Impaired TLR5 Functionality Is Associated with Survival in Melioidosis


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**Supplementary Material**

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Impaired TLR5 Functionality Is Associated with Survival in Melioidosis

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Melioidosis is infection caused by the flagellated saprophyte Burkholderia pseudomallei. TLR5 is a pathogen recognition receptor activated by bacterial flagellin. We studied a genetic variant that encodes a defective TLR5 protein, TLR5<sub>1174C>T, to elucidate the role of TLR5 in melioidosis. We measured NF-κB activation induced by B. pseudomallei in human embryonic kidney–293 cells transfected with TLR5 and found that B. pseudomallei induced TLR5<sub>1174C</sub> but not TLR5<sub>1174T</sub>-dependent activation of NF-κB. We tested the association of TLR5<sub>1174C>T with outcome in 600 Thai subjects with melioidosis. In a dominant model, TLR5<sub>1174C>T was associated with protection against in-hospital death (adjusted odds ratio: 0.20; 95% confidence interval: 0.08–0.50; p = 0.001) and organ failure (adjusted odds ratio: 0.37; 95% confidence interval: 0.19–0.71; p = 0.003). We analyzed blood cytokine production induced by flagellin or heat-killed B. pseudomallei by TLR5<sub>1174C>T genotype in healthy subjects. Flagellin induced lower monocyte-normalized levels of IL-6, IL-8, TNF-α, IL-10, MCP-1, IL-1ra, G-CSF, and IL-1β in carriers of TLR5<sub>1174C</sub> B. pseudomallei induced lower monocyte-normalized levels of IL-10 in carriers of TLR5<sub>1174T</sub>. We conclude that the hypofunctional genetic variant TLR5<sub>1174C>T is associated with reduced organ failure and improved survival in melioidosis. This conclusion suggests a deleterious immunoregulatory effect of TLR5 that may be mediated by IL-10 and identifies this receptor as a potential therapeutic target in melioidosis. The Journal of Immunology, 2013, 190: 3373–3379.
Materials and Methods

Bacteria

*B. pseudomallei* BP-1, *B. pseudomallei* 1026b, or *B. pseudomallei* K96243 organisms were grown in Luria–Bertani broth for 6 h (log phase) or 19 h (stationary phase) and heat killed as previously described (3).

Human subjects

The melioidosis case–control study cohort has been previously described (13). Clinical data and blood were obtained prospectively from patients with culture-proven melioidosis admitted to Sappasithiprasong Hospital, Ubon Ratchathani, Thailand, from 1999 to 2005. Consent for enrollment into clinical studies of melioidosis was obtained from subjects or their representatives at the time of recruitment.

For studies using whole blood, fasting blood samples were obtained from healthy white participants in a Harbormview Medical Center inflammatory response research study. Enrollment criteria and blood processing have been previously described (14). Thai subjects donating blood at the blood donation center at Sappasithiprasong Hospital in 2010 were recruited for participation in a similar study. Subjects were included if they were between the ages of 18 and 60 and did not report any history of immunodeficiency or inflammatory conditions, chronic diseases, pregnancy in the past 6 mo, anti-inflammatory medication use in the past week, antibiotic use in the past month, vaccination in the past 6 mo, heavy exercise or alcohol consumption in the past 24 h, or smoking in the past month. Those who met enrollment criteria gave written informed consent to participate and provided a postdonation blood sample.

These studies were approved by the University of Washington Human Subjects Division Institutional Review Board; the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand; and the Ethical Review Committee for Research in Human Subjects, Sappasithiprasong Hospital, Ubon Ratchathani, Thailand; and the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Assays

NF-κB activation was determined with a luciferase reporter assay in Chinese hamster ovary (CHO) cells stably expressing huTLR5 and in human embryonic kidney–293 cells (HEK-293) transiently transfected with huTLR5
t174C or huTLR5
t174F, as previously described (6, 15, 16).

