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Toll-Like Receptor 4 Region Genetic Variants are Associated with Susceptibility to Melioidosis

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Abstract

Melioidosis is a tropical infection caused by the Gram-negative soil saprophyte *Burkholderia pseudomallei*. Despite broad exposure of northeast Thais, disease develops in only a small proportion of individuals. Although diabetes is a risk factor, the mechanisms of host susceptibility to melioidosis are still poorly understood. We postulated that Toll-like receptors (TLRs) regulate host susceptibility to disease, and that genetic variation in TLRs is associated with melioidosis. We analyzed the frequency of eight previously described TLR pathway polymorphisms in 490 cases compared to 950 non-hospitalized controls or 458 hospitalized controls. Based on these results, we then analyzed the frequency of additional *TLR4* or *TLR6-1-10* region polymorphisms in cases and controls. We found that the $TLR4_{1196C>T}$ variant was associated with protection from melioidosis when compared to non-hospitalized controls. The $TLR1_{742A>G}$ and $TLR1_{-7202A>G}$ variants were associated with melioidosis when compared to hospitalized controls. In further analyses, we found that two additional *TLR4* region polymorphisms were associated with disease. In diabetics, three other *TLR6-1-10* region polymorphisms were associated with disease when compared to hospitalized controls. We conclude that TLR genetic variants may modulate host susceptibility to melioidosis. Confirmation of these findings and further investigation of the mechanisms is required.

Keywords

melioidosis; *Burkholderia pseudomallei*; infection; Toll-like receptor; innate immunity; genetic variation

Introduction

Melioidosis is a tropical infection caused by the Gram-negative soil saprophyte *Burkholderia pseudomallei*. Disease may occur after bacterial inhalation, ingestion or cutaneous inoculation. Clinical manifestations are diverse but lung involvement is very common.[1] Mortality from melioidosis in northeast Thailand is 40%. Exposure to *B. pseudomallei* in northeast Thais appears widespread as seropositivity occurs in ~70% of children by the age four but annual incidence is about 21 cases per 100 000.[2, 3] Diabetes, present in about 50% of cases, is the main risk factor, but whether there are other explanations for the low incidence of disease given the high exposure to the bacterium remains unclear.[1]

A genetic influence on susceptibility to infection has been clearly established.[4] Two small studies implicate genetic variation in the development of melioidosis.[5, 6] Previous human genetic studies of Gram-negative infections have predominantly examined sepsis with heterogeneous microbial etiologies rather than large populations with infections caused by a single bacterium. Genetic studies of pneumonia have similarly been limited by small sample sizes for any single pathogen. Study of host genetics in a large cohort of melioidosis subjects, including a sizable number of pneumonic cases, is therefore pertinent.

Toll-like receptors (TLRs) are pathogen recognition receptors that initiate an inflammatory response upon ligation by conserved motifs on invading pathogens.[7] TLR pathway genetic

variation is associated with susceptibility to various infections or altered outcome from infection in numerous studies.[8] Experimental data indicate that TLRs 2, 4, and 5 modulate the host response to *B. pseudomallei*, likely activated by bacterial lipopeptides, lipopolysaccharide, and flagellin, respectively.[9, 10] (West, unpublished data). TLRs 1 and 6 form heterodimers with TLR2.[7] TIRAP (also known as MAL) is an adaptor molecule that is recruited upon ligation of TLRs 2 or 4.[7] We performed a case-control candidate gene study at a large referral hospital in northeast Thailand to test the hypothesis that variation in TLR pathway genes is associated with the development of melioidosis in a widely exposed population. The study was undertaken in two parts: First, a primary list of well characterized single nucleotide polymorphisms (SNPs) in TLR pathway genes was defined and analysed. Second, significant SNP associations prompted the analysis of additional variants from the pertinent gene regions.

Results

Four hundred and ninety *B. pseudomallei* culture-positive cases, 950 non-hospitalized controls presenting to outpatient clinic or the blood donation center, and 458 *B. pseudomallei* culture-negative hospitalized controls with clinical signs of infection were identified at Sappasithiprasong Hospital, Ubon Ratchathani, Thailand. Characteristics of cases and controls are shown in Table 1.

