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Accessibility
The Role of \textit{TLR4} 896 A>G and 1196 C>T in Susceptibility to Infections: A Review and Meta-Analysis of Genetic Association Studies

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Abstract

\textbf{Background:} Toll-like receptor 4 plays a role in pathogen recognition, and common polymorphisms may alter host susceptibility to infectious diseases.

\textbf{Purpose:} To review the association of two common polymorphisms (\textit{TLR4} 896A>G and \textit{TLR4} 1196C>T) with infectious diseases.

\textbf{Data Sources:} We searched PubMed and EMBASE up to March 2013 for pertinent literature in English, and complemented search with references lists of eligible studies.

\textbf{Study Selection:} We included all studies that: reported an infectious outcome; had a case-control design and reported the \textit{TLR4} 896A>G and/or \textit{TLR4} 1196C>T genotype frequencies; 59 studies fulfilled these criteria and were analyzed.

\textbf{Data Extraction:} Two authors independently extracted study data.

\textbf{Data Synthesis:} The generalized odds ratio metric (OR\textsubscript{G}) was used to quantify the impact of \textit{TLR4} variants on disease susceptibility. A meta-analysis was undertaken for outcomes reported in >1 study. Eleven of 37 distinct outcomes were significant. \textit{TLR4} 896 A>G increased risk for all parasitic infections (OR\textsubscript{G} 1.59; 95%CI 1.05-2.42), malaria (1.31; 95%CI 1.04-1.66), brucellosis (2.66; 95%CI 1.66-4.27), cutaneous leishmaniasis (7.22; 95%CI 1.91-27.29), neurocysticercosis (4.39; 95%CI 2.53-7.61), \textit{Streptococcus pyogenes} tonsillar disease (2.93; 95%CI 1.24-6.93), typhoid fever (2.51; 95%CI 1.18-5.34) and adult urinary tract infections (1.98; 95%CI 1.04-3.98), but was protective for leprosy (0.36; 95%CI 0.22-0.60). \textit{TLR4} 1196 C>T effects were similar to \textit{TLR4} 896 A>G for brucellosis, cutaneous leishmaniasis, leprosy, typhoid fever and \textit{S. pyogenes} tonsillar disease, and was protective for bacterial vaginosis in pregnancy (0.55; 95%CI 0.31-0.98) and \textit{Haemophilus influenzae} tonsillar disease (0.42; 95%CI 0.17-1.00). The majority of significant associations were among predominantly Asian populations and significant associations were rare among European populations.

\textbf{Conclusions:} Depending on the type of infection and population, \textit{TLR4} polymorphisms are associated with increased, decreased or no difference in infectious disease. This may be due to differential functional expression of \textit{TLR4}, the co-segregation of \textit{TLR4} variants or a favorable inflammatory response.

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**Introduction**

Toll-like receptors (TLRs) are a class of highly conserved membrane bound pattern recognition receptors (PRRs) that play an integral role in the regulation of the immune system through the recognition of pathogen-associated molecular patterns (PAMPs) and the activation of immune response genes [1,2]. Toll-like receptor 4 (TLR4), is a well-studied TLR, specifically recognizing lipopolysaccharide from Gram-negative bacteria [3,4] and initiating intracellular signal cascades, that involve the adaptor protein encoded by the myeloid differentiation primary response gene 88 (MyD88), which ultimately activates nuclear factor kappa B [5] and leads to interferon production [6]. TLR4 has also been shown to recognize mannans of fungal pathogens [7], Mycobacterium tuberculosis [8], and the fusion protein of respiratory syncytial virus [9].

Two single nucleotide polymorphisms (SNPs), TLR4 896 A>G (corresponding to an Asp299Gly substitution mutation ; SNP ID: rs 4986790) and TLR4 1196 C>T (corresponding to a Thr399Ile substitution mutation; SNP ID: rs 4986791), have been shown to be associated with LPS hypersensitivity [10,11]. In whites, the two SNPs are in linkage disequilibrium (D=1 and r2=0.791, HapMap accessible at: http://hapmap.ncbi.nlm.nih.gov/). Structurally, these mutations are found outside of the ligand binding domain of TLR4 and crystal structures have shown that these mutations have no effect on LPS binding. Instead, they do cause local conformational changes around the area of the mutation that may affect folding efficiency, cell surface expression, protein stability, as well as interaction with downstream messenger proteins [12]. At the molecular level, it has been shown that the TLR4 896 A>G mutation interferes with TLR4 interaction with MyD88 and other downstream messengers [13]. These mutations also appear to affect the levels of functional TLR4 expression, leading to a 2-fold reduction [14]. This reduction is further amplified to 10-fold in the absence of myeloid differentiation factor 2 (MD-2) which forms a complex with TLR4 and LPS [14,15].

There has been great interest regarding the association of the TLR4 SNPs TLR4 896 A>G and TLR4 1196 C>T to susceptibility for infection and other non-infectious disease states. Clinical studies associating these SNPs to infectious disease susceptibility have produced mixed results [16-19]. The present study aims to reassess the association of the TLR4 SNPs TLR4 896 A>G and TLR4 1196 C>T with infectious disease susceptibility using the Generalized Odds Ratio (ORG), which can elucidate the magnitude and association of individual genotypes with susceptibility to disease [20].

**Materials and Methods**

**Study Selection**

We conducted searches on Pubmed and EMBASE up to March, 2013 (last access on March 3, 2013). The search terms included: "(toll AND like AND receptor AND 4 AND polymorphism) OR (TLR4 AND polymorphism) OR Asp299Gly OR D299G OR Thr399Ile OR T399I" for PubMed; "(tlr4'/exp OR 'tlr4') AND ('polymorphism OR asp299gly OR d299g OR thr399ile OR t399i')" for EMBASE. The titles and abstracts of the studies were reviewed; titles that included TLR4 polymorphisms and risk for infectious disease were included for more detailed evaluation. Studies that reviewed TLR4 polymorphisms and their association with non-infectious disease were excluded, as were studies that were not published in English. An eligible study fulfilled all of the following three criteria: (i) the study reported an infectious disease outcome, (ii) the study was performed using a case-control design, where “cases” refer to subjects with a disease outcome and controls refer to a healthy population (without the disease outcome), and, (iii) the study reported genotype frequencies for TLR4 896 A>C, TLR4 1196C>T, or both.