For immunoassay studies, 380 μl fresh whole blood in citrate mixed 1:1 with RPMI 1640 media was added to preprepared plates containing 20 μl stimulants. For the Harbormview Medical Center study, the stimulant was log phase heat-killed *B. pseudomallei* 1026b 2.5 × 10⁶ CFU/ml. For the Sappasithiprasong Hospital study, the stimulants were E. coli 0111:B4 LPS 10 ng/ml, *S. typhimurium* flagellin 500 ng/ml, log phase heat-killed *B. pseudomallei* 1026b 2.5 × 10⁶ CFU/ml, or heat-killed *B. pseudomallei* K96243 2.5 × 10⁶ CFU/ml. Plates were incubated at 37°C on a shaking incubator under 5% CO₂ for 6 h before being spun down and the plasma was removed and frozen. For the Harbormview Medical Center study, plasma IFN-γ, IL-10, IL-1p70, IL-1β, IL-6, IL-8, and TNF-α were subsequently assayed in duplicate, using an electrochemiluminescence imager (Mesoscale Discovery, Gaithersburg, MD). For the Sappasithiprasong Hospital study, IL-6, IL-8, TNF-α, IL-10, MCP-1, IL-1-ra, G-CSF, and IL-1β were later assayed in duplicate on a multiplex bead system (LumineX, Austin, TX) using reagents from R&D Systems (Minneapolis, MN) or by ELISA (IL-1β for *B. pseudomallei* only) (14, 15). For each subject, a complete blood count with differential was performed in the clinical laboratory at the time of phlebotomy.

Genetic methods

DNA was extracted from whole blood using Nucleon BACC3 (GE Healthcare, Buckinghamshire, U.K.) or QIAamp DNA Blood Midi (Qiagen, Hilden, Germany) kits (13). Genotyping was performed using an allele-specific primer extension method (Sequenom, San Diego, CA) with reads by a MALDI-TOF mass spectrometer (13) or was performed using ABI TaqMan assays on an ABI Prism 7900 (Applied Biosystems, Carlsbad, CA).

Statistics

Continuous clinical data are reported as mean values. The association between genotype and outcome was evaluated using the Fisher exact test or by logistic regression. Survival analyses were performed with the log-rank test. For tests of population stratification, allele counts between cases and controls were compared with the χ² test. Continuous in vitro data expected to follow a normal distribution are reported as mean ± SD. Comparisons between two groups were made using the t test, and comparisons between three or more groups were made using ANOVA and the Bonferroni post-test. Data not normally distributed are reported as median ± interquartile range. Plasma cytokine levels were analyzed in raw form or following normalization to monocyte counts. Given their mostly nonnormal distributions, cytokine values were log10 transformed before analysis by linear regression, adjusting for sex in the Thai cohort (14). Statistics were performed with Stata 11.1 (College Station, TX), incorporating the function genthw. A two-sided p ≤ 0.05 was considered significant.

Results

**TLR5 mediates activation of NF-κB by *B. pseudomallei***

We first tested whether *B. pseudomallei* activates NF-κB, a transcription factor in the TLR pathway, in a TLR5-dependent manner. As wild-type CHO cells do not respond to flagellin (5), we stimulated CHO cells stably transduced with huTLR5 and an NF-κB luciferase reporter with stationary and log phase killed *B. pseudomallei* BP-1 (Fig. 1) (16). We examined bacteria from different growth phases because of lost or reduced expression of bacterial flagellin by *B. pseudomallei* in the stationary phase (S. Peacock, unpublished observations). Although stimulation with only the higher concentrations of stationary phase bacteria resulted in NF-κB activation, we detected robust NF-κB activation upon stimulation with lower concentrations of log phase bacteria. These findings indicated TLR5-mediated innate immune activation by *B. pseudomallei*.