We first tested the association between eight well characterized TLR pathway gene SNPs and susceptibility to melioidosis in cases compared to non-hospitalized controls. These SNPs are either well defined functionally or have been associated with susceptibility or outcome to infection in multiple studies.[8, 11–26] Only $TLR4_{1196C>T}$ was significantly associated with melioidosis in a general genetic model comparing genotype frequencies (p=0.05) (Table 2). In a dominant model adjusted for age, sex, and diabetes status, the variant significantly conferred over a three fold protective effect (OR 0.29, 95% CI: 0.09–0.99, p=0.05). Unlike in Caucasian populations, $TLR41_{196C>T}$ and $TLR4_{896A>G}$ were not in strong LD ($r^2 = 0.69$) and the MAF for each variant was ~1%. In the subgroups of cases with bacteremia or pulmonary involvement, no variants were significantly associated with disease (Supplemental table 1).

We then tested the association of the primary SNPs with melioidosis compared to hospitalized controls. As most melioidosis cases were identified by screening hospitalized patients, this control group may better represent the source population. None of the SNPs was significantly associated with disease in unadjusted models but in recessive models adjusted for age, sex, and diabetes, associations of $TLR1_{742A>G}$ and $TLR1_{-7202A>G}$ with disease were significant (Table 2). In adjusted analyses, both of these variants were significantly associated with bacteremia and $TLR1_{742A>G}$ was associated with pulmonary involvement compared to hospitalized controls (Table 3). $TLR1_{742A>G}$ and $TLR1_{-7202A>G}$ were in high LD (r^2= 0.84) and had similar allele frequencies to $TLR1_{742A>G}$ in a Vietnamese population.[26] In these adjusted models, the effect of diabetes on case control status was very strong (OR ~3–4) (Tables 2 and 3), prompting us to evaluate whether a diabetes-specific effect of *TLR1* variants existed. Further analyses were performed to test the association of each *TLR1* SNP in melioidosis cases compared to hospitalized controls,

stratifying by diabetes status. No significant associations were observed (Supplemental Table 2).

To test for population stratification between cases and controls, 25 independent SNPs were genotyped.[27] We conducted allelic analyses calculating the χ^2 statistic for 24 these SNPs (rs169476 had no variation) and determined the mean χ^2 (Supplemental Table 3). For cases compared to non-hospitalized controls and to hospitalized controls, respectively, the mean χ^2 was 1.18 and 0.96. The proximity of these numbers to one suggested that minimal population stratification exists.[28]

Asian populations are underrepresented in studies of TLR pathway genetic variation and disease. Given the initial findings of associations of *TLR4* and *TLR1* variants with melioidosis, in the second phase of the study additional coding SNPs and haplotype tagging SNPs in the *TLR4* and *TLR6-1-10* regions in Asian populations (selected as described in the methods) were analyzed. Eight *TLR4* region SNPs and 18 *TLR6-1-10* region SNPs (Supplemental Table 4) were tested for associations with melioidosis compared to each of the two control groups.

Two of the eight *TLR4* region SNPs showed an association with melioidosis (Table 4). rs10818066 was significantly associated with melioidosis in an unadjusted model when tested versus non-hospitalized controls but not when compared to hospitalized controls. In an adjusted model, the effect of the variant was significantly protective for both sets of control groups. When compared to each control group, rs960312 was significantly associated with disease in unadjusted models and the variant significantly increased susceptibility in adjusted models. Applying a conservative Bonferroni correction for multiple comparisons, several associations remained significant (Table 4). In subsequent adjusted analyses, rs10818066 was associated with bacteremic or pulmonary melioidosis when compared to either control group (Supplemental Table 5). rs960312 was associated with bacteremic and pulmonary melioidosis in unadjusted analyses when compared to hospitalized controls. In adjusted models, the variant was associated with pulmonary melioidosis compared to either control group.

None of the three *TLR4* region variants, $TLR4_{1196C>T}$, rs10818066, or rs960312, was in high LD with each other (Fig. 1). To examine the combined effects of these polymorphisms on melioidosis susceptibility, haplotypes with frequencies >1% were constructed (Table 5). The association with disease was examined compared to each of the two control groups using additive, dominant, and recessive models. The effects observed were comparable to those attributable to rs10818066 or rs960312 in isolation.