**Data Extraction**

Two authors (PDZ and MLP) independently extracted data from the final included articles. Any discrepancies were reviewed and resolved by consensus. The information extracted included name of first author, origin of population being studied, number of cases and controls being studied subdivided by genotype frequencies (homozygous wild-type, heterozygous, and homozygous mutant), the disease being studied, and the conclusions reportedly drawn from each study.

**Data Synthesis**

We used the generalized odds ratio (ORG) along with its 95% Confidence Interval (95% CI) to address the association of TLR4 896 A>C and TLR4 1196 C>T polymorphisms with outcomes of interest (disease susceptibility). The ORG provides a model-free approach of estimating the genetic risk in genetic association studies (GAS) and meta-analysis of GAS, depending on the mutational load [20]. The ORG is defined as follows: for any two subjects, one diseased (case) and one non-diseased (control), the ORG estimates the odds of being diseased relative to the odds of being non-diseased when the diseased subject has higher mutational load than the non-diseased subject, i.e. the risk of disease is proportional to the increased genetic exposure. Alternatively, the ORG shows how many diseased-healthy pairs exist in the study for which the diseased have the larger mutational load, relative to the number of pairs for which the non-diseased have the larger mutational load [20][21]. The ORG estimates the overall genetic risk effect by utilizing the complete genotype distribution whereas the OR of conventional genetic models (additive, dominant, recessive, co-dominant) is calculated by merging genotypes. In addition, the conventional genetic models are not independent and thus, the interpretation of results is difficult when more than one model is significant [22]. In the meta-analysis of GAS, heterogeneity was quantified using the Cochran’s Q and I² metric [20]. The existence of the differential magnitude of effect in large versus small studies was checked using the Harbord’s test [23] for meta-analysis involving at least four studies. Also, the Hardy-Weinberg equilibrium (HWE) was used as a quality criterion for control populations. HWE deviations may result in biased estimations as they can influence type-I error in single study effects, and may alter statistical significance in meta-analysis of gene-disease associations.
TLR4 896 A>G and disease susceptibility

For outcomes with more than 1 available study, a meta-analysis was performed for chronic periodontitis (10 studies) [61,63,65-72], Helicobacter pylori infection (2 studies) [52,53], malaria (3 studies) [54-56], meningococcal disease (4 studies) [57-60], sepsis (3 studies) [75-77], respiratory syncytial virus (2 studies) [73,74], tuberculosis (5 studies) [78-82] and urinary tract infections in children (3 studies) [83-85]. Combined effects were also calculated for all Gram negative infections [30,31,33,35,39,46,47,52,53,57-60], all Gram positive infections [43,45,46] and all parasitic infections [36,37,40,50,51,54-56] (Table 3). A significant risk was found for all parasitic infections combined (OR_0.159; 95% CI 1.05-2.42), effect derived from Asian, African and South American populations; Figure 1) and malaria (OR_0.131; 95% CI 1.04-1.66, a combined effect for African and Asian studies; Figure 2). The effect on malaria was of marginal significance across African studies [54,56] (OR_0.129; 95% CI 0.99-1.69). All other effects were insignificant, namely all Gram negative infections (OR_1.10; 95% CI 0.90-1.38), all Gram positive infections (OR_1.28; 95% CI 0.43-3.81), Chagas disease (OR_1.06; 95% CI 0.53-2.14), H. pylori (OR_0.91; 95% CI 0.61-1.36), meningococcal disease (OR_1.10; 95% CI 0.90-1.34), aggressive or chronic periodontitis (OR_1.04; 95% CI 0.53-2.04 and OR_0.94; 95% CI 0.75-1.18, respectively), respiratory syncytial virus (OR_1.02; 95% CI 0.72-1.44), sepsis (OR_0.81; 95% CI 0.41-1.56) and tuberculosis (OR_1.18; 95% CI 0.80-1.73). The meta-analysis results are summarized in Table 3. Statistical heterogeneity varied from absent to moderate. The Harbord’s test indicated that there is no differential magnitude of effect in large versus small studies for all outcomes (p<0.05). Across populations of European ancestry, the risk of meningococcal disease [57-59] (OR_1.12; 95% CI 0.85-1.49) and chronic periodontitis (excluding the two non-European studies [66,68]; OR_1.06; 95% CI 0.53-2.14) remained insignificant. The effects on meningococcal disease and aggressive periodontitis did not alter after removing from analysis the two studies not in HWE equilibrium (data not shown) [58,62]. Effects on tuberculosis remained insignificant across Indian [78,81] (OR_1.34; 95% CI 0.62-2.90) or S. American [80,82] populations (OR_1.30; 95% CI 0.39-4.33). For outcomes with a single available study, a significant risk was present for brucellosis (OR_2.66; 95% CI 1.66-4.27) [30], cutaneous leishmaniasis (OR_7.22; 95% CI 1.91-27.29) [36], neurocysticercosis (OR_4.39; 95% CI 2.53-7.61) [40], and typhoid fever (OR_2.51; 95% CI 1.18-5.34) [47]. All the significant single-study effects are summarized in Table 4.

Of note, all these effects were derived from Asian studies. Increased risk for tonsillar infection due to Streptococcus pyogenes (OR_2.93; 95% CI 1.24-6.93) [46] was noted in the Greek pediatric population, as was an increased risk for urinary tract infections in adults (OR_1.98; 95% CI 1.04-3.98) in a Chinese population [48]. Interestingly, not all outcomes were negative and the TLR4 896 A>G polymorphism was associated with significant protection against leprosy (OR_0.36; 95% CI 0.22-0.60) in East Africa [38]. The use of the OR_0 metric resulted in more conservative estimates of associations, as two reportedly significant associations (1 reporting increased risk for Gram-negative osteomyelitis [41] and 1 reporting a protective effect for Streptococcus pneumoniae in children [45]) were downgraded to non-significant. Six control populations deviated for HWE equilibrium [30,35,39,53,58,62], and associations of TLR4 variants with disease were readdressed after correcting genotypes with their expected frequencies. These effects did not change (they appear in brackets in Tables 1,2). Specifically, the association of TLR4 896 A>G and brucellosis [30] remained significant after HWE correction (OR_2.69; 95% CI 1.67-4.33).