**TLR5
t174F is associated with protection against death in human melioidosis**

To explore the importance of TLR5 signaling in human melioidosis, we selected a common hypofunctional human polymor-
TLR5, TLR51174C>T (rs5744168), for study. In a case–control study, we analyzed the association of TLR51174C>T with outcome in a cohort of Thai subjects with culture-proven melioidosis admitted to Sappasitiprasong Hospital. Of 614 subjects with blood available for genotyping who passed quality control checks, survival at hospital discharge was known for 600. Mortality was 23.8%. The characteristics of the melioidosis subjects are listed in Table I. We successfully genotyped TLR51174C>T in 592 individuals (98.7% call rate); 514 (86.8%) were genotype C/C, 76 (12.8%) C/T, and 2 (0.3%) T/T (MAF 6.8%). After confirming Hardy–Weinberg equilibrium in the survivors (p = 0.8), we tested the association of the variant with in-hospital death. We found that 15.9% of survivors were heterozygotes or minor homozygotes, compared with 4.3% of nonsurvivors (contingency table p = 0.0004) (Table II). In a dominant genetic model (grouping carriers of the rare allele—TLR51174CT and TLR51174TT—together), we observed a very strong association of the TLR51174C>T variant with protection against death [odds ratio (OR): 0.24; 95% confidence interval (CI): 0.10–0.56; p = 0.001].

We next performed an adjusted association of the TLR51174C>T variant with in-hospital death. To identify possible confounders to include in the model, we tested whether TLR51174C>T was associated with any pre-existing condition (diabetes, chronic liver disease, renal insufficiency, and steroid use). We found no association of the variant with any of these factors. However, we considered in the model factors that were independently associated with death: age, diabetes, renal insufficiency, pneumonia, bacteremia, and urinary tract infection. The appropriateness of the antimicrobial regimen did not differ between survivors and nonsurvivors. As pathogen recognition is characterized by cross talk and redundancy (17, 18), we also considered potential confounding effects of other TLR pathway genetic variants. We have previously observed that TLR4 and adapter molecule MyD88 modulate innate immune activation by B. pseudomallei (3). TIRAP is an adapter molecule that cooperates with MyD88. We chose two coding variants, one in TLR4 and one in TIRAP, to analyze in this population: TLR4896A>G (rs4986790) and TIRAP558C>T (rs7932766). Both variants have been previously associated with infection, with functional effects demonstrated by some authors (19–24). We have shown that the TLR4896A>G variant is rare in Thais (MAF < 1%) (13). In melioidosis patients, we identified a trend toward the independent association of TLR4896G with death (OR of death: 4.97; 95% CI: 0.82–30.0; p = 0.08 for a dominant model). TIRAP558C>T has a MAF ~5% (13) but in melioidosis patients, TIRAP558T was not significantly associated with death (OR of death: 0.83; 95% CI: 0.44–1.58; p = 0.58 for a dominant model). In the model of the association of TLR51174T with death, neither TLR4896G nor TIRAP558T was significant (p = 0.06 or p = 0.49, respectively) or changed the main effect of TLR51174T. In the final model of the association of TLR51174T with death, adjusting for age, diabetes, renal insufficiency, pneumonia, and bacteremia, the protective effect remained very strong (OR: 0.20; 95% CI: 0.08–0.50; p = 0.001).

We plotted Kaplan–Meier in-hospital survival curves by TLR51174C>T genotype for all melioidosis cases (Fig. 2). A significant difference was noted in the risk of death between 0 and 30 d for individuals having TLR51174CC genotypes compared with those having TLR51174CT or TLR51174TT genotypes by the log-rank test (p = 0.001).

To test whether systemic ancestry differences (population stratification) between cases and controls contributed substantially to our findings, we examined the frequency of 25 unrelated single nucleotide polymorphisms from across the genome (13, 25). We compared the allele frequency of these variants in HapMap European ancestry and Han Chinese Beijing populations and selected the 17 variants with a χ² > 1.5 (mean χ² = 22.3). These variants were considered maximally variable between the two populations and might therefore serve as markers of population variation in our cohort. We genotyped these variants in the subset of 434 melioidosis subjects whose DNA was assayed on the Sequenom platform and compared allele frequencies in nonsurvivors with those in survivors (Supplemental Table I). The mean χ² of all 17 variants was 1.14, suggesting little population stratification (26).

**TLR51174T is associated with protection against organ failure in human melioidosis**

To determine whether the TLR51174C>T variant was associated with an intermediate phenotype in melioidosis, we identified subjects in our cohort whose hospitalizations were complicated by organ failure, defined as shock or respiratory failure. Of genotyped subjects, 32% (177 of 552) developed organ failure during their hospitalization. Of the subjects with organ failure, 7.9% were...