The associations with disease of *TLR6-1-10* region SNPs were examined, stratifying by diabetes status based on our initial analysis. There were few significant associations among subjects without diabetes (Supplementary Table 6) or in diabetics when comparing melioidosis cases to non-hospitalized controls (Table 6). Comparing diabetic melioidosis cases to hospitalized diabetic controls, three of 16 SNPs in Hardy Weinberg equilibrium were strongly associated with disease: rs2087465, rs3924112, and rs5743794 (Table 6). In adjusted models, these associations persisted. The association of rs2087465, a non-coding

SNP in the *TLR6* region was significant even after applying a Bonferroni correction. In an adjusted analysis the magnitude of protection conferred by this variant was particularly large (OR 0.13, 95% CI: 0.03–0.48, p=0.002).

None of these three SNPs was in strong LD with each other (r^2 for each pair <0.7). Haplotypes were constructed with all three SNPs and the association with disease was tested in hospitalized diabetics. The additive model haplotype comprised of the rare allele at all three loci resulted in the largest significant effect (OR 0.49, 95% CI: 0.33–0.75, p=0.001).

Discussion

This study of nearly 1 900 Thais at risk for melioidosis is largest study of human genetic variants and susceptibility to melioidosis, and the first to examine genetic variations in pattern recognition receptor pathways. This study is also notable as one of the few large investigations of host genetic factors underlying Gram-negative infection. Based on an abundant literature implicating TLR pathway variants in susceptibility to or outcome from infection, this pathway was targeted in our analysis of melioidosis patients. Our main findings are that *TLR4* region gene variants are associated with melioidosis, and in hospitalized diabetics, *TLR6-1-10* gene variants are associated with differential susceptibility to melioidosis than to other illnesses.

As the primary host receptor for LPS, TLR4 is the canonical TLR for Gram-negative pathogens. *B. pseudomallei* is a Gram negative pathogen that induces an inflammatory response that is TLR4-dependent and *B. pseudomallei* LPS is a TLR4 agonist.[9] Therefore there is compelling *in vitro* evidence for the importance of TLR4 in melioidosis. The role of TLR4 in mouse models of respiratory *Burkholderia* infection is less apparent[10, 29] but in human cases of melioidosis, TLR4, MD2, and CD14 are all expressed at higher levels than in controls.[10]

Polymorphisms in *TLR4* have been extensively studied.[8] The two best known SNPs are at positions 896 and 1196. They are typically in high LD in Caucasian populations, where they occur more frequently than in Asian populations. Several studies suggest that these SNPs are associated with susceptibility to sepsis[18, 30] but not to meningococcal or pneumococcal infection.[31–35] The SNPs are linked with resistance to legionellosis and to recurrent UTIs[23, 36] Thus their role may be population- and infection-specific. In this study, a substantial protective effect of the extremely rare $TLR4_{1196C>T}$ allele was observed when compared to non-hospitalized controls but not in comparison to hospitalized controls. A likely explanation is that many hospitalized control subjects had other infections or undiagnosed melioidosis that are similarly associated with a lower frequency of the minor allele. A role for TLR4 in human melioidosis is greatly supported by our additional findings of an association with disease attributable to two other *TLR4* region variants, regardless of control group chosen for comparison. The rs10818066 variant also conferred protection against melioidosis but the rs960312 variant was associated with susceptibility to disease. A comparable pattern of effect for $TLR4_{1196C>T}$ and rs960312 was observed in a study of genetic associations with liver fibrosis in Caucasians.[37] That rs10818066 and rs960312 are located in intergenic regions and not in LD with $TLR4_{1196C>T}$ suggests that these SNPs are

in LD with other unidentified causative variants. We hypothesize that altered TLR4-dependent host responses to *B. pseudomallei* LPS in carriers of these causative variants modulate host susceptibility to successful infection by the invading pathogen. Resequencing of the *TLR4* region in this little studied population and careful assessments of functional effects of variants will be required to further test this hypothesis. In aggregate, these data provide the strongest evidence to date that TLR4 is an important element of host defense in human melioidosis.

