INTRODUCTION

The innate immune system provides early recognition of microbial pathogens important to host defense. Toll-like receptors (TLRs) play a key role in host defense, providing a mechanism to respond to highly conserved pathogen-associated molecular patterns. In humans, there are 10 unique TLR genes coding for receptors that initiate responses to pathogen-associated molecular pattern ligands with a robust inflammatory response. TLR2 heterodimerizes with TLR6, TLR1 and possibly TLR10, and these combinations facilitate the recognition of multiple distinct bacterial patterns diversifying innate immune sensing. The importance of TLR2 in host defense has been well established in mice where its deficiency has been associated with increased susceptibility to mycobacterial infection, pneumococcal meningitis and sepsis due to Staphylococcus aureus and Listeria monocytogenes. TLR1/2 and TLR2/6 heterodimers can discriminate the acylation of bacterial lipopeptides recognizing triacyl- and diacyl-lipopeptides, respectively. The synthetic triacyl lipopeptide N-palmitoyl-S-dipalmitoylglyceryl Cys-Ser-(Lys)4 (Pam3CSK4) and diacyl lipopeptide fibroblast stimulating ligand-1 (FSL-1) derived from Mycoplasma salivarium have been shown to stimulate via TLR1/2 and TLR2/6 heterodimers. Additionally, TLR2/6 heterodimers recognize peptidoglycan (PGN) and a yeast cell wall particle, Zymosan.

A role for TLR1/2 and TLR2/6 in human disease has been suggested by candidate gene studies. We and others have demonstrated that there exists high interindividual variability in terms of human leukocyte inflammatory responses to pathogen-associated molecular patterns, and that a portion of this variability is attributable to common genetic variants. Genetic variation in TLR2 has been shown to confer reduced responses to PGN and heat-killed S. aureus in vitro. More recently, we have demonstrated that variants in TLR1 are highly associated with Pam3CSK4-induced whole blood cytokine production. We reported that common genetic variants in TLR1 conferred marked hyperresponsiveness to Pam3CSK4 and these same variants were associated with increased risk of organ dysfunction and death in septic shock. Other studies have demonstrated associations between genetic variation in TLR1 with susceptibility to leprosy and tuberculosis. These data support a role for TLR1/2-mediated responses in human disease. However, to date, our understanding of the role for genetic variation in TLR-mediated responses has been based on targeted candidate gene studies. Thus, in order to more comprehensively assess the genetic factors controlling TLR2-mediated responses in the healthy human population, we undertook a genome-wide association study (GWAS) to identify loci modifying Pam3CSK4-induced cytokine production in whole blood ex vivo.

RESULTS

We employed samples from 360 healthy Caucasian subjects who had an average age of 35 ± 14 years and were 39% male. Given that many innate immunity genes demonstrate population differences in allele frequencies including the genes coding for TLRs, we performed principal component analysis (PCA) to address the possibility that there might exist population admixture within our genotyped subjects. PCA revealed that...
Subjects who self-reported as Caucasian cluster with Caucasians from Utah (CEU) and the Toscani in Italia (TSI) populations from the HapMap3 collection\textsuperscript{22} (Supplementary Figure S1). However, we did identify associations between eigenvalues from the first three principal components and TLR agonist-induced cytokine production and hence these eigenvalues were used as covariates in the multiple linear regression models for the GWAS.

We used a genome-wide association test adjusted for age, gender and the first three principal components, and identified 19 single-nucleotide polymorphisms (SNPs) within the TLR10/1/6 locus on chromosome 4 that were associated with Pam\textsubscript{3}CSK\textsubscript{4}-induced interleukin (IL)-6 (Figure 1a, Table 1). No other loci achieved associations at a genome-wide level of significance including SNPs found in genes involved in TLR1R/2 signaling such as \textit{TIRAP}, \textit{IRAK4} and \textit{IRAK1} that we had anticipated \textit{a priori} to be associated with the cytokine-induced phenotypes (Table 2). Notably, all cytokine values obtained from the whole blood assay were normalized to a monocyte count obtained from the donor at the time of phlebotomy. In this way we mitigated the chances of identifying variation that merely affected the number of circulating monocytes.