TLR4 1196 C>T and disease susceptibility

A meta-analysis of GAS was performed for malaria (2 studies) [55,56], aggressive periodontitis (4 studies) [61,62,64,65], chronic periodontitis (9 studies) [61,63,65,67,69-72,87], and tuberculosis (3 studies) [78,80,81] and revealed no significant effects. Statistical heterogeneity varied from absent to moderate. Specifically, the combined effects were OR_1.30 (95% CI 0.64-2.65) for malaria, OR_0.78 (95% CI 0.42-1.65) for aggressive and OR_1.12 (0.83-1.52) for chronic periodontitis, and OR_1.07 (95% CI 0.81-1.42) for tuberculosis. Effects were also insignificant for all Gram negative infections combined [OR_1.11 (95% CI 0.66-1.87)][33,35,39,46,47,52], all Gram positive infections combined [OR_1.09 (95% CI 0.13-9.09)] [45,46] and all parasitic infections combined [OR_1.50 (95% CI 0.88-2.56)] [36,37,40,51,55,56]. The meta-analysis results are summarized in Table 3. The Harbord’s test indicated that there
Table 1. Genotypic frequencies reported for the TLR4 896 A>G SNP and association with disease outcome; significant effects are in bold; outcomes that have been studied more than once have been grouped together in the table, with the overall effect described in the shaded area† genotypic frequencies of controls that did not satisfy Hardy Weinberg Equilibrium, [effects in brackets after correction of HWE deviations].

| Name                        | Population | A/A | A/G | G/G | Disease Outcome          | Conclusion Reported | ORG (95% CI)   |
|-----------------------------|------------|-----|-----|-----|---------------------------|---------------------|---------------|
| Carvalho et al [29]         | England    | 70  | 10  | 58  | 18  | 0 Aspergillosis           | Overall susceptibility not studied | 2.10 (0.92-4.81) |
| Rezazadeh et al [30]        | Iran       | 65  | 46  | 68  | 127 | 3 Brucellosis             | Increased risk†       | 2.66 (1.66-4.27) [2.69 (1.67-4.33)] |
| Doorduy et al [31]          | Netherlands | 608 | 72  | 3   | 405 | 49 1 Campylobacter        | No association        | 1.00 (0.68-1.46) |
| Plantinga et al [32]        | Tanzania   | 99  | 9   | 107 | 10  | 0 Oropharyngeal candidiasis in HIV | No association | 1.02 (0.41-2.55) |
| Laisk et al [33]            | Estonia    | 287 | 35  | 1   | 61  | 9  0 C.trachomatis(women) | No association        | 1.24 (0.58-2.67) |
| Szibeni et al [34]          | Hungary    | 108 | 10  | 0   | 37  | 4  0 NecEnterocolitis in LBW infants | No association | 1.26 (0.40-4.00) |
| Lee, et al [35]             | United States | 431 | 11  | 2   | 103 | 2  3 Gram –ve infections in liver transplant | No association† | 0.66 (0.26-1.70) [0.42 (0.16-1.66)] |
| Ajdary, et al [36]          | Iran       | 73  | 2   | 0   | 102 | 26 0 Leishmaniasis (Cutaneous) | Increased risk        | 7.22 (1.91-27.29) |
| Rasouli et al [37]          | Iran       | 137 | 18  | 0   | 110 | 11 1 Leishmaniasis (Visceral) | No association        | 0.81 (0.38-1.75) |
| Bochud et al [38]           | East Africa | 155 | 37  | 2   | 375 | 32 2 Leprosy | Protective | 0.36 (0.22-0.60) |
| West, et al [39]            | Thailand   | 1377| 20  | 1   | 484 | 5  0 Meliodosis            | No association†       | 0.74 (0.29-1.92) [0.70 (0.27-1.78)] |
| Verma, et al [40]           | India      | 127 | 22  | 1   | 77  | 61 2 Neurocystercerosis   | Increased risk        | 4.39 (2.53-7.61) |
| Montes et al [41]           | Spain      | 135 | 20  | 0   | 65  | 12 3 Osteomyelitis        | Increased risk        | 1.55 (0.76-3.20) |
| Emonis et al [42]           | Netherlands | 374 | 58  | 1   | 293 | 42 2 Otitis media (acute) | Overall susceptibility not studied | 0.96 (0.63-1.45) |
| Moens et al [43]            | Belgium    | 161 | 16  | 1   | 84  | 13 2 Invasive pneumococcal infection | No association       | 1.69 (0.81-3.54) |
| Mrazek et al [44]           | Czechoslovakia | 217 | 34  | 1   | 89  | 9  0 Prosthetic joint infection | No association | 0.66 (0.31-1.42) |
| Doorduy et al [31]          | Netherlands | 608 | 72  | 3   | 173 | 20 0 Salmonella gastroenteritis | No association | 0.96 (0.57-1.60) |
| Yuan et al [45]             | Australia  | 364 | 44  | 1   | 82  | 3  0 S. pneumoniae        | Protective            | 0.35 (0.12-1.07) |
| Liadaki, et al [46]         | Greece     | 195 | 27  | 0   | 99  | 6  0 Tonsillar Disease (H.influenzae) | No association | 0.47 (0.19-1.14) |
| Liadaki, et al [46]         | Greece     | 264 | 25  | 0   | 30  | 8  0 Tonsillar Disease (S.pyogenes) | Increased risk        | 2.93 (1.24-6.93) |
| Bhuvanendran, et al [47]    | Malaysia   | 241 | 9   | 0   | 277 | 27 0 Typhoid Fever        | Increased Risk        | 2.51 (1.18-5.34) |
| Yin, et al [48]             | China      | 227 | 21  | 0   | 109 | 20 0 UTT (Adults)         | Increased risk        | 1.98 (1.04-3.98) |
| Hawn et al [49]             | United States | 274 | 33  | 6   | 585 | 65 2 UTT (Women)         | No association        | 0.79 (0.52-1.20) |
| Weitzel, et al [50]         | Northern Chile | 42  | 3   | 0   | 114 | 11 0 Chagas Disease      | No association        | 1.06 (0.53-2.14) |
| Zafra et al [51]            | Colombia   | 191 | 9   | 0   | 262 | 10 3 Chagas Disease      | No association        | 1.20 (0.35-4.14) |
| Achyut et al [52]           | India      | 168 | 32  | 0   | 110 | 20 0 H. pylori            | No association        | 0.91 (0.61-1.36) |
| Moura et al [53]            | Brazil     | 222 | 28  | 4   | 206 | 25 1 H. pylori            | No association†       | 0.87 (0.50-1.50) [0.81 (0.47-1.40)] |
| Esposito, et al [54]        | Burundi    | 300 | 36  | 1   | 528 | 72 2 Malaria (children)   | No association        | 1.13 (0.74-1.73) |
| Zakeri, et al [55]          | Iran       | 287 | 33  | 0   | 276 | 39 5 Malaria (all ages)   | No association        | 1.38 (0.86-2.22) |
| Mockenhaupt et al [56]      | Ghana      | 239 | 47  | 4   | 444 | 129 7 Malaria (pregnancy) | Overall susceptibility not studied | 1.42 (0.99-2.02) |
| Biebl et al [57]            | Austria     | 678 | 88  | 3   | 167 | 18 0 Meningococcal disease (all ages) | No association | 1.10 (0.90-1.34) |

