)                 | (0.399)            | (0.008)           |
| -5531A/G | 0.648              | 0.182             | 0.714                   | **0.004**          | 0.501             |
|          | (0.808)            | (0.188)           | (0.741)                 |                    | 0.224             |
| -2299C/T | 0.818              | 0.845             | 0.067                   | 0.803              | 0.111             |
|          | (1.000)            | (1.086)           | (0.982)                 |                    | 0.935             |
| -2076C/T | 0.327              | 0.405             | 0.738                   | **0.004**          | 0.551             |
|          | (0.524)            | (0.618)           | (0.732)                 |                    | 0.372             |
| 238C/G   | 0.080              | 0.181             | 0.622                   | 0.151              | 1.000             |
|          | (0.108)            | (0.110)           | (0.661)                 |                    | 1.000             |
| 742A/G   | 0.530              | 0.090             | 0.438                   | **0.037**          | 0.009             |
|          | (0.399)            | (0.382)           | (0.481)                 |                    | 0.481             |
| 1804G/T  | 0.164 (0.208)      | 0.142 (0.343)     | 0.129                   | 0.309 (0.677)      | 0.024             |
|          | (0.399)            | (0.399)           | (0.273)                 | (0.941)            | 0.557             |

In parenthesis, $p$-value for a recessive model; nominal significances in bold.

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negative bacterial products [14]. On the other hand, CRP and IL-10 levels varied significantly among genotypes for four of the TLR1 variants, with \( p \)-values ranging from 0.002 to 0.047. However, given the assessment used in this study to explore TLR1 SNP relationships with biomarkers, statistical power was limited for most comparisons, particularly for those involving IL-6 and IL-1\( \beta \).

Table 4. P-values and statistical power (%) from the longitudinal analysis of differences in serum biomarker levels by TLR1 tSNPs.

| tSNP       | IL-1\( \beta \) | IL-6 | CRP | IL-10 |
|------------|-----------------|------|-----|-------|
|            | \( p \)-value   | Power| \( p \)-value | Power | \( p \)-value | Power |
| -7202A/G   | 0.714           | 6.5  | 0.277 | 19.0  | 0.036 | 56.1  | 0.012 | 72.2  |
| -5531A/G   | 0.614           | 7.9  | 0.433 | 12.1  | 0.995 | 5.0   | 0.009 | 76.3  |
| -2299C/T   | 0.527           | 9.6  | 0.408 | 13.0  | 0.288 | 18.4  | 0.413 | 12.8  |
| -2076C/T   | 0.916           | 5.1  | 0.148 | 30.2  | 0.002 | 88.8  | 0.076 | 42.7  |
| 238C/G     | 0.380           | 14.0 | 0.186 | 26.1  | 0.476 | 10.9  | 0.640 | 7.5   |
| 742A/G     | 0.902           | 5.2  | 0.307 | 17.3  | 0.011 | 73.5  | 0.047 | 51.6  |
| 1804G/T    | 0.685           | 6.9  | 0.320 | 16.7  | 0.129 | 32.8  | 0.117 | 34.7  |

Nominal significances in bold.

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Figure 1. IL-6, IL-1\( \beta \), IL-10, and CRP serum levels according to the TLR1 248Ser-602Ile haplotype status. Values represent mean ± SEM biomarker levels at inclusion, at 48 hours, and at 7th day for patients showing homozygosity for the TLR1 248Ser-602Ile haplotype (n = 8) and for the rest of patients (n = 52). Note that values represented correspond to those from the 60 severe septic patients with serum measures available at the three time points. Significance of biomarker differences by the haplotype status was obtained using general linear models (GLM) for repeated measures in a longitudinal analysis assuming a recessive model (CRP, \( p = 0.024 \); IL-10, \( p = 0.015 \); for IL-1\( \beta \) and IL-6, \( p < 0.45 \)).