We next sought to identify loci associated with responses to TLR2/6 ligands FSL-1, PGN and Zymosan in 167 subjects for which we had measured whole blood responses to these ligands. We did not identify any associations reaching genome-wide significance (Figure 1b) and, notably, no SNPs within the TLR10/1/6 locus or TLR2 were even nominally associated \((P>0.05)\) with responses to these ligands. Nonetheless, there were several moderately strong associations detected at other genomic loci with these cytokine responses ranging from \(P=1.55 \times 10^{-6}\) (Zymosan-induced IL-6), \(P = 3.30 \times 10^{-6}\) (FSL-induced IL-6) to \(P = 4.37 \times 10^{-6}\) (PGN-induced IL-6). As these analyses included fewer subjects than the GWAS of Pam\textsubscript{3}CSK\textsubscript{4}-induced responses, we re-ran the GWAS of Pam\textsubscript{3}CSK\textsubscript{4}-induced IL-6 using only these 167 subjects. This analysis still identified multiple SNPs that were associated at a genome-wide level of significance \((P<4.7 \times 10^{-12})\), demonstrating that although statistical power for this sub-study may have been limiting, the associations with Pam\textsubscript{3}CSK\textsubscript{4}-induced responses are

\[P \approx 1.55 \times 10^{-6}\]  

\[P = 3.30 \times 10^{-6}\]  

\[P = 4.37 \times 10^{-6}\]

---

\*All SNPs meeting genome-wide significance \((P<1 \times 10^{-8})\).  
\*Effect size and direction associated with copy number of minor allele (change in mean log\textsubscript{10}[IL-6] with each copy of minor allele).
orders of magnitude stronger than any associations with TLR2/6 agonist-induced responses.

In order to identify SNPs within the TLR10/1/6 locus not directly genotyped by our platform that may be driving the observed associations with Pam3CSK4-induced cytokine production, we used imputation to infer missing genotypes on chromosome 4 using 1000 genomes NCBI Build 3723 as a reference population. These imputed SNPs were tested for association with the Pam3CSK4-induced cytokine phenotypes. We observed a 222-kb region across the TLR10/1/6 locus that was associated with Pam3CSK4-induced IL-6 at a genome-wide level of significance (Figure 2). The SNP most highly associated with hypermorphic responses was rs67719080 (p = 1.27 × 10^{-27}), an intergenic SNP between TLR10 and TLR1. Of the SNPs that fell within genes, SNPs within TLR10 were most highly associated with hypermorphic cytokine responses (Figure 2). The most highly associated TLR10 coding SNP was rs4129009 (TLR10:3232A>G), a nonsynonymous polymorphism that causes an amino acid change in the highly conserved Toll/Interleukin-1 receptor (TIR) domain. Individuals homozygous for the rare allele had increased IL-6 production consistent with a hypermorphic response (Figure 3). In addition to the TIR domain SNP, we also identified a missense SNP in TLR10, rs11096955 (I369L), near leucine-rich repeat 9 (LRR9: amino acids 349–368) of TLR10 that was strongly associated with hypermorphic responses to Pam3CSK4 (p = 5.36 × 10^{-16}).

Coding SNPs within TLR1 were also highly associated with the Pam3CSK4-induced cytokine phenotype including rs4833095 (TLR1:742A>G) and rs5743618 (TLR1:1805G>T) but were not in high linkage disequilibrium with the TLR10 coding SNP rs4129009 (Table 3), suggesting a distinct association. Notably, rs5743551, a SNP found 5' to TLR1 that we have previously shown to be highly associated with death and organ dysfunction in sepsis, was also highly associated (p = 2.8 × 10^{-15}). Finally, we also found a strong association with a nonsynonymous variant in TLR6 (rs5743818, TLR6:1925T>G) and Pam3CSK4-induced responses (p = 1.28 × 10^{-7}). This SNP was not found to be in high linkage disequilibrium with the other most highly associated coding SNPs in TLR1 (r² = 0.11) and TLR10 (r² = 0.08) (Table 3).