Our data also suggest that in diabetics, *TLR6-1-10* region variants regulate differential susceptibility to melioidosis compared to other illnesses. While the function of TLR10 in humans remains unclear, TLRs 1 and 6 form heterodimers with TLR2 to permit signaling upon ligation by bacterial cell wall components. Both TLR1 and 6 augment TLR2-dependent signaling upon stimulation with heat killed *B. pseudomallei*.[9] TLR2-deficiency may heighten the cytokine response to *B. pseudomallei* in macrophages and confers protection in murine studies of respiratory infection.[9, 10] In Caucasians, the high LD *TLR1* SNPs *TLR1*$_{742A>G}$, *TLR1*-$_{7202A>G}$, and TLR11804G>T are linked with immunomodulatory effects and sepsis outcomes.[13, 26] Diabetes is a defined risk factor for melioidosis, and studies have demonstrated *B. pseudomallei*-specific defects in neutrophil function such as phagocytosis, reduced chemotaxis, and resistance to apoptosis.[38] Altered TLR signaling occurs in diabetics[39] but this has not been extensively studied during infection. Here, we demonstrate that in diabetics, *TLR6-1-10* region variants are associated with differential susceptibility to melioidosis than to other illnesses. We did not find that these variants are associated with susceptibility to melioidosis compared to otherwise healthy subjects. Homozygosity of the rs2087465 minor allele confers a nearly eight-fold protective effect against melioidosis in hospitalized diabetics. As the frequency of this allele is comparable in diabetics with melioidosis and in otherwise healthy diabetics, however, an alternative explanation is that this allele markedly heightens susceptibility to non-melioidosis illness in diabetics. Review of available diagnoses in the hospitalized diabetic controls revealed a large spectrum of infectious illnesses, including pneumonia, sepsis, tuberculosis, leptospirosis, as well as non-infectious processes. Thus, our data provide evidence that the relative importance of different innate immune receptors may vary depending on underlying diseases as well as on specific infection. Additional study of genetic variation in *TLR6-1-10* in both non-diabetics and diabetics in this population is indicated.

There are several limitations to this study. It is possible that our cases were not selected from the same population as controls – a rationale for selecting two control groups – but we did not observe significant population stratification. The exposure of the cases and controls may have differed but studies have shown widespread seropositivity to *B. pseudomallei* in the local population.[2] While replicating genetic associations in another cohort is desirable, there are few locations where a comparable study of melioidosis host genetics could be undertaken. Thus, as large a study as possible with multiple control groups was designed. The results underscore the importance of generating additional genetic data in Thai and other southeast Asian populations.

In conclusion, *TLR4* genetic variants are associated with melioidosis in a Thai population. In diabetic populations, *TLR6-1-10* variants are associated with differential susceptibility to

melioidosis compared to other illnesses. Further investigations of causative genetic variants and mechanisms of susceptibility in this population are required.

Methods

Clinical study design

Cases (n=490) were identified among inpatients at Sappasithiprasong Hospital, Ubon Ratchathani, northeast Thailand from 1999 to 2005. A study team screening patients with clinical signs of infection cultured blood, urine, and other relevant samples (e.g. abscess aspirates) for *B. pseudomallei*.[40] Case status was defined by a positive culture for *B. pseudomallei* from a sample collected by the study team or independently by hospital clinicians. Two separate groups of control subjects were defined. The first group totaled 950 non-hospitalized subjects. As the majority of melioidosis cases have underlying diabetes, this control group combined 475 healthy individuals who presented to the blood donation center and 475 otherwise healthy diabetics recruited from the outpatient diabetes clinic at the hospital between 2007 and 2008. A second control group was comprised of 458 hospitalized subjects with clinical signs of infection who were screened for melioidosis by the study team but were culture negative for *B. pseudomallei*. An exclusion criterion for all control subjects was a previous history of melioidosis. The University of Washington Human Subjects Division Institutional Review Board, Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand, and the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand approved this study.

Genomic methods

From the literature single nucleotide polymorphisms in TLR pathway genes (*TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, TIRAP*) with well defined functional effects or associated with altered susceptibility to or outcome from infection were identified. Due to little published data on genetic variation in Thais, additional SNP identification and election was performed using the Genome Variation Server (http://gvs.gs.washington.edu/GVS/). Coding SNPs in candidate genes were selected. Within the region encompassed by 50,000 bases upstream and downstream of each candidate gene, SNPs with a minor allele frequency (MAF) ≥ 2% in populations identified as Japanese, Chinese, and Asian were binned into groups with $r^2 \geq 0.8$ to identify haplotype tagging SNPs. DNA was extracted from whole blood using Nucleon BACC3 kits (Amersham Biosciences). Genotyping was performed using an allele-specific primer extension method (Sequenom, Inc.) with reads by a MALDI-TOF mass spectrometer.[41]