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**Table I. Characteristics of subjects with melioidosis**

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>All (n = 600)</th>
<th>Nonsurvivors (n = 143)</th>
<th>Survivors (n = 457)</th>
</tr>
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<tr>
<td>Mean age</td>
<td>48.6</td>
<td>51.7</td>
<td>47.7</td>
</tr>
<tr>
<td>Female sex</td>
<td>46</td>
<td>43</td>
<td>47</td>
</tr>
<tr>
<td>Pre-existing conditionb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>52</td>
<td>44</td>
<td>55</td>
</tr>
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<td>Chronic liver disease</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>7</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Steroid use</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteremia</td>
<td>50</td>
<td>73</td>
<td>43</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>38</td>
<td>57</td>
<td>33</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>12</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Clinical course</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td>26</td>
<td>61</td>
<td>15</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>18</td>
<td>68</td>
<td>2</td>
</tr>
<tr>
<td>Organ failurec</td>
<td>32</td>
<td>81</td>
<td>17</td>
</tr>
</tbody>
</table>

Numbers are a percentage except for mean age.

*Missing data for 4 subjects (bacteremia), 4 subjects (pneumonia), 4 subjects (urinary tract infection), 39 subjects (shock), 29 subjects (respiratory failure), 40 subjects (organ failure).

bUnknown pre-existing conditions are considered negative.
cOrgan failure is defined as presence of shock or respiratory failure.
genotype $\text{TLR5}_{1174T}$ or $\text{TLR5}_{1174C}$, compared with 16.3% of subjects who did not develop organ failure (Table II). In an unadjusted dominant model of the association of the $\text{TLR5}_{1174C}$ with organ failure, the OR was 0.44 (95% CI: 0.24–0.81; $p = 0.009$). Age, diabetes, pneumonia, bacteremia, and urinary tract infection were independently associated with organ failure, and so were considered in the adjusted model. In the final model incorporating diabetes, pneumonia, and bacteremia, the OR for the association of $\text{TLR5}_{1174C}$ with organ failure was 0.37 (95% CI: 0.19–0.71; $p = 0.003$).

$\text{TLR5}_{1174T}$ does not mediate activation of NF-\(\kappa\)B by B. pseudomallei

We next assessed how $\text{TLR5}_{1174T}$ modulates innate immune activation by B. pseudomallei in isolation in vitro. As HEK-293 cells do not endogenously express TLRs (27), we transiently transfected HEK-293 cells with either $\text{TLR5}_{1174C}$ or $\text{TLR5}_{1174T}$ and an NF-\(\kappa\)B luciferase reporter before stimulating the cells with heat-killed log phase B. pseudomallei (Fig. 3). In contrast to $\text{TLR5}_{1174C}$, $\text{TLR5}_{1174T}$ was unable to mediate B. pseudomallei–induced NF-\(\kappa\)B activation.

![Graph showing Survival from melioidosis](image)

**FIGURE 2.** Survival from melioidosis is enhanced for carriers of $\text{TLR5}_{1174T}$. Kaplan–Meier in-hospital survival curves are plotted for melioidosis subjects, grouped by genotype. Curves are significantly different by the log rank test ($p = 0.001$).

B. pseudomallei induces reduced blood cytokine responses ex vivo in carriers of $\text{TLR5}_{1174T}$

Together, these findings suggested that carriers of $\text{TLR5}_{1174T}$ may generate impaired inflammatory responses during melioidosis infection that result in reduced organ failure and lower mortality. To establish whether $\text{TLR5}_{1174C}$ is associated with altered cytokine production in response to B. pseudomallei in humans, we obtained blood from 11 healthy white subjects of known $\text{TLR5}_{1174C}$ genotype. We stimulated fresh whole blood ex vivo with heat-killed B. pseudomallei 1026b. We measured IFN-\(\gamma\), IL-10, IL-12p70, IL-1\(\beta\), IL-6, IL-8, and TNF-\(\alpha\) in plasma. We found no $\text{TLR5}_{1174C}$–dependent differences in raw cytokine production induced by B. pseudomallei. We have found that cytokine levels induced by B. pseudomallei are dependent on leukocyte count (N. Chantratita, submitted for publication). The $\text{TLR5}_{1174C}$ genotype was not associated with total leukocyte count ($p = 0.92$), but heterozygous or homozygous carriers of $\text{TLR5}_{1174T}$ had significantly higher monocyte counts than did wild-type subjects (643 ± 177 versus 369 ± 24 cells per microliter, $p = 0.029$). As monocytes are central drivers of the innate immune response, we normalized cytokine values to monocyte count and compared responses by genotype (Fig. 4). We found that IL-10 and IL-6 levels were significantly lower in carriers of $\text{TLR5}_{1174T}$ ($p = 0.04$ and $p = 0.009$, respectively). A trend toward lower IL-8 levels ($p = 0.059$) was noted. Although the sample size was small, these data supported a functional effect of $\text{TLR5}_{1174T}$ in innate immune responses to B. pseudomallei.