TLR4 Polymorphisms and Infectious Diseases

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Table 1 (continued).

| Name                        | Population | A/A | A/G | G/G | A/A | A/G | G/G | Disease Outcome                  | Conclusion Reported | ORG (95% CI)        |
|-----------------------------|------------|-----|-----|-----|-----|-----|-----|----------------------------------|---------------------|---------------------|
| Read et al [58]             | England    | 787 | 81  | 11  | 924 | 110 | 13 | Meningococcal disease (all ages) | No association      | 1.13 (0.86-1.51)    |
|                            |            |     |     |     |     |     |     |                                  |                     | [1.05-0.79-1.38]    |
| Faber et al [59]            | Europe     | 190 | 23  | 1   | 165 | 27  | 5  | Meningococcal disease (infants)  | Increased risk      | 1.55 (0.89-2.72)    |
| Allen et al [60]            | Gambia     | 198 | 51  | 2   | 198 | 51  | 3  | Meningococcal meningitis (children) | No association       | 1.02 (0.67-1.56)    |
|                            |            |     |     |     |     |     |     |                                  |                     |                     |
|                            |            |     |     |     |     |     |     | Periodontitis (aggressive)      |                     | 1.04 (0.53-2.04)    |
| Brett et al [61]            | England    | 90  | 7   | 0   | 37  | 8   | 0  | Aggressive periodontitis        | No association      | 2.73 (0.96-7.76)    |
| Emingil et al [62]          | West Europe| 147 | 7   | 1   | 86  | 4   | 0  | Aggressive periodontitis        | No association      | 0.96 (0.30-3.12)    |
|                            |            |     |     |     |     |     |     |                                  |                     | [0.81-0.26-2.54]    |
| James et al [63]            | West Europe| 103 | 20  | 0   | 69  | 4   | 0  | Aggressive periodontitis        | No association      | 0.33 (0.12-0.97)    |
| Noack et al [64]            | Germany    | 71  | 9   | 0   | 100 | 11  | 0  | Aggressive periodontitis        | No association      | 0.86 (0.35-2.13)    |
| Schulz et al [65]           | Germany    | 73  | 7   | 0   | 52  | 8   | 0  | Aggressive periodontitis        | No association      | 1.58 (0.56-4.47)    |
|                            |            |     |     |     |     |     |     | Periodontitis (chronic)         |                     | 0.94 (0.75-1.18)    |
| Garlet, et al [66]          | Brazil     | 131 | 74  | 12  | 135 | 56  | 6  | Chronic periodontitis           | No association      | 0.70 (0.47-1.03)    |
| Noack et al [67]            | Germany    | 68  | 8   | 0   | 96  | 12  | 0  | Chronic periodontitis           | No association      | 1.04 (0.42-2.61)    |
| Sahingur et al [68]         | United States | 59  | 17  | 1   | 95  | 19  | 0  | Chronic periodontitis           | No association      | 0.67 (0.33-1.37)    |
| Schulz et al [65]           | Germany    | 73  | 7   | 0   | 66  | 7   | 0  | Chronic periodontitis           | No association      | 1.10 (0.38-3.19)    |
| Izakovcicova Holla et al [69]| Czechoslovakia | 195 | 23  | 0   | 147 | 24  | 0  | Chronic periodontitis           | No association      | 1.38 (0.76-2.53)    |
| Berdeli et al [70]          | Turkey     | 100 | 6   | 0   | 79  | 4   | 0  | Chronic periodontitis           | No association      | 0.88 (0.26-3.01)    |
| James et al [63]            | West Europe| 78  | 16  | 0   | 77  | 17  | 1  | Chronic periodontitis           | No association      | 1.11 (0.53-2.31)    |
| Brett et al [61]            | England    | 90  | 7   | 0   | 47  | 6   | 0  | Chronic periodontitis           | No association      | 1.66 (0.55-4.97)    |
| Laine et al [71]            | Netherlands| 90  | 8   | 1   | 90  | 10  | 0  | Chronic periodontitis           | No association      | 1.16 (0.46-2.93)    |
| Folwaczyny et al [72]       | Germany    | 236 | 8   | 0   | 234 | 10  | 0  | Chronic periodontitis           | No association      | 1.24 (0.50-3.12)    |
|                            |            |     |     |     |     |     |     | Respiratory Syncytial Virus     |                     | 1.02 (0.72-1.44)    |
| Lofgren, et al [73]         | Finland    | 290 | 59  | 7   | 251 | 55  | 6  | Respiratory Syncytial Virus     | No association      | 1.06 (0.73-1.66)    |
| Paulus et al [74]           | Canada     | 97  | 9   | 0   | 218 | 17  | 1  | Respiratory Syncytial Virus     | No association      | 0.84 (0.37-1.91)    |
|                            |            |     |     |     |     |     |     | Sepsis                           |                     | 0.81 (0.42-1.56)    |
| Ahmad-Nejad et al [75]      | Germany    | 99  | 12  | 1   | 31  | 6   | 1  | Sepsis (ICU)                    | No association      | 1.72 (0.64-4.63)    |
| Carregaro et al [76]        | Brazil     | 178 | 26  | 1   | 88  | 9   | 0  | Sepsis (ICU)                    | No association      | 0.71 (0.33-1.56)    |
| Feterowski et al [77]       | Germany    | 135 | 19  | 0   | 143 | 10  | 0  | Sepsis (ICU)                    | No association      | 0.51 (0.23-1.19)    |
|                            |            |     |     |     |     |     |     | Tuberculosis                     |                     | 1.18 (0.80-1.73)    |
| Najmi et al [78]            | India      | 206 | 44  | 0   | 95  | 34  | 6  | Tuberculosis                     | Increased association | 2.00 (1.23-3.25)   |
| Newport et al [79]          | Gambia     | 235 | 58  | 5   | 241 | 62  | 4  | Tuberculosis                     | No association      | 1.01(0.69-1.49)     |
| Sanchez, et al [80]         | Colombia   | 270 | 29  | 1   | 429 | 36  | 1  | Tuberculosis                     | No association      | 0.78 (0.47-1.28)    |
| Selvaraj et al [81]         | South India| 151 | 53  | 3   | 153 | 47  | 4  | Tuberculosis                     | No association      | 0.91 (0.59-1.40)    |
| Rosas-Taraco et al [82]     | Mexico     | 110 | 4   | 0   | 94  | 10  | 0  | Tuberculosis                     | No association      | 2.70 (0.87-8.39)    |
|                            |            |     |     |     |     |     |     | UTI                              |                     | 1.41 (0.70-2.84)    |
| Akill, et al [83]           | Turkey     | 79  | 14  | 0   | 97  | 14  | 1  | UTI-children                     | No association      | 0.85 (0.39-1.84)    |
| Erfan, et al [84]           | Turkey     | 29  | 1   | 0   | 28  | 2   | 0  | UTI-children                     | No association      | 1.70 (0.22-13.37)   |
| Karoly et al [85]           | Hungary    | 218 | 17  | 0   | 88  | 15  | 0  | UTI-children                     | Increased risk      | 2.18 (1.06-4.52)    |