Statistical methods

The study analysis was undertaken in two phases. First, a primary list of SNPs was defined: $TIRAP_{537C>T}$, $TIRAP_{558C>T}$, $TLR1_{1804G>T}$, $TLR2_{597C>T}$, $TLR2_{2258G>A}$, $TLR4_{896A>G}$, $TLR4_{1196C>T}$, and $TLR5_{1174C>T}$. Each SNP has either been associated with susceptibility to infection or outcome from infection in a prior study or has been shown to regulate cell function.[11–25] In Caucasian populations, $TLR1_{1804G>T}$ is in high linkage disequilibrium (LD) with two other *TLR1* SNPs: $TLR1_{742A>G}$, another non-synonymous coding SNP and $TLR1\text{-}_{7202A>G}$, a tagging SNP[13, 26], although $TLR1_{742A>G}$ and $TLR1_{1804G>T}$ are not in LD

in a Vietnamese population.[26] *TLR1*$_{1804G>T}$ could not be readily accommodated in our plex design so both *TLR1*$_{742A>G}$ and *TLR1*-$_{7202A>G}$ were genotyped instead. The MAF for *TLR2*$_{2258G>A}$ was 0% in our population. Therefore, the eight final SNPs analyzed were: *TIRAP*$_{537C>T}$ (rs8177374), *TIRAP*$_{558C>T}$ (rs7932766), *TLR1*$_{742A>G}$ (rs4833095), *TLR1*-$_{7202A>G}$ (rs5743551), *TLR2*$_{597C>T}$ (rs3804099), *TLR4*$_{896A>G}$ (rs4986790), *TLR4*$_{1196C>T}$ (rs4986791), and *TLR5*$_{1174C>T}$ (rs5744168). The frequency of these SNPs was compared in cases versus controls. Based on the initial results suggesting hits for *TLR4* and *TLR1* SNPs, additional variants in these genes were examined in the second phase of the analysis. *TLR1* is part of a locus comprising *TLR6*, *TLR1*, and *TLR10*, so SNPs from this entire locus were selected. The secondary SNPs are listed in Supplemental Table 4.

SNPs were first examined for deviation from Hardy-Weinberg equilibrium in the control populations studied using the Fischer exact test. The association between genotype and disease was analyzed using χ^2 test except when cell values in the table < 10, in which case the Fisher exact test was chosen. Logistic regression was performed with an appropriate genetic model (dominant or recessive), adjusting for age, sex, and (where suitable) diabetes status. In the initial study phase, two additional analyses were performed defining cases as the subgroup of patients with bacteremia or as those with lung abnormalities or pleural effusions. No adjustment was made for multiple comparisons in this initial phase because of previously demonstrated associations or functional effect of each of the eight primary SNPs. Twenty five unrelated SNPs from across the genome were genotyped and the mean χ^2 statistic for the comparison of allele frequencies between cases and non-hospitalized controls was examined as a measure of population stratification.[27, 28] In the subsequent study phase, a conservative Bonferroni correction was applied to multiple comparisons to maintain the desired family-wise type I error rate. All analyses were performed with Stata version 11.1 (College Station, Texas) incorporating genhw and haplologit functions. P-values ≤0.05 were considered significant. LD mapping was performed with Haploview v4.2.[42]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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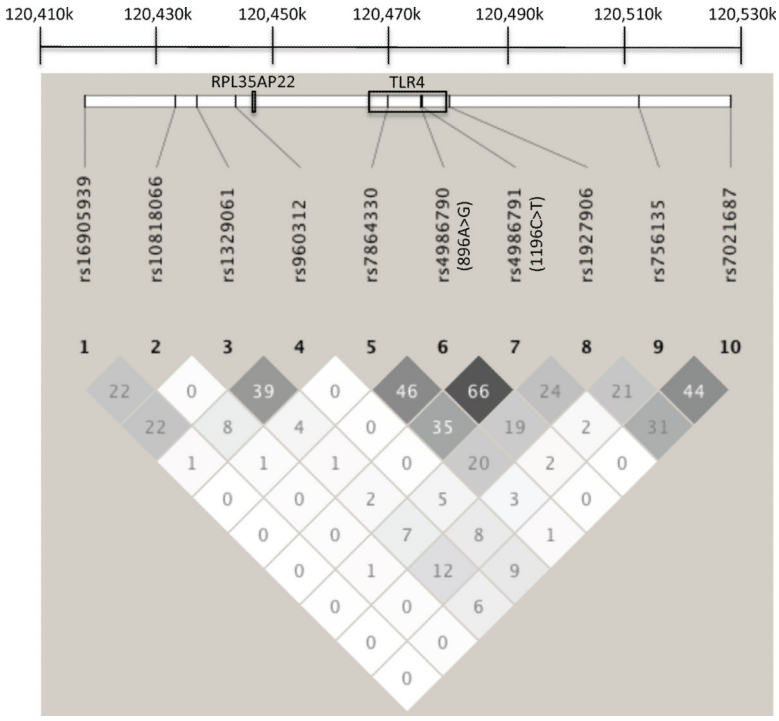

Figure 1.