To confirm this effect in a larger cohort and to determine whether this effect was also apparent in a Thai population susceptible to melioidosis, we obtained blood from 300 healthy blood donors at Sappasithiprasong Hospital. We stimulated fresh whole blood with LPS, flagellin, or heat-killed B. pseudomallei 1026b or K96243. We quantified IL-6, IL-8, TNF-\(\beta\), IL-10, MCP-1, IL-1\(\alpha\), G-CSF, and IL-1\(\beta\) levels in plasma. We genotyped the subjects at $\text{TLR5}_{1174C}$ and found that 269 (90%) subjects were major homozygotes, 31 (10%) were heterozygotes, and 0 (0%) were homozygotes (MAF 5.2%). In this cohort, we did not observe any $\text{TLR5}_{1174C}$–dependent differences in total leukocyte count ($p = 0.22$) or monocyte count ($p = 0.54$). Flagellin stimulation

![Graph showing NF-\(\kappa\)B activation](image)

**FIGURE 3.** NF-\(\kappa\)B activation induced by B. pseudomallei is abrogated by $\text{TLR5}_{1174T}$. HEK-293 cells transiently transfected with human $\text{TLR5}_{1174C}$ or $\text{TLR5}_{1174T}$, NF-\(\kappa\)B–dependent firefly ELAM luciferase, and control thymidine kinase–driven Renilla luciferase were stimulated with media alone, IL-1\(\beta\) 20 ng/ml, S. typhimurium flagellin 100 ng/ml, or log phase heat-killed B. pseudomallei 1026b at various concentrations in colony-forming units per milliliter. NF-\(\kappa\)B activation was determined by the ratio of ELAM to Renilla light emission after 24 h. Data plotted are means ± SDs of triplicate or quadruplicate conditions that represent one of two similar experiments performed independently. ***$p \leq 0.001$ by ANOVA with the Bonferroni posttest for comparisons between similarly stimulated cells transfected with empty vector $\text{TLR5}_{1174C}$ or $\text{TLR5}_{1174T}$.
of blood from subjects with the TLR5<sub>1174CT</sub> genotype, compared with TLR5<sub>1174CC</sub> subjects, induced significantly lower levels of all raw cytokine responses except IL-1ra (e.g., median TNF-α concentrations were 260.6 pg/ml compared with 632.7 pg/ml, \( p < 0.001 \)) and significantly lower levels of all monocyte-normalized cytokine responses (Fig. 5). Stimulation with LPS, a TLR4 agonist, induced no TLR5<sub>1174C</sub>T-dependent difference in raw or normalized cytokine levels. Stimulation with both strains of B. pseudomallei induced no significant differences in raw cytokine responses, but both strains induced significantly less monocyte-normalized IL-10, and a trend toward lower monocyte-normalized IL-8 levels was observed (\( p = 0.057 \) and \( p = 0.084 \)) in TLR5<sub>1174CT</sub> subjects. K96243 also induced significantly less G-CSF in TLR5<sub>1174CT</sub> subjects after normalization for monocyte count.

**Discussion**

The results of this study provide compelling evidence of the importance of TLR5 as a regulator of the host response in melioidosis. In the largest investigation to date of genetic variation as a determinant of outcome from melioidosis, we show that B. pseudomallei induces a TLR5-dependent innate immune response and that a TLR5 genetic variant that encodes a defective TLR5 protein is associated with marked protection against death or organ failure in Thais. We further demonstrate a functional effect of this variant in the blood cytokine response to flagellin and to killed B. pseudomallei.