is no differential magnitude of effect in large versus small studies for all outcomes (p≥0.05).

For outcomes with a single available study, a significant risk was present for cutaneous leishmaniasis in Iran (OR= 10.14; 95% CI 1.90-54.16) [36], neurocysticercosis in India (OR= 3.10; 95% CI 1.45-6.67) [40], S. pyogenes tonsillar disease in Greece (OR= 3.12; 95% CI 1.36-7.13) [46] and typhoid fever in Malaysia (OR= 2.26; 95% CI 1.01-5.07) [47]. A significant protection was conferred for bacterial vaginosis in pregnancy (OR= 0.55;95% CI 0.31-0.98) in the United States (notably,
Table 2. Genotypic frequencies reported for the *TLR4* 1196 C>T SNP and association with disease outcome; significant effects are in bold; outcomes that have been studied more than once have been grouped together in the table, with the overall effect described in the shaded area.

| Name                                  | Population  | Case Genotype | Control Genotype | Disease Outcome                           | Conclusion Reported | ORg (95% CI) |
|----------------------------------------|-------------|---------------|------------------|------------------------------------------|---------------------|--------------|
| Goepfert et al [86]                    | United States | 316 | 28 | 0 | 435 | 21 | 0 | Bacterial Vaginosis in Pregnant | Protective | 0.55 (0.31-0.98) |
| Lalik et al [33]                       | Estonia      | 287 | 35 | 1 | 61 | 9 | 0 | C. trachomatis(women) | No association | 1.24 (0.58-2.67) |
| Szebeni et al [34]                     | Hungary      | 108 | 10 | 0 | 37 | 4 | 0 | NecEnteroColitis in LBW infants | No association | 1.26 (0.39-4.00) |
| Lee, et al [35]                        | United States | 395 | 64 | 4 | 89 | 18 | 1 | Gram—ve infections in liver transplant | No association | 1.23 (0.71-2.15) |
| Achuty et al [52]                      | India        | 188 | 11 | 1 | 115 | 9 | 6 | H pylori | No association | 2.08 (0.95-4.54) |
| Afdary, et al [36]                     |             | 74  | 1  | 0 | 105 | 21 | 2 | Leishmaniasis (Cutaneous) | Increased risk of infection | 10.14 (1.90-54.16) |
| Rasouli et al [37]                     | Iran         | 137 | 18 | 0 | 112 | 9 | 1 | Leishmaniasis (Visceral) | No association | 0.67 (0.30-1.49) |
| Bochud et al [38]                      | East Africa  | 179 | 15 | 1 | 407 | 8 | 0 | Melioidosis | Protective | 0.23 (0.10-0.55) |
| West, et al [39]                       | Thailand     | 1379 | 22 | 1 | 486 | 3 | 0 | Prosthetic joint infection | No association | 0.43 (0.14-1.33) |
| Verma, et al [40]                      | India        | 140 | 9  | 1 | 114 | 25 | 1 | Neurocyticercosis | Increased risk | 3.13 (1.46-6.73) |
| Montes et al [41]                      | Spain        | 133 | 22 | 0 | 67 | 10 | 3 | Osteomyelitis | Increased risk | 1.19 (0.57-2.47) |
| Mrazek et al [44]                      | Czechoslovakia | 219 | 33 | 0 | 88 | 0 | 0 | Tonsillar Disease (H.influenzae) | Protective | 0.42 (0.17-1.00) |
| Ahmad-Nejad et al [75]                 | Germany      | 98  | 13 | 1 | 31 | 6 | 1 | Sepsis (ICU) | No association | 1.58 (0.60-4.23) |
| Yuan et al [45]                        | Australia    | 365 | 43 | 1 | 82 | 3 | 0 | S. pneumoniae | Protective | 0.36 (0.12-1.09) |
| Liadaki, et al [46]                    | Greece       | 192 | 30 | 0 | 99 | 6 | 0 | Tonsillar Disease (S.pyogenes) | Increased risk | 3.12 (1.36-7.13) |
| Bhuvanendran, et al [47]               | Malaysia     | 242 | 8  | 0 | 282 | 22 | 0 | Typhoid Fever | Increased Risk | 2.26 (1.01-5.07) |
| Hawn et al [49]                        | United States | 277 | 35 | 4 | 589 | 69 | 0 | UTI - Women | No association | 0.83 (0.55-1.26) |
|                                        | Chagas Disease | 1    | 0  | 0 | 114 | 11 | 0 | Chagas Disease | No association | 1.19 (0.35-4.14) |
