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Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious diseases

C. Skevaki,* M. Pararas,†
K. Kostelidou,*‡ A. Tsakris† and
J. G. Routsias†
*Research Laboratories, Second Department of Pediatrics, †Department of Microbiology, School of Medicine, University of Athens, Athens, and ‡Department of Food Science and Nutrition, University of the Aegean, Lemnos, Greece

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Correspondence: C. Skevaki, Research Laboratories, Second Department of Pediatrics, 41 Fidipidou Street, 11527 Athens, Greece.
E-mail: cskevaki@allergy.gr

Summary

Toll-like receptors (TLRs) are the best-studied family of pattern-recognition receptors (PRRs), whose task is to rapidly recognize evolutionarily conserved structures on the invading microorganisms. Through binding to these patterns, TLRs trigger a number of proinflammatory and anti-microbial responses, playing a key role in the first line of defence against the pathogens also promoting adaptive immunity responses. Growing amounts of data suggest that single nucleotide polymorphisms (SNPs) on the various human TLR proteins are associated with altered susceptibility to infection. This review summarizes the role of TLRs in innate immunity, their ligands and signalling and focuses on the TLR SNPs which have been linked to infectious disease susceptibility.

Keywords: infection, innate immunity, TLR proteins, SNPs

Introduction

Most living organisms have developed efficient mechanisms of defence to protect them from encounters with pathogens. These defensive mechanisms constitute a phylogenetically preserved immunity, known as innate immunity [1]. Innate immunity is of paramount importance in the initial recognition of invading pathogens, as the ability of a host to perceive invasion by pathogenic organisms and to react appropriately to control infection. It is often synonymous to survival, as the result of delayed detection of pathogens can be devastating infections, exaggerated systemic responses and production of life-threatening tissue damage, organ dysfunction and death.

Innate immunity relies mainly on the recognition of evolutionarily conserved structures on pathogens, which are termed pathogen-associated molecular patterns (PAMPs), through a limited number of germline-encoded pattern recognition receptors (PRRs), of which the family of Toll-like receptors (TLRs) has been studied most extensively [2].

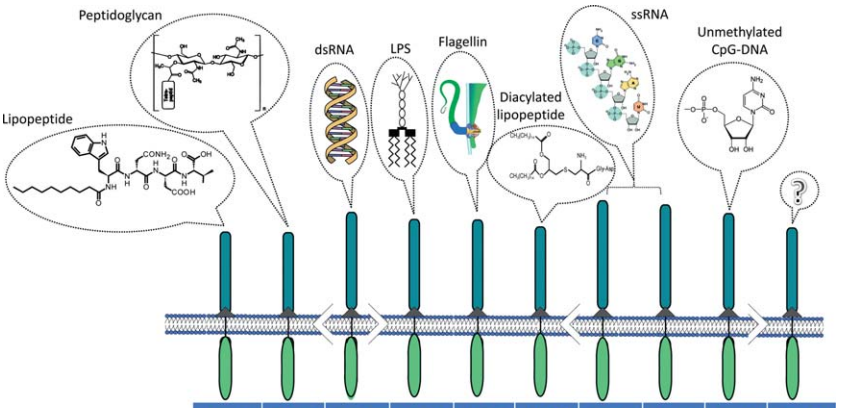
Genetic variations such as single nucleotide polymorphisms (SNPs) greatly influence innate immune responses towards pathogenic challenges and disease outcome; therefore, a range of susceptibility to infections appears among people, with some of them being predisposed to certain infections while others are being protected [3]. In this review, we present TLRs and their function and describe SNPs traced in TLRs and how these are affecting susceptibility to infectious diseases.

Innate immunity and TLRs

Innate and adaptive immunity are required to eliminate pathogens from the host. The innate immune system is based on imposing barriers to the entry of infection, but also on the recruitment of cellular components to identify and activate anti-microbial responses. In the battery of arsenal in the anti-microbial response, the host also relies on the activity of the TLR protein family which, through recognition of PAMPs and subsequent signalling pathways, triggers/orchestrates the inflammatory response in an attempt to clear the offending pathogen and leads to the specific adaptive response.

The family of TLRs is the major and most extensively studied class of PRRs. TLRs are evolutionarily conserved between insects and vertebrates. TLRs derived their name and were discovered originally based on homology to the *Drosophila* Toll protein [4], which was discovered as a gene involved in the control of dorsoventral axis formation in fruit-fly embryos, but was also shown to function in immunity of the insect [5]. It was this observation which opened the way for the subsequent description of TLRs in mammalian cells.