### Table 2. Genes anticipated *a priori* to be associated with Pam3CSK4-induced IL-6 phenotype

| Gene | Chromosome | Loci (Mb) | SNP | SNP gene | P-value |
|------|------------|-----------|-----|----------|--------|
| TLR2 | 4          | 154.84–154.85 | rs2405432* | RNF175 | 0.17   |
| NFKB1 | 4          | 103.54–103.76 | rs2085548 | Intergenic | 0.25      |
| CD14 | 5          | 139.99–139.99 | rs1583005 | Intergenic | 0.02      |
| MYD88 | 3          | 38.16–38.16 | rs9825655 | DLEC1 | 0.02   |
| IRAK4 | 12         | 42.43–42.46 | rs7972025 | Intergenic | 0.04      |
| LY96 | 8          | 75.07–75.10 | rs10504553 | Intergenic | 0.04      |
| TIRAP | 11         | 125.66–125.66 | rs478309 | FOXRED1 | 0.11      |

**Abbreviations:** IL-6, interleukin-6; Pam3CSK4, N-palmitoyl-S-dipalmitoylglyceryl Cys-Ser-(Lys)4; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor. **a**TLR and TLR signaling genes anticipated to be associated with the agonist-induced cytokine concentration. **b**For each gene, a window 50 kb from either end of the gene was included to select the most highly associated SNP. **c**SNP most highly associated within the gene range. Asterisk signifies the SNP was imputed. **d**Gene in which the SNP was located.
DISCUSSION

In this GWAS, we found that the TLR10/1/6 region on chromosome 4 is the dominant common genetic locus controlling interindividual variation in responses to Pam3CSK4 in whole blood from healthy subjects ex vivo. Although the genes coding for TLRs are distributed throughout the genome, TLR10, TLR1, and TLR6 cluster at a locus on chromosome 4p14. Evidence suggests that this tandem arrangement arose from a gene duplication event.24 Notably, all three of these genes have significant allelic heterogeneity with an abundance of rare variants that may indicate an influence of purifying selection.24 In addition, there exist significant geographic differences in genetic variation between European populations within the TLR10/1/6 locus.21 However, our PCA shows that our subjects clustered with Caucasian populations in HapMap3 and our adjustment with principal components in the linear regression suggests that the association testing is not confounded by cryptic population substructure.

Among the SNPs within TLR1 showing the strongest associations in our study were several that have been previously associated with susceptibility to leprosy (rs5743618),25 risk for prostate cancer and placental malaria (rs4833095).26,27 These findings are consistent with the assertion that functional responses mediated by TLR1/2 heterodimers might drive important biologic responses and alter risk for disease.24 We were more surprised to find strong associations with coding SNPs within TLR10 as there is no known ligand specific for TLR10 and it is not known that TLR10 ligation actually generates an intracellular response.4,28 These findings suggest that SNPs within TLR10 may contribute to associations between disease susceptibility and the TLR10/1/6 locus.

Table 3. Coding SNPs in TLR10/1/6 locus most highly associated with Pam3CSK4-induced responses