|                                        | Malaria      | 282 | 9  | 0 | 267 | 8 | 0 | Chagas disease | No association | 0.95 (0.37-2.42) |
|                                        | Zakeri, et al [55] | 270 | 50 | 0 | 271 | 49 | 0 | Malaria (all ages) | No association | 0.98 (0.64-1.50) |
|                                        | Mockenhaupt et al [56] | 283 | 7 | 0 | 550 | 28 | 2 | Malaria (pregnancy) | Overall susceptibility not studied | 2.06 (0.91-4.62) |
|                                        | Periodontitis (aggressive) | 10.14 (1.90-54.16) |
|                                        | Periodontitis (chronic) | 0.78 (0.42-1.65) |
|                                        | Tuberculosis | 1.07 (0.81-1.42) |
|                                        | Najmi et al [56] | 206 | 43 | 1 | 105 | 26 | 4 | Tuberculosis | No association | 1.37 (0.82-2.28) |
|                                        | Sanchez, et al [80] | 272 | 26 | 1 | 429 | 36 | 1 | Tuberculosis | No association | 0.87 (0.52-1.46) |
Table 2 (continued).

| Name                          | Population | Control Genotype | Case Genotype | C/C | C/T | T/T | C/C | C/T | T/T | Disease Outcome | Conclusion Reported | OR$_0$ (95% CI) |
|-------------------------------|------------|-----------------|--------------|-----|-----|-----|-----|-----|-----|-----------------|-------------------|-----------------|
| Selvaraj et al [81]           | South India| 152             | 46           | 4   | 150 | 49  | 4   | 150 | 49  | 4 Tuberculosis  | No association    | 1.04 (0.58-1.61) |

*†* genotypic frequencies of controls that did not satisfy Hardy Weinberg Equilibrium, [effects in brackets after correction of HWE deviations].

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Table 3. Summary of disease associations derived from meta-analysis of case-control studies.

| Disease Outcome | Studies | Polymorphism | Effect (OR$_0$; 95% CI) | $P_Q$ | $I^2$ | $P_H$ |
|-----------------|---------|--------------|-------------------------|-------|-------|-------|
| All Gram - infections | 13 | TLR4 896 A>G | 1.10 (0.90-1.38) | 0.01 | 52% | 0.32 |
| 6               | TLR4 1196 C>T | 1.11 (0.66-1.67) | 0.02 | 61% | 0.59 |
| Helicobacter pylori | 2     | TLR4 896 A>G | 0.91 (0.61-1.36) | 0.79 | -    | -    |
| Meningococcal Disease | 4 | TLR4 896 A>G | 1.10 (0.90-1.34) | 0.43 | 0    | 0.93 |
| All Gram + infections | 3 | TLR4 896 A>G | 1.28 (0.43-3.81) | 0.01 | 77% | -    |
| 2               | TLR4 1196 C>T | 1.09(0.13-9.09) | 0.002 | -   | -    |
| All parasitic infections | 8 | TLR4 896 A>G | 1.59 (1.05-2.42) | <0.001 | 72% | 0.72 |
| 7               | TLR4 1196 C>T | 1.50 (0.88-2.56) | 0.01 | 64% | 0.5  |
| Chagas Disease    | 2     | TLR4 896 A>G | 1.06 (0.53-2.14) | 0.82 | -    | -    |
| 2               | TLR4 1196 C>T | 1.03 (0.49-2.18) | 0.76 | -   | -    |
| Malaria           | 3     | TLR4 896 A>G | 1.31 (1.04-1.66) | 0.71 | 0    | -    |
| 2               | TLR4 1196 C>T | 1.30 (0.64-2.65) | 0.11 | -   | -    |
| Periodontitis(Aggressive) | 5 | TLR4 896 A>G | 1.04 (0.53-2.04) | 0.07 | 52% | 0.16 |
| 4               | TLR4 1196 C>T | 0.78 (0.42-1.65) | 0.29 | 20% | 0.92 |
| Periodontitis (Chronic) | 10 | TLR4 896 A>G | 0.94 (0.75-1.18) | 0.68 | 0   | 0.74 |
| 9               | TLR4 1196 C>T | 1.12 (0.83-1.52) | 0.74 | 0   | 0.93 |
| RSV              | 2     | TLR4 896 A>G | 1.02 (0.72-1.44) | 0.61 | -    | -    |
| Sepsis           | 3     | TLR4 896 A>G | 0.81 (0.41-1.56) | 0.16 | 45% | -    |
| Tuberculosis      | 5     | TLR4 896 A>G | 1.18 (0.80-1.73) | 0.03 | 63% | 0.43 |
| 3               | TLR4 1196 C>T | 1.07 (0.81-1.42) | 0.47 | 0   | -    |
| LUTI (Children)  | 3     | TLR4 896 A>G | 1.41 (0.70-2.84) | 0.21 | 35% | -    |