TLRs are type 1 integral membrane glycoproteins; the extracellular domain is characterized by the presence of varying numbers of leucine-rich repeats (LRRs) which form a 'horseshoe' structure interacting with nucleic acids and proteinaceous ligands [6] (Figs 1 and 2) and are therefore



	TLR-1	TLR-2	TLR-3	TLR-4	TLR-5	TLR-6	TLR-7	TLR-8	TLR-9	TLR-10
Gene ID	7096	7097	7098	7099	7100	10333	51284	51311	54106	81793
Chromosomal location	4p14	4q32	4q35	9q33.1	1q41	4p14	Xp22.3	Xp22	3p21.3	4p14
Number of aa	786	784	904	839	858	796	1049	1041	1032	811
Molecular weight (kD)	84	84	97	90	91	91	121	120	116	95

Fig. 1. Toll-like receptor (TLR) proteins. GeneBank Accession numbers, chromosomal locations, number of amino acids and molecular weight for each of the TLR proteins are provided. The pictures above the TLRs depict their main known ligands.

responsible for the TLR–ligand interaction. The LRR domains of the TLRs consist of 19–25 tandem copies of repeats that are 24–29 amino acids in length and contain *xLxxLxLxx* pattern. Each unit consists of a beta strand and an alpha-helix connected by loops. The cytoplasmic signalling domain is homologous to that of the interleukin (IL)-1 receptor, designated the Toll/IL-1R (TIR) domain. The TLRs can recognize a range of elements from bacteria, fungi, protozoa and viruses (ligands), which can be categorized into lipid, protein and nucleic acid components. Ligand binding to TLRs through PAMP–TLR interaction induces receptor oligomerization, homodimerization or heterodimerization, which subsequently triggers intracellular signal transduction.

To date, a total of 11 TLR homologues have been discovered in the human gene database [7], 10 of which are functional (TLR-1–TLR-10; see Fig. 1), and their ligands have been identified. TLR-11, which binds profilin in mice and recognizes uropathogenic bacteria, has been shown to be non-functional in humans, due to a premature stop codon [8,9]. Crystal structures of the human TLRs or their ectodomains, either as single proteins or as complexes with other receptors or ligands, have emerged in recent years, providing an insight into their dimerization, ligand-binding and signalling; specifically, the available structures are those of the TLR-1–TLR-2 heterodimer [10], TLR-3 [11], TLR-4–myeloid differentiation factor 2 (MD2)-2 with bound endotoxin antagonist eritoran [12] and TLR-5-flagellin [13].

Different approaches are used to classify human TLRs. Based on primary structure and function, they are subdivided into five subfamilies: TLR-2, TLR-3, TLR-4, TLR-5 and TLR-9 [14]. The TLR-2 subfamily comprises four members; namely, TLR-1, TLR-2, TLR-6 and TLR-10, which are highly homologous, and work in a pairwise combination in the presence of their respective ligands. The TLR-9 cluster includes TLR-7, TLR-8 and TLR-9. Subfamilies