| SNP               | P-value | Gene | Alleles | Feature             | LD² |
|-------------------|---------|------|---------|---------------------|-----|
| rs4833095         | 1.15e–25| TLR1 | C/T     | Missense: S248N     | 1.0 |
| rs4129009         | 5.04e–25| TLR10| A/G     | Missense            | 0.66|
| rs4274855         | 5.04e–25| TLR10| A/G     | UTR-5'              | 0.66|
| rs5743566         | 9.71e–25| TLR1 | C/G     | UTR-5'              | 0.73|
| rs5743565         | 9.71e–25| TLR10| A/G     | UTR-3'              | 0.73|
| rs9715841         | 1.61e–24| TLR10| C/T     | Coding-synonymous   | 0.61|
| rs10776482        | 1.61e–24| TLR10| C/T     | Coding-synonymous   | 0.61|
| rs10776483        | 1.61e–24| TLR10| C/T     | Coding-synonymous   | 0.61|
| rs11096956        | 1.61e–24| TLR10| G/T     | Coding-synonymous   | 0.61|
| rs5743618         | 7.10e–24| TLR10| G/T     | Missense: I602S     | 0.86|
| rs11466661        | 5.36e–16| TLR10| A/C     | UTR-3'              | 0.61|
| rs11096955        | 5.36e–16| TLR10| A/C     | Missense: L369I     | 0.61|
| rs11096957        | 5.36e–16| TLR10| A/C     | Missense: N241H     | 0.61|
| rs5743818         | 1.28e–09| TLR6  | G/T     | Coding-synonymous   | 0.11|

Abbreviations: LD, linkage disequilibrium; Pam3CSK4, N-palmitoyl-S-dipalmitoylglyceryl Cys-Ser-(Lys)4; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor; UTR, untranslated region. *Most highly associated coding SNPs to PAM3CSK4-induced interleukin-6 (IL-6). **Adjusted for age, gender and eigenvalues from first three principal components.ptional population substructure.

Figure 3. Minor alleles in TLR1 and TLR10 are associated with hypermorphic effects on Pam3CSK4-induced IL-6. Coding SNPs for TLR1 (a, b) and TLR10 (c) most highly associated with Pam3CSK4-induced IL-6 showing hypermorphic responses with the rare genotype.

Table 3. Coding SNPs in TLR10/1/6 locus most highly associated with Pam3CSK4-induced responses

| SNP               | P-value | Gene | Alleles | Feature             | LD² |
|-------------------|---------|------|---------|---------------------|-----|
| rs4833095         | 1.15e–25| TLR1 | C/T     | Missense: S248N     | 1.0 |
| rs4129009         | 5.04e–25| TLR10| A/G     | Missense            | 0.66|
| rs4274855         | 5.04e–25| TLR10| A/G     | UTR-5'              | 0.66|
| rs5743566         | 9.71e–25| TLR1 | C/G     | UTR-5'              | 0.73|
| rs5743565         | 9.71e–25| TLR10| A/G     | UTR-3'              | 0.73|
| rs9715841         | 1.61e–24| TLR10| C/T     | Coding-synonymous   | 0.61|
| rs10776482        | 1.61e–24| TLR10| C/T     | Coding-synonymous   | 0.61|
| rs10776483        | 1.61e–24| TLR10| C/T     | Coding-synonymous   | 0.61|
| rs11096956        | 1.61e–24| TLR10| G/T     | Coding-synonymous   | 0.61|
| rs5743618         | 7.10e–24| TLR10| G/T     | Missense: I602S     | 0.86|
| rs11466661        | 5.36e–16| TLR10| A/C     | UTR-3'              | 0.61|
| rs11096955        | 5.36e–16| TLR10| A/C     | Missense: L369I     | 0.61|
| rs11096957        | 5.36e–16| TLR10| A/C     | Missense: N241H     | 0.61|
| rs5743818         | 1.28e–09| TLR6  | G/T     | Coding-synonymous   | 0.11|