P$_Q$: p value for Q homogeneity test; P$_H$: p value for Harbord’s small study effects test, --not applicable

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African Americans comprised 78% of the cases [86], leprosy in East Africa (OR$_0$ 0.23; 95% CI 0.10-0.55) [38], and Haemophilus influenzae tonsillar disease in a Greek pediatric population (OR$_0$ 0.42; 95% CI 0.17-1.00) [46]. The significant results are summarized in Table 4. Only 1 control population deviated from HWE equilibrium that assessed the risk of melioidosis [39], a risk that did not change after correction with the expected genotype frequencies (Table 2). Two reportedly significant associations for Gram-negative osteomyelitis (increased risk) and S. pneumoniae (protection) were not confirmed in this analysis with the use of the OR$_0$ metric.

The significant effects were unidirectional and similar in magnitude when both TLR4 896 A>G and 1196 C>T were examined (Table 4), that is if TLR4 896 A>G was protective then 1196 C>T was also protective. When TLR4 896 A>G increased risk, then 1196 C>T increased risk. Specifically, the point estimates for 896 A>G and 1196 C>T variants were (respectively): 7.22 and 10.14 for cutaneous leishmaniasis, 4.39 and 3.13 for neurocysticercosis, 2.93 and 3.12 for S. pyogenes tonsillar disease, 2.51 and 2.26 for typhoid fever, 0.36 and 0.23 for leprosy. An exception to the rule was H. influenzae tonsillar disease, where the protective effect of TLR4 896 A>G did not reach statistical significance (OR$_0$ 0.47; 95% CI 0.19-1.14), while 1196 C>T showed significant association (OR$_0$ 0.42; 95% CI 0.17-1.00).

Discussion

We performed a systematic literature review to address the potential association of 2 common TLR4 single nucleotide polymorphisms (TLR4 896 A>G, TLR4 1196 C>T) with infectious diseases. An increased risk was documented for all parasitic infections combined, malaria [54-56], brucellosis [30], cutaneous leishmaniasis [36], typhoid fever [47], neurocysticercosis [40] and adult urinary tract infections [48]. Interestingly, all these effects were reported in populations of Asian descent, with the exception of parasitic infections and malaria where the effect was a combined effect from Asian,
African and South American populations. This finding is more striking when we consider that European populations comprised the majority of GAS data (28 out of 59 studies, 48%) and a significant risk was found only for TLR4 polymorphisms and *S. pyogenes* tonsillitis among Greek children [46]. Another notable finding is that, for some infections, these single nucleotide polymorphisms were associated with lower infection rates. Overall, these effects sum to a total of 11 significant SNPs-disease associations that represent almost one third (30%) of all outcomes addressed in the eligible studies and there was consistency of effects (risk or protection) between 896 A>G and 1196 C>T variants when both associations were studied.

In this study we utilized the generalized odds ratio (OR₉) metric to quantify the magnitude of associations. This metric provides a straightforward interpretation of the relative risk effect, based solely on genotype distribution [20]. The generalized odds ratio overcomes this problem by directly quantifying the magnitude of association of a gene with disease [20]. Implementing the OR₉ obviates the need for selecting, estimating and interpreting individual genotype contrasts (dominant, recessive and co-dominant) and their effect. OR₉ can also be used in meta-analysis of GAS to summarize effects and produce robust results, avoiding the shortcomings of multiple model testing, namely the lack of biologic justification and non-independency of effects [20,88,89]. For example, for TLR4 896 A>G association with malaria, the combined OR₉ showed that the probability of having malaria might be 31% higher for subjects having higher mutational load relative to those with lower mutational load (subjects who are homozygous for G allele have the highest mutational load, those homozygous for A allele have the lowest, and heterozygous have an intermediate level). The application of the OR₉ metric also resulted in a more conservative estimate of
associations, given that associations for infections such as osteomyelitis (39) and *S. pneumonia* (43) were downgraded to insignificant. The associations derived from tuberculosis data were insignificant similar to those reported [90].

In our analysis, TLR4 polymorphisms were associated with susceptibility to a diverse spectrum of infections including Gram-negative, Gram-positive bacteria as well as parasitic infections, such as cutaneous leishmaniasis and neurocysticercosis. This wide spectrum of associations correlates with the spectrum of recognition molecules by TLR4. Indeed, TLR4 is involved in induction of cell-mediated immunity to *Brucella abortus* in mice [7] and TLR4 signaling also upregulates macrophage anti-leishmanial activity [91]. Similarly, binding of the *Salmonella typhi* porin OmpS1 to TLR4 leads to overexpression of MHCII and CD40 molecules and activation of dendritic cells [92]. TLR4 can recognize LPS of Gram-negative bacteria [3,4], glycans of the helminth *Taenia solium* [93] as well as the fusion protein of respiratory syncytial virus [9].

Interestingly, our analysis also confirmed that these polymorphisms are also protective for certain types of infection, such as leprosy. It is not clear why such polymorphisms confer increased susceptibility to some infection, but protect from others. It could be speculated that in some infections the immune response leads to an inflammatory response that is protective, whereas in others such response may be essential in the pathogenesis of the infectious process. An example is *Mycobacterium leprae* where the TLR4-mediated immune response to the pathogen may modulate inflammatory processes that influence disease manifestations but are not attributable to direct stimulation by M. leprae. Indeed, Bochud et al [38] found that the stimulation of monocytes with M. leprae inhibited their subsequent response to TLR4 stimulation with LPS.