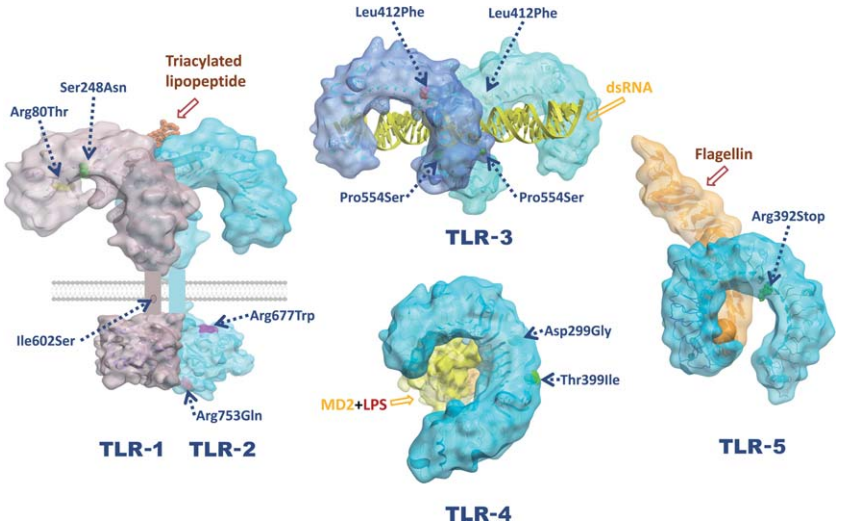


Fig. 2. Three-dimensional structure of Toll-like receptor (TLR) proteins. Block arrows show the main ligand for each TLR protein (single protein, homo- or heterodimer of two TLR proteins) as positioned on the protein. Dashed arrows show the positions of amino acids which change as a result of common single nucleotide polymorphisms (SNPs). The cell membrane and the intracellular domain is also depicted in the TLR-1/TLR-6 heterodimer.

TLR-3, TLR-4 and TLR-5 comprise a single member which works alone or in association with other receptors or molecules. TLRs are positioned in the cell as a function of the nature of their ligands. Members of the TLR-2, TLR-4 and TLR-5 subfamilies are located mainly in the plasma membrane and recognize extracellular ligands. The TLR-3 and TLR-9 subfamilies are located in the membranes of intracellular vesicles such as endosomes. Messenger RNA for at least one TLR is expressed (constitutively or induced following infection) in most tissues, while several tissues express them all [15].

The major importance of TLRs is also manifested by their ability to regulate cell proliferation and survival in a variety of biological settings [16]. This newly comprehended fact is in agreement with the TLR role, as the innate immune response should be able to expand populations of useful immune cells and integrate inflammatory responses to tissue repair procedures.

TLR ligands and signalling

TLRs can recognize a variety of components derived mainly from bacteria, fungi, protozoa and viruses. Known TLR ligands are presented in Fig. 1 and can be categorized into lipid, protein and nucleic acid constituents.

Of all the mammalian TLRs, TLR-2 is capable of detecting the widest PAMP repertoire within a range of pathogens, including Gram-positive and Gram-negative bacteria, mycobacteria, fungi, viruses and parasites [17]. This attribute is mainly the result of the heterodimerization ability of TLR-2 with either TLR-1 or TLR-6. The fact that TLR-2 is crucial for the recognition of Gram-positive bacteria and mycobacteria is of particular clinical importance. Gram-positive bacteria now represent the most common cause of severe infections linked to organ dysfunction or septic shock in the intensive care unit [18], while tuberculosis still remains a principal cause of death worldwide.

TLR-4 recognizes lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria [19]. TLR-5 recognizes flagellin, an important structural protein for motile bacteria [20]. TLR-3, TLR-7, TLR-8 and TLR-9 sense oligonucleotides from microbes or host cells: TLR-3 recognizes double-stranded RNA from viruses [21,22], while TLR-7 and TLR-8 detect viral and non-viral single-stranded RNA [23]. Finally, TLR-9 recognizes DNA from herpes simplex virus (HSV)-1/2 and unmethylated cytosine–phosphate–guanosine (CpG) motifs from bacteria and viruses [24–26].

A large number of ligands are recognized by TLRs. Accordingly, the signalling pathways following the binding of a ligand to its cognate TLR protein differ from one another. Despite the differences, however, we could describe a 'core' signalling pathway where, following ligand binding, TLRs dimerize and undergo conformational changes in order to recruit adaptor molecules via the TIR-domain [27]. The selective use of adaptor proteins is one of the main mechanisms for the differential signalling downstream of TLRs. The first adaptor molecule to be identified was myeloid differentiation factor 88 (MyD88) [28], which is involved in signalling triggered by all TLRs except TLR-3 (TLR-4 can activate both MyD88-dependent and MyD88-independent signalling). Using MyD88-deficient mice, it was shown that TLR-signal transduction can also occur independently of MyD88, resulting in the identification of additional adaptor molecules, such as MAL, MyD88 adaptor-like protein (which is also called TIRAP: TIR domain-containing adapter protein), TRIF, TRIF-related adaptor molecule (TRAM) and sterile alpha- and armadillo motif-containing protein (SARM).

In the pathways stimulated through TLR-2, -4 and -5, MyD88-dependent signalling leads to the activation of nuclear factor kappa B (NF-κB) via the IL-1 receptor kinases and TRAF6 resulting in expression of inflammatory genes, while in the pathways stimulated through TLR-7 and TLR-9, MyD88 signalling drives type I interferon (IFN) production.