Although TLR5<sub>1174C</sub>T is associated with susceptibility to Legionnaires’ disease (Legionella pneumophila pneumonia) and recurrent urinary tract infections (6, 28), our study is the first, to our knowledge, to identify an association of the variant with outcome from infection. TLR5<sub>1174T</sub> has previously been shown to blunt flagellin-dependent IL-6 and IL-8 release in whole blood (6, 14). In addition, we show that levels of IL-10, TNF-α, MCP-1, G-CSF, IL-1ra, and IL-1β induced by flagellin are also reduced in carriers of the variant. It is therefore tempting to postulate that the hypofunctional TLR5 variant broadly impairs the inflammatory response to flagellated infection, resulting in reduced sepsis-induced organ failure and death. This idea is consistent with other studies showing that carriage of the variant is protective in inflammatory conditions, including systemic lupus erythematosus, cystic fibrosis (the variant is associated with a higher body mass index), and Crohn’s disease (29–31). However, IL-10 is the sole cytokine that is consistently reduced in carriers of TLR5<sub>1174T</sub> from...
two different populations upon stimulation of blood with whole B. pseudomallei, suggesting an effect of the variant that is restricted to IL-10 production when innate immunity is simultaneously activated by multiple ligands. IL-10 is an anti-inflammatory cytokine clearly implicated in sepsis, although its role in modulating the host response during this complex and dynamic process remains poorly understood (32, 33). In a clinical trial of antibiotic therapy in melioidosis, baseline IL-10 levels were increased in non-survivors of melioidosis, compared with those in survivors, and the IL-10 level was an independent predictor of mortality (34). A recent study of inhalation melioidosis in African green monkeys found that increased IL-10 levels correlated with reduced survival time on day four following infection (35). These data are concordant with our findings. Conceivably, impaired IL-10 release in carriers of TLR5<sub>1174C</sub>T may, in fact, enhance inflammation and control of the invading pathogen in the early stages of infection, resulting in improved organ function and survival. Further investigation is required to elucidate the TLR5-driven and IL-10-dependent mechanisms at play during melioidosis.

We have previously examined the association of TLR genetic variants with susceptibility to melioidosis (13) but did not detect any relationship between TLR5<sub>1174C</sub>T and disease. These contrasting observations underscore the very different roles the innate immune system plays in modulating host susceptibility to infection versus governing outcome once infection is established. It is often difficult to ascertain the specific route of infection—percutaneous versus inhaled—in clinical cases of melioidosis. However, the differential associations of TLR5<sub>1174C</sub>T with melioidosis compared with Legionella pneumonia further highlight the importance of additional immune modulators in governing clinical responses to airborne infection caused by flagellated organisms. The protective effect of TLR5<sub>1174T</sub> on outcome from melioidosis and in susceptibility to various inflammatory conditions may explain the relatively high frequency of the hypofunctional allele in a variety of populations.

Our genetic association study has several limitations. Despite our best efforts to select cases and controls appropriately and test for population stratification, unmeasured population stratification may bias our analyses. A second limitation is that the observed mortality in this cohort was lower than in previous descriptions of outcome from melioidosis at this hospital (36). This observation is most likely due to a bias in this study against subjects who died extremely rapidly after admission and did not have blood collected for genotyping by the study team. In addition, our genetic association will require replication in a second, independent population. However, as Sappasitprasong Hospital is one of the few sites with sufficient infrastructure to perform such a genetic study in melioidosis, we designed as large a clinical study as possible at this single site.

Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Table I: Frequency distribution of population stratification SNPs in melioidosis subjects

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome</th>
<th>Status</th>
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<th>Allele 2 Count</th>
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<td>rs1028184</td>
<td>2</td>
<td>Survivors</td>
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<td>364</td>
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<td>0.78</td>
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<td></td>
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<td>Non-survivors</td>
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<td>113</td>
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<td>606</td>
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<td>179</td>
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Non-survivors
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