![Figure 2. Malaria: Random effects (RE) generalized odds ratio (OR, G) estimates with the corresponding 95% confidence interval (CI) for the variant TLR4 896 A>G. The horizontal axis is plotted on a log scale.](image-url)

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Table 4. Summary of significant associations with disease outcomes, derived from single case-control studies.

| Study           | Population | Disease Outcome     | Polymorphism            | ORG (95% CI) |
|-----------------|------------|---------------------|-------------------------|--------------|
| Goepfert [66]   | USA        | Bacterial vaginosis (pregnancy) | TLR4 896 C>T          | 0.55         |
|                 |            |                     |                         | (0.31-0.98)  |
| Rezazadeh [30]  | Iran       | Brucellosis         | TLR4 896 A>G           | 2.66         |
|                 |            |                     |                         | (1.66-4.27)  |
| Ajdary [36]     | Iran       | Cutaneous leishmaniasis | TLR4 896 A>G           | 7.22         |
|                 |            |                     |                         | (1.91-27.29) |
| Bochud [38]     | East Africa | Leprosy             | TLR4 896 A>G           | 0.36         |
|                 |            |                     |                         | (0.22-0.60)  |
|                 |            |                     | TLR4 1196 C>T          | 0.23(0.10-0.55) |
| Verma [40]      | India      | Neurocysticercosis  | TLR4 896 A>G           | 4.39         |
|                 |            |                     |                         | (2.53-7.61)  |
|                 |            |                     | TLR4 1196 C>T          | 3.13         |
|                 |            |                     |                         | (1.46-6.73)  |
| Liadaki [46]    | Greece     | H. influenzae (tonsillitis) | TLR4 1196 C>T          | 0.42         |
|                 |            |                     |                         | (0.17-1.00)  |
| Liadaki [46]    | Greece     | S. pyogenes (tonsillitis) | TLR4 896 A>G           | 2.93         |
|                 |            |                     |                         | (1.24-6.93)  |
|                 |            |                     | TLR4 1196 C>T          | 3.12         |
|                 |            |                     |                         | (1.36-7.13)  |
| Bhuvanedran [47]| Malaysia   | Typhoid fever       | TLR4 896 A>G           | 2.51         |
|                 |            |                     |                         | (1.18-5.34)  |
|                 |            |                     | TLR4 1196 C>T          | 2.26         |
|                 |            |                     |                         | (1.01-5.07)  |
| Yin [48]        | China      | UTI (Adults)        | TLR4 896 A>G           | 1.98         |
|                 |            |                     |                         | (1.04-3.98)  |

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Among Indo-European populations, 6–14% of the individuals are double heterozygous for both polymorphisms [94]. It is suggested that the double heterozygous TLR4 896 A>G/TLR4 1196 C>T haplotype does not functionally differ from wild type TLR4. Therefore, co-segregation may result in a functionally neutral phenotype and, as seen in European populations, lead to the lack of significant associations. Conversely, TLR4 896 A>G was frequently found (10-18%) among African populations, with only 2% having TLR4 1196 C>T co-segregation. Two studies (on typhoid fever and leprosy) indicated weak linkage disequilibrium in Malaysian [47] and East African populations [38]. These differences between Europeans (co-segregation) compared to Asian and African population (lack of co-segregation) may explain why the majority of significant associations were noted for endemic diseases of Asia and Africa.

Our analysis on the impact of these polymorphisms in periodontitis illustrates the different impact of polymorphisms based on the population. More specifically, despite the bulk of studies on aggressive and chronic periodontitis, TLR4 variants did not show any significant association, even though TLR4 has been shown to be overexpressed in gingival epithelial cells and gingival fibroblasts [95-97] in association with periodontal inflammation involving pathogens related to periodontitis, such as Porphyromonas gingivalis, Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans [98-101]. One possible explanation is that this finding was because all relevant studies were almost exclusively confined to European ancestry populations and the lack of susceptibility may be related to the strong linkage disequilibrium, that is the non-random association between 896 A>G and 1196 C>T in Europeans [94].

Importantly, our analysis highlights the need to evaluate the impact of these polymorphisms in different populations and various clinical conditions. Moreover, the absence of significant associations in meta-analysis data for periodontitis, tuberculosis, meningococcal disease and sepsis, signifies that the functional alterations related to polymorphic TLR4 variants may not be critical to produce the clinical phenotype. Lack of reproducibility stands as a barrier for conclusive evidence, and design, sample size and environmental and genetic heterogeneity between populations may affect results. Finally, the presence of a significant effect may rely on the magnitude of functional expression of TLR4. Protection or risk may be moderated by the level of TLR4 functional expression, which is modulated by TLR4 polymorphism and MD-2 presence [14,15]. Therefore, it is essential to explore whether MD-2 is important in the response to some infections, but not others, or that levels of TLR4 vary in one infection compared to another.

The heterogeneity of the populations studied along with multiple endpoints should also be considered as potential study limitation that may influence statistical power. Moreover, different populations mount diverse immunologic responses and the clinical relevance of polymorphisms is not always straightforward. The lack of association for a disease phenotype highlights that gene-to-gene interactions and gene-environment interactions may be influential parameters of disease association. Case-control design of individual GAS precludes adjusted analysis for gene-gene-environment interactions and may have reduced the efficiency of genetic risk estimates, though it is unlikely to inflate false-positive results [89].

Despite these limitations, genetic markers of immune response such as TLR4 variants, are valuable not only to classify high-risk patients based on disease susceptibility but also to predict disease severity and other sequelae. The associations of TLR4 896A>G with hearing loss in survivors of bacterial meningitis [102] and the increased risk of tympanostomy among toddlers with history of bronchiolitis [103] are indicative examples.

In conclusion, our analysis highlights the complex effect of TLR4 variants in susceptibility to infectious disease. Some of the effects, such as in malaria, are validated in a variety of studies, whereas single case-control studies should be cautiously interpreted until more information on the specific outcomes is added. Taken in their totality, our results indicate that depending on the infection and the population studied, the same polymorphism may be associated with risk, protection or have no effect. In this context, our analysis provides the rationale for understanding the protective or adverse effect of
TLR4 polymorphisms and may provide a basis to explain the maintenance of these polymorphisms.

Supporting Information

Checklist S1. PRISMA checklist. (DOC)

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