The MyD88-independent pathway is regulated by TRIF [29]; TRIF activates the transcription factors IRF-3 and IRF-7 (interferon regulatory factors 3 and 7), which become phosphorylated, form hetero- or homodimers and translocate to the nucleus, where they trigger off the production of type I IFNs [30,31]. Therefore, TRIF is considered to be linked closely to anti-viral signalling, as TRIF-mediated signals result in IFN production.

While TLR-3 only uses TRIF as an adaptor molecule, TLR-4 uses TRIF under limited conditions in a MyD88-independent manner. Recent findings suggest that the preference between the MyD88- and TRIF-dependent signalling mechanisms may be attributed to the 'smooth' and 'rough' forms of LPS [32].

Common SNPs of Toll-like receptors and their potential association with infectious diseases

SNPs are DNA sequence variations occurring when a single nucleotide in the sequence genome is altered. Some SNPs, termed synonymous polymorphisms, do not result in any amino acid change in the protein, due to genetic code redundancy. In other cases (non-synonymous SNPs), the polymorphism results in an altered amino acid which may or may not affect protein structure or function. In this study, we present the common SNPs studied on the human TLRs and their association to infectious diseases.

TLR-1

TLR-1 heterodimerizes with TLR-6 to mediate host responses to lipopeptides from different classes of pathogens. TLR-1 and TLR-6 SNPs, as well as their association with infectious diseases, is given in Table 1. The SNP TLR-1–7202A>G (rs5743551, occurring in the 5′-near gene of

Table 1. Single nucleotide polymorphisms in Toll-like receptor (TLR)-1/TLR-6 and association with human diseases.

TLR	SNP id	SNP	Amino acid change	Association studied	Frequency*	Reference
TLR-1	rs5743551	−7202A>G	– (promoter region)	Increased mortality and organ dysfunction Increased susceptibility to Gram-positive infection in sepsis and septic shock	0·4324	[33]
TLR-1	rs5743618	1805G>T	Ser602Ile	Protection by 602Ser allele against leprosy†	n.a.‡	[34]
				Association of 602Ile with *Chlamydia trachomatis* infection among women with pelvic inflammatory disease		[38]
				Protection by 602Ser from pyelonephritis		[37]
TLR-1	rs4833095	A743G	Asn248Ser	Susceptibility to malaria in pregnancy exhibited by 248Asn allele (study presents data from African population, where 248Asn is not common)	0·200§	[39]
TLR-1	rs4833095	A743G	Asn248Ser	Susceptibility to tuberculosis	0·4372	[42]
TLR-1	rs5743618	G1805T	Ser602Ile		0·3227	
TLR-1	rs4833095	A743G	Asn248Ser	Susceptibility to *Atopobium vaginae*	0·4372	[43]
TLR-1	rs4833095	A743G	Asn248Ser	Susceptibility to invasive aspergillosis in allogeneic haematopoietic stem cell transplant recipients	0·4372	[85]
TLR-6	rs5743810	T745C	Ser249Pro		0·1971	
TLR-1	rs5743611	G239C	Arg80Thr		0·0366	

*Frequencies given are taken from the National Center for Biotechnology Information (NCBI) database, unless a comment is made. †602Ser is the major genotype in white individuals (75% frequency) but displays a decreased allele frequency in individuals of Turkish origin (43%), with whom the study was performed. ‡n.a. = not available in NCBI database. §The presented frequency for this single nucleotide polymorphism (SNP) is from the original paper.

TLR-1), was associated strongly with a higher risk for death in sepsis and organ dysfunction in a large prospective cohort of patients with sepsis and septic shock [33] and patients with septic shock, bearing that homology in TLR-1 −7202G had a markedly higher prevalence of Gram-positive infection. TLR-1 1805G>T(Ser602Ile, rs5743618) is a non-synonymous polymorphism, affecting the transmembrane domain of TLR (Fig. 2) that presents a high level of linkage disequilibrium (LD) with TLR-1 −7202A>G. Carriers of the isoleucine allele demonstrated an increased cell surface expression of TLR-1 on peripheral monocytes [34], while carriers of the serine allele have decreased signalling ability and produce decreased IL-6 levels after lipopeptide stimulation [35]. The SNP 1805G>T (Ser602Ile TLR-1) was shown to protect against leprosy among leprosy patients and asymptomatic control subjects recruited from areas where this disease was endemic. It was found that the 602Ser allele was significantly under-represented in the studied leprosy patient population. Interestingly, genotyping revealed that 602Ser was the most prevalent allele found in white individuals (75% frequency), with a decreased allele frequency observed in individuals of Turkish (43%) and African descent (26%) [34]. These data indicate that more than half of white individuals are homozygous for the 602Ser allele. All 21 donors of East Asian ancestry genotyped in the same study were homozygous for the 602Ile allele; the absence of the 602Ser allele in this group suggests that any strong selection for the appearing 602 allele was linked to either genetic/environmental factors, geographically confined specific pathogens or past epidemics [34]. Given the diverse range of pathogens recognized by TLR-1/TLR-2 heterodimers, the potential clinical impact of this SNP is high [36].