Linkage disequilibrium of *TLR4* region single nucleotide polymorphisms in Thais. Genetic map indicates location of SNPs relative to *TLR4* on chromosome 9. Numbers within LD map denote R^2 values. Figure generated by Haploview and modified.

Table 1

Characteristics of cases and controls

	Cases	Non-hospitalized Controls	Hospitalized Controls
Number	490	950	458
Median age (IQR)	49 (39–60)	47 (29–60)	58 (47–68)
Male (%)	51	48	51
Diabetes (%)	56	50	28
Bacteremia (%)	51		
Lung infection (%)	41		

Table 2

Associations of TLR pathway genetic variants with melioidosis

SNP		Unadjusted[1]				Adjusted[2]		
		Genotype			p	Model	OR (95% CI)	p
		G/G	G/A	A/A				
TLR1 742A>G	Cases	126	246	117				
	Non-hospitalized controls	271	459	215	0.50	Rec	1.09 (0.84–1.41)	0.53
	Hospitalized controls	133	230	93	0.31	Rec	1.44 (1.02–2.03)	0.04
		G/G	G/A	A/A				
TLR1 −7202A>G	Cases	132	227	128				
	Non-hospitalized controls	263	462	223	0.51	Rec	1.20 (0.93–1.55)	0.16
	Hospitalized controls	128	227	96	0.20	Rec	1.52 (1.08–2.13)	0.02
		T/T	T/C	C/C				
TLR2 597C>T	Cases	302	160	23				
	Non-hospitalized controls	603	301	37	0.68	Dom	1.05 (0.83–1.32)	0.68
	Hospitalized controls	294	147	14	0.39	Dom	1.10 (0.82–1.48)	0.51
		A/A	A/G	G/G				
TLR4 896A>G	Cases	484	5	0				
	Non-hospitalized controls	923	17	1	0.58	Dom	0.55 (0.20–1.49)	0.24
	Hospitalized controls	454	3	0	0.73	Dom	1.44 (0.30–6.84)	0.65
		C/C	C/T	T/T				
TLR4 1196C>T	Cases	486	3	0				
	Non-hospitalized controls	925	19	1	0.05	Dom	0.29 (0.09–0.99)	0.05
	Hospitalized controls	454	3	0	1.00	Dom	1.10 (0.19–6.35)	0.92
		C/C	C/T	T/T				
TLR5 1174C>T	Cases	425	59	1				
	Non-hospitalized controls	835	103	4	0.66	Dom	1.09 (0.77–1.54)	0.63
	Hospitalized controls	404	50	1	0.81	Dom	0.97 (0.62–1.51)	0.88
		C/C	C/T	T/T				
TIRAP 539C>T	Cases	461	25	0				
	Non-hospitalized controls	898	45	1	0.87	Dom	1.02 (0.61–1.70)	0.94

SNP		Unadjusted[1]				Adjusted[2]		
		Genotype			p	Model	OR (95% CI)	p
TIRAP 558C>T	Hospitalized controls	436	21	0	0.76	Dom	1.39 (0.72–2.71)	0.33
		C/C	C/T	T/T				
	Cases	439	45	2				
	Non-hospitalized controls	838	104	4	0.56	Dom	0.86 (0.60–1.24)	0.43
	Hospitalized controls	405	48	1	0.66	Dom	0.84 (0.53–1.33)	0.46

[1] χ^2 or, if cell count <10, Fisher's exact tests of association were performed for cases versus each control group

[2] Dominant or recessive logistic regression models adjusted for age, sex, and diabetes status were performed for cases versus each control group

Table 3

Associations of *TLR1* variants with bacteremic or pulmonary melioidosis compared to hospitalized controls