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for which it was below 30% (Table 4). Nevertheless, congruent with our previous results, homozygote patients for -7202G or 248Ser alleles, both constituting risk alleles for sepsis-associated complications, showed significantly increased levels for CRP ($p=0.036$ and $p=0.012$, respectively) and lower levels for the compensatory anti-inflammatory cytokine IL-10 ($p=0.011$ and $p=0.047$, respectively) (Table 4), regardless of the in vitro findings suggesting higher IL-10 levels for the -7202GG genotype in response to TLR1/TLR2 agonists [14]. This sustained pro-inflammatory state was also observed for patients homozygous for the risk haplotype 248Ser-602Ile (n = 8), associated with the highest NF-kB activation upon TLR1 stimulation [17], compared to the rest of patients (n = 52; $p=0.024$ and $p=0.015$ for CRP and IL-10 serial measures, respectively; $p=0.45$ for IL-1β and IL-6) (Figure 1), slightly improving significance levels over individual SNP comparisons. Results for the longitudinal analysis barely changed when tests were performed under additive models or when adjusted for age, gender, and APACHE II (not shown).

It is worth noting that no demographical or clinical differences were found between samples considered in the GLM analysis of TLR1 SNP genotypes and serial measures of biomarkers, i.e. those patients who were not discharged from the ICU or were not dead before seven days, and patients without serum measures at the three time points, except for mortality ($p=0.033$). Thus, it is reasonable to assume that this GLM biomarker analysis might be biased towards the expected for survivor patients. We explored such possibility by a post hoc analysis of differences in biomarker levels at individual time points among genotypes using all available serum measures. This analysis supported the strong correlation of TLR1 SNPs with CRP and IL-10 levels observed before, while revealed barely significant relationships of TLR1 SNPs with IL-6 and IL-1β levels (Table S3), indicating that GLM results were not as biased as predicted.

Due to the remarkable effects on NF-kB activation, Ser602Ile has been put forward as the principal functional variant affecting TLR1 activity across the whole chromosome region encompassing TLR10-TLR1-TLR6 genes [18]. Nevertheless, we found stronger associations for SNPs -7202G/A and Asn248Ser with sepsis complications and with biomarker serum levels, in agreement with previous association studies [14,36], albeit no functional properties evidences suggest that a more complex scenario, possibly involving variant in LD with it) might be relevant for susceptibility to this devastating clinical condition.

Leu, etc) might be needed to be able to detect Ser602Ile effects on mediated responses (e.g. TLR4 Apv399Gly, TIRAP/Mal Ser180-Leu, etc) might be needed to be able to detect Ser602Ile effects on this devastating clinical condition.

In conclusion, despite the limited power of the study, here we have replicated the association of TLR1 gene variants, with established increased NF-kB activation in vitro, with risk for circulatory dysfunction during severe sepsis, and associated TLR1 variants with elevated CRP and decreased IL-10 levels in serum during the first week of sepsis development. In line with previous evidences, our results suggest that genetic factors favouring an exacerbated host pro-inflammatory response to an infection predisposes to sepsis complications.

Figure 2. Linkage disequilibrium (LD) plot of $r^2$ values between TLR1 SNPs. The plot was created with the genotype data from the Spanish population-based controls. Each diamond of the LD plot represents a pair-wise SNP comparison with its $r^2$ value indicated and schematically symbolized by a color gradient ranging from black ($r^2=100$, corresponding to complete LD) to grey (100-$r^2$=0, moderate LD) to white ($r^2=0$, corresponding to absence of LD).

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Supporting Information

Table S1 Genotype counts for the TLR1 SNPs used in meta-analysis. Found at: doi:10.1371/journal.pone.0013759.s001 (0.04 MB DOC)

Table S2 Mean (±SEM) serum levels of IL-6, IL-1β, IL-10 and CRP stratified by TLR1 SNP genotypes. Found at: doi:10.1371/journal.pone.0013759.s002 (0.05 MB DOC)

Table S3 P-values from the post hoc analysis of differences in IL-1β, IL-6, CRP and IL-10 levels among TLR1 genotypes at individual time points using all available serum measures from each time point (at inclusion, 48 hours, and 7th day). Found at: doi:10.1371/journal.pone.0013759.s003 (0.04 MB DOC)

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