Abbreviations: LD, linkage disequilibrium; Pam3CSK4, N-palmitoyl-S-dipalmitoylglyceryl Cys-Ser-(Lys)4; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor; UTR, untranslated region. *Most highly associated coding SNPs to PAM3CSK4-induced interleukin-6 (IL-6). **Adjusted for age, gender and eigenvalues from first three principal components. *Linkage disequilibrium (R²) between each SNP and the highest TLR1 coding SNP rs4833095.
The most highly associated nonsynonymous SNP in TLR10, rs4129009, causes an amino-acid change in the TIR domain of the intracellular portion of the protein. The TIR domain is critical for intracellular signaling in other TLR family members. A recent study has shown that a chimeric receptor containing the extracellular domain of TLR10 and the intracellular domain of TLR1 (including the TIR domain) induced a cellular response to Pam3CSK4 comparable to wild-type TLR1. This study suggests that the extracellular portion of TLR10 recognizes Pam3CSK4 but that the intracellular portion of TLR10 does not translate this recognition event to an intracellular signal. Our study shows that individuals homozygous for the rare allele of rs4129009 in TLR10 have increased cytokine responses, suggesting that this genetic alteration of the TIR domain may result in a functionally active TLR10 molecule. Of note, this SNP has previously been reported to be associated with decreased risk of atopic asthma. In addition to this SNP in the TIR domain, we identified another highly associated missense SNP in TLR10, rs11096955 (I369L), near LRR9, that could alter ligand binding. In order to best identify whether the TLR10 signal is an independent association, future research should be aimed at other racial groups where haplotype blockings in these region are smaller. Future work will need to more finely delineate whether SNPs in TLR10 or TLR1 (or both) are causally responsible for the associations observed. However, because of moderate linkage disequilibrium, conditional regression analysis adjusting for the top SNPs in this analysis was underpowered to detect independent associations.

The importance of genetic variation in TLR genes and downstream TLR signaling genes is highlighted by candidate gene studies that have demonstrated associations between variants in these genes and diseases for which host defense and inflammation is pathologic. With respect to genes encoding the TLR1/2 family, functional polymorphisms within the TLR10/1/6 locus and TLR2 have been associated with altered susceptibility to the mucobacterial infections of leprosy and tuberculosis. A TLR1 polymorphism (rs5743618, Ser602Ile) that mediates higher levels of signaling and cell surface expression is associated with protection from recurrent urinary tract infection and pyelonephritis. In sepsis, where severe infection leads to overwhelming inflammation and end-organ dysfunction, a TLR1 polymorphism (rs5743551) associated with marked hyper-responsiveness has been associated with the risk of death and organ dysfunction and sepsis-induced acute lung injury. Outside of infectious diseases, polymorphisms within the TLR10/1/6 locus have been variably associated with prostate cancer, non-Hodgkin’s lymphoma, Crohn’s disease, asthma and chronic sarcoidosis. Our findings that the TLR10/1/6 locus explains a large portion of population variance in TLR1/2-mediated responses in vitro provides additional support for the importance of this locus in human disease.

Several previous reports have demonstrated associations between disease risk and genetic variation in TLRs and genes of the TLR intracellular signaling pathway including TLR2, TIRAP, IRAK4 and IRAK1. In spite of these previous findings, we detected only nominally significant associations with variants in some TLR-related genes (Table 2). It should be noted that this study was designed to have adequate statistical power to detect associations with common genetic factors (minor allele frequency > 5%). This study is inadequately powered for detection of associations with rare genetic variants (minor allele frequency < 1%) and, therefore, we cannot exclude the possibility that rare variants within these or other genes may also play a role in modulating these effects. Nonetheless, our findings suggest that common genetic variation in TLR pathway genes outside of the TLR10/1/6 locus play only a minor role in modifying TLR1/2 responses in the Caucasian population.

In summary, our study shows that genetic variation within the TLR10/1/6 locus is the major common genetic factor explaining interindividual variation in TLR1/2-mediated cytokine responses to Pam3CSK4 in vitro. We find that the most highly associated SNPs fall within TLR10 and that some of these SNPs are located in or near important functional domains (TIR domain and LRR9) of TLR10, suggesting that this receptor might have functional relevance. Overall, this study supports ongoing efforts to understand the importance of this locus to human diseases involving innate immunity.

**MATERIALS AND METHODS**

**Study subjects**

We used DNA samples and innate immune response phenotypes collected from 360 healthy Caucasian volunteers recruited from the Seattle metropolitan area from whom written informed consent was obtained. This was approved by the University of Washington Human Subjects Committee. This population has been previously described by our group.