SNP		Unadjusted[1]				Adjusted[2]		
		Genotype			p	Model	OR [95% CI)	p
		G/G	G/A	A/A				
TLR1 742A>G	Bacteremic cases	74	114	60	0.42	Rec	1.52 (1.00–2.30)	0.05
	Hospitalized Controls	133	230	93				
		G/G	G/A	A/A				
TLR1 −7202A>G	Bacteremic cases	74	107	65	0.17	Rec	1.55 (1.03–2.34)	0.04
	Hospitalized Controls	128	227	96				
		G/G	G/A	A/A				
TLR1 742A>G	Pulmonary cases	46	111	39	0.27	Dom	1.59 (1.04–2.41)	0.03
	Hospitalized Controls	133	230	93				
		G/G	G/A	A/A	0.74	Dom	1.40 (0.93–2.12)	0.11
TLR1 −7202A>G	Pulmonary cases	51	105	40				
	Hospitalized Controls	128	227	96				

[1] χ^2 or, if cell count <10, Fisher's exact tests of association were performed

[2] Dominant or recessive logistic regression models were adjusted for age, sex, and diabetes status

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Table 4

Associations of *TLR4* region variants with melioidosis

SNP		Unadjusted[1]				Adjusted[2]		
		Genotype			p[3]	Model	OR [95% CI)	p
rs10818066		T/T	T/C	C/C				
	Cases	178	256	55	**0.006**	Rec	0.58 (0.42–0.81)	**0.001**
	Non-hospitalized controls	345	434	163				
	Hospitalized controls	159	222	76	0.06	Rec	0.59 (0.40–0.89)	0.012
rs7864330		T/T	T/G	G/G				
	Cases	477	10	0	0.50	Dom	0.64 (0.31–1.33)	0.23
	Non-hospitalized controls	918	30	1				
	Hospitalized controls	441	14	0	0.41	Dom	0.58 (0.24–1.40)	0.23
rs1329061		T/T	T/C	C/C				
	Cases	269	188	32				
	Non-hospitalized controls	549	334	64	0.49	Dom	1.11 (0.89–1.39)	0.35
	Hospitalized controls	264	171	22	0.44	Rec	1.27 (0.69–2.33)	0.44
rs1690593		A/A	A/G	G/G				
	Cases	375	109	4				
	Non-hospitalized controls	719	208	19	0.25	Rec	0.41 (0.14–1.23)	0.11
	Hospitalized controls	350	97	9	0.32	Rec	0.30 (0.09–1.03)	0.06
rs1927906		A/A	A/G	G/G				
	Cases	467	18	1				
	Non-hospitalized controls	890	58	1	0.09	Dom	0.65 (0.38–1.11)	0.11
	Hospitalized controls	434	21	0	0.57	Dom	0.75 (0.38–1.49)	0.41
rs7021687		G/G	G/A	A/A				
	Cases	458	27	1				
	Non-hospitalized controls	889	57	1	0.83	Rec	1.76 (0.11–28.3)	0.69
	Hospitalized controls	422	32	0	0.42	Dom	0.88 (0.49–1.56)	0.65
rs756135		A/A	G/A	G/G				
	Cases	428	61	1				
	Non-hospitalized controls	823	119	4	0.93	Rec	0.43 (0.05–3.87)	0.45

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SNP		Unadjusted[1]				Adjusted[2]		
		Genotype			p[3]	Model	OR [95% CI)	p
rs960312	Hospitalized controls	394	60	3	0.58	Rec	0.36 (0.30–4.42)	0.43
		A/A	A/G	G/G				
	Cases	358	122	9				
	Non-hospitalized controls	750	177	15	0.02	Dom	1.39 (1.07–1.81)	0.01
	Hospitalized controls	370	85	2	**0.005**	Dom	1.45 (1.03–2.04)	0.03

[1] χ^2 or, if cell count <10, Fisher's exact tests of association were performed for cases versus each control group

[2] Dominant or recessive logistic regression models adjusted for age, sex, and diabetes status were performed for cases versus each control group

[3] Tests meeting significance after Bonferroni correction (0.05/8 = 0.00625) for multiple comparisons are in bold.