The TLR-1 Ser602Ile polymorphism has also been linked to urinary tract infections, with 1805TT (602Ile homozygous) showing protection from pyelonephritis when compared to the 1805GT (Ser602Ile heterozygous) or GG genotypes (Ser602 homozygous) [37], and protection from *Chlamydia trachomatis* infection in African American women with pelvic inflammatory disease [38].

Two TLR-1 polymorphisms, rs4833095 (Asn248Ser) and rs5743618 (Ser602Ile), were assessed among 302 primiparous Ghanaian women for their association with *Plasmodium falciparum* infection and manifestation; TLR-1 Asn248Ser variant was identified as being involved in the recognition of *P. falciparum* and indicated its role in susceptibility to and manifestation of malaria in pregnancy [39]. Indeed, recent data demonstrate that the glycosylphosphatidylinositols (GPIs) of *P. falciparum*, which activate macrophages and produce inflammatory responses, are engaged preferentially by TLR-2–TLR-1 dimers [40,41].

Alleles of these SNPs (248Ser and 602Ile) were also associated with tuberculosis disease in African American patients [42]. Asn248, which is common in European Americans, is a conserved residue in the extracellular domain of TLR-1 and TLR-6 (Fig. 2) and a putative glycosylation site; its replacement by Ser might result in altered glycosylation, potentially changing TLR-1 folding or function, e.g. in PAMP recognition or signal transduction [42].

Table 2. Single nucleotide polymorphisms in Toll-like receptor (TLR)-2 and association with human diseases.

TLR	SNP id	SNP	Amino acid change	Association studied	Frequency*	Reference
TLR-2	rs121917864	C2029T	Arg677Trp	Susceptibility to leprosy	n.a.†	[46]
TLR-2	rs121917864	C2029T	Arg677Trp	Susceptibility to tuberculosis	n.a.†	[47]
TLR-2	rs5743708	G2258A	Arg753Gln	Susceptibility to staphylococcal septic shock	0·0119	[48]
TLR-2	rs5743708	G2258A	Arg753Gln	Susceptibility to tuberculosis (Turkish population)	0·0119	[49]
TLR-2	rs5743708	G2257A	Arg753Gln	Increased risk of infective endocarditis	0·0119	[50]
TLR-2	rs5743708	G2257A	Arg753Gln	Protection from Lyme disease	0·0119	[51]
TLR-2	rs3804099	T597C	Asn199Asn	Susceptibility to filariasis by *Wuchereria bancrofti*	0·4491	[53]
	rs3804100	T1350C	Ser450Ser		0·1176	
TLR-2	rs3804099	T597C	Asn199Asn	Increased susceptibility to tuberculous meningitis	0·4491	[55]

*Frequencies given are taken from the National Center for Biotechnology Information (NCBI) database, unless a comment is made. †n.a. = not available in NCBI database; SNP = single nucleotide polymorphism.

Carriage of one of the key species in bacterial vaginosis, *Atopobium vaginae*, in the first half of pregnancy was associated significantly with the presence of TLR-1 743A>G variation (rs4833095) and associated marginally with the TLR-1 promoter −7202A>G variation [43]. However, even if these associations constitute a genuine biological phenomenon, overall, the attributable risk of these polymorphisms appears to be limited [43].

TLR-2

TLR-2, as a heterodimer with TLR-1 or TLR-6, recognizes a large number of common bacterial motifs, including lipopeptides, peptidoglycan, glycosylphosphatidylinositol (GPI)-linked proteins and zymosan. Table 2 summarizes the TLR-2 SNPs that are described below. Two non-synonymous SNPs in TLR-2 