**Cytokine assays**

Innate immune responses were measured in whole blood ex vivo as previously described. Whole blood collected from each subject was exposed to Pam3CSK4 (360 subjects at 100 ng ml⁻¹), FSL-1, PGN (167 subjects at 100 ng ml⁻¹) and Zymosan (179 subjects at 100 μg ml⁻¹) for 6 h, supernatants were collected and produced of tumor necrosis factor-α, IL-1β, IL-6, IL-8, IL-10, granulocyte colony-stimulating factor, IL-1 receptor antagonist and monococyte-chemotactic protein-1 was measured by cytometric bead-based immunoassay (Luminex, Austin, TX, USA). A complete blood count with differential cell counts was obtained at the time of blood sampling for the stimulation assays and cytokine concentrations were normalized to monocyte counts.

**Genotyping and imputation**

Genomic DNA was genotyped using the Illumina (San Diego, CA, USA) Human 1M Beadchip array. In addition, we imputed genotypes on chromosome 4 not present on the array with the BEAGLE software package version 3.3.4 using EUR genotypes from 1000 Genomes as a reference.

**Quality control**

Quality control was performed as described by Anderson et al. We assessed for discordance between reported sex and genotype-determined sex, excess autosomal heterozygosity, excess relatedness (identity by descent of > 0.1875) and population substructure using PCA and removed 14 subjects, resulting in a total of 346 subjects. All subjects had a genotype call rate of over 97%. The 561,491 SNPs were filtered to remove all SNPs with a minor allele frequency < 0.05, Hardy–Weinberg equilibrium P < 0.001 or a call rate ≤ 0.90, resulting in 493,197 SNPs that were used for association testing. Imputed SNPs for chromosome 4 were filtered for an allelic R² of 0.85.

**Data analysis**

We tested for associations between genome-wide genotypes and log10-transformed, monocyte-normalized, cytokine values by multiple linear regression assuming additive effects. Subjects and SNPs passing quality control filtering were tested for association with Pam3CSK4-induced, monocyte-normalized, whole blood cytokine production, adjusting for covariates including age, gender and eigenvalues from the first three principal components generated by PCA clustering subjects with samples from HapMap3 (Release 3, NCBI build 36). Correcting for multiple tests, we considered a P < 1 x 10⁻⁸ to be indicative of genome-wide significance. We assigned P-values to TLR signaling genes anticipated a priori to be associated with the cytokine phenotype by choosing the P-value of the highest SNP within a 50-kb range from the 5’ and 3’ end of the gene. All above analyses were performed and linkage disequilibrium calculated using the Golden Helix (Bozeman, MT, USA) software package.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.
ACKNOWLEDGEMENTS

This work was supported by the National Heart, Lung, and Blood Institute Grant R01 HL089807-01 (to MMW), National Institute of Allergy and Infectious Diseases Grant U54 AI057141 (to MMW), National Institute of Aging Longevity Consortium Grant U19AG023122 (to APR), and the NIH NHLBI training grant T32 HL 72873-33 (CM).

REFERENCES

1 Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001; 2: 675–680.
2 Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z et al. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipopolysaccharides. J Immunol 2002; 169: 10–14.
3 Takeuchi O, Kawai T, Miyahara PF, Moro M, Radolf JD, Zychlinsky A et al. Discrimination of bacterial lipopolysaccharides by Toll-like receptor 6. Int Immunol 2001; 13: 933–940.
4 Guan Y, Ranoa DRE, Jiang S, Mutha SK, Li X, Baudry J et al. HumanTLR 10 and 1 share common mechanisms of innate immune sensing but not signaling. J Immunol 2010; 184: 5094–5103.

Drennan MB, Nicolle D, Queniaux VIF, Jacobs M, Allie N, Mpangi J et al. Toll-like receptor 2-deficient mice succumb to Mycobacterium tuberculosis infection. Am J Pathol 2004; 166: 49–57.