Table 5

TLR4 region haplotype

***TLR4*$_{1196C>T}$_rs10818066_rs960312**			
Haplotype[1]	**000**	**001**	**010**
Estimated frequency in non-hospitalized controls	0.48	0.11	0.40
Odds ratio (95% CI)[2]	1.01 (0.87–1.18)	1.40 (1.09–1.80)	0.64 (0.48–0.88)
p	0.89	0.009	0.004
Estimated frequency in hospitalized controls	0.49	0.09	0.41
Odds ratio (95% CI)[2]	0.96 (0.80–1.14)	1.58 (1.18–2.14)	0.62 (0.45–0.86)
p	0.62	0.003	0.004

[1] 1 indicates presence of rare allele at each locus. Frequency of all other haplotypes <1%

[2] Additive model for 000, dominant model for 001, recessive model for 010

Table 6

Associations of *TLR6-1–10* region variants with melioidosis in diabetics

SNP	Control group[1]	Unadjusted P[2,3]	Model[4]	Adjusted OR [95% CI)	P[3]
rs721653	Non-hospitalized	0.97	Dom	1.05 (0.75–1.46)	0.78
	Hospitalized	0.72	Dom	0.86 (0.54–1.38)	0.54
rs2087465	Non-hospitalized	0.97	Rec	0.92 (0.26–3.14)	0.90
	Hospitalized	**0.002**	Rec	0.13 (0.03–0.48)	**0.002**
rs3775073	Non-hospitalized	0.50	Dom	1.24 (0.85–1.82)	0.25
	Hospitalized*	0.01	Dom	2.31 (1.39–3.83)	**0.001**
rs3924112	Non-hospitalized	0.88	Dom	0.83 (0.60–1.16)	0.27
	Hospitalized	0.04	Rec	0.31 (0.13–0.73)	0.007
rs4274855	Non-hospitalized	0.74	Dom	0.74(0.50–1.10)	0.14
	Hospitalized	0.27	Dom	0.61 (0.36–1.03)	0.06
rs4321646	Non-hospitalized	0.97	Rec	1.23 (0.49–3.06)	0.66
	Hospitalized	0.65	Dom	1.30 (0.78–2.18)	0.32
rs5743794	Non-hospitalized	0.59	Rec	0.66 (0.31–1.42)	0.29
	Hospitalized	0.02	Rec	0.30 (0.11–0.78)	0.01
rs5743808	Non-hospitalized	0.09	Dom	1.37 (0.95–1.98)	0.09
	Hospitalized	0.18	Dom	1.68 (0.97–2.90)	0.07
rs5743831	Non-hospitalized	0.32	Dom	0.80 (0.58–1.11)	0.19
	Hospitalized*	0.79	Dom	1.18 (0.74–1.89)	0.49
rs11096957	Non-hospitalized	0.83	Dom	0.87 (0.60–1.26)	0.87
	Hospitalized	0.40	Dom	0.69 (0.40–1.21)	0.20
rs11096964	Non-hospitalized	0.13	Dom	1.48 (0.96–2.27)	0.07
	Hospitalized	0.11	Dom	1.76 (0.92–3.36)	0.09
rs11466651	Non-hospitalized	0.58	Dom	0.79 (0.55–1.15)	0.22
	Hospitalized	0.22	Dom	0.69 (0.41–1.16)	0.16
rs11466653	Non-hospitalized	0.63	Dom	0.82 (0.57–1.19)	0.30
	Hospitalized	0.23	Dom	0.73 (0.44–1.22)	0.23
rs11466655	Non-hospitalized	0.90	Rec	1.18 (0.66–2.10)	0.58
	Hospitalized	0.30	Rec	2.71 (0.96–7.64)	0.06

SNP	Control group[1]	Unadjusted P[2,3]	Model[4]	Adjusted OR [95% CI)	P[3]
rs11944159	Non-hospitalized	0.09	Dom	1.56(1.02–2.39)	0.04
	Hospitalized	0.10	Dom	1.18 (0.95–3.47)	0.07
rs17429224	Non-hospitalized	0.20	Dom	1.39 (1.00–1.94)	0.05
	Hospitalized	0.12	Rec	0.40 (0.16–1.00)	0.05
rs17429273	Non-hospitalized	0.67	Dom	1.86 (0.32–10.65)	0.49
	Hospitalized	1.00	Dom	0.71 (0.07–7.36)	0.78
rs17616434	Non-hospitalized	0.65	Rec	1.22 (0.82–1.81)	0.32
	Hospitalized	0.19	Rec	1.54 (0.85–2.80)	0.15

[1] * denotes deviation from Hardy-Weinberg equilibrium

[2] χ^2 or, if cell count <10, Fisher's exact tests of association were performed for cases versus controls

[3] Tests meeting significance after Bonferroni correction (0.05/16 = 0.003) for multiple comparisons are in bold

[4] Dominant or recessive logistic regression models were adjusted for age and sex