Echchannaoui H, Frei K, Schnell C, Lebl SL, Zimmerli W, Landmann R. Toll-like receptor 2-deficient mice are highly susceptible to Streptococcus pneumoniae meningoitis because of reduced bacterial clearing and enhanced inflammation. J Infect Dis 2002; 186: 788–796.

Takeuchi O, Hoshiko K, Akira S. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J Immunol 2000; 165: 5392–5396.

Torres D, Barrier M, Bilh F, Queniaux VIF, Maillet I, Akira S et al. Toll-like receptor 2 is required for optimal control of Listeria monocytogenes infection. Infect Immun 2004; 72: 2131–2138.

Takeuchi O, Kaufmann A, Grote K, Kawai T, Hoshino K, Moro M et al. Cutting edge: preferentially the R-stereocomer of the mycoplasmal lipoprotein macrophage-activating lipopeptide-2 activates immune cells through a toll-like receptor 2- and MyD88-dependent signaling pathway. J Immunol 2000; 164: 554–557.

Brightbill HD, Librati DH, Krutzik SR, Yang RB, Belisle JI, Blehrarski JR et al. Host defense mechanisms triggered by microbial lipopolysaccharides through toll-like receptors. Science 1999; 285: 732–736.

Aliprantis AO, Yang RB, Mark MR, Suggett S, Devaux B, Radolf JD et al. Cell activation and apoptosis by bacterial lipopolysaccharides through toll-like receptor-2. Science 1999; 285: 736–739.

Okusawa T, Fujita M, Nakamura J-I, Intö T, Yasuda M, Yoshimura A et al. Relationship between structures and biological activities of mycoplasmal diacylated lipoproteids and their recognition by toll-like receptors 2 and 6. Infect Immun 2004; 72: 1657–1665.

Takeuchi O, Hoshiko K, Kawai T, Sanjo H, Takahda H, Ogawa T et al. Differential roles ofTLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 1999; 11: 443–451.

Sato M, Sano H, Iwaki D, Kudo K, Konishi M, Takahashi H et al. Direct binding of Toll-like receptor 2 to zymosan, and zymosan-induced NF-kappa-B activation and TNF-alpha secretion are down-regulated by lung collectin surfactant protein A. J Immunol 2003; 171: 417–425.

Wulfel MM, Gordon AC, Holden TD, Radella F, Strout J, Kajikawa O et al. Toll-like receptor 1 polymorphisms affect innate immune responses and outcomes in sepsis. Am J Respir Crit Care Med 2008; 178: 710–720.

Wulfel MM, Park WY, Radella F, Ruzinski J, Sandstrom A, Strout J et al. Identification of high and low responders to lipopolysaccharide in normal subjects: an approach to identify modulators of innate immunity. J Immunol 2005; 175: 2570–2578.

Mrabet-Dahi S, Dalpke AH, Niebuhr M, Frey M, Draing C, Brand S et al. The Toll-like receptor 2 R753Q mutation modifies cytokine production and Toll-like receptor expression in atopic dermatitis. J Allergy Clin Immunol 2008; 121: 1031–1039.

Pino-Yanes M, Corrales A, Casula M, Blanco J, Muriel A, Espinosa E et al. Common variants ofTLR1 associate with organ dysfunction and sustained pro-inflammatory responses during sepsis. PLoS One 2010; 5: e13759.

Johnson CM, Lyle EA, Omueti KD, Stepanovsky VA, Yegin O, Alpsoy E et al. Cutting edge: a common polymorphism impairs cell surface trafficking and functional responses ofTLR5 but protects against leprosy. J Immunol 2007; 178: 7520–7524.

Ma X, Liu Y, Gowan BB, Graviss EA, Clark AG, Musser JM. Full-exon resequencing reveals toll-like receptor variants contribute to human susceptibility to tuberculosis disease. PLoS One 2007; 2: e1318.

Supplementary Information accompanies the paper on Genes and Immunity website (http://www.nature.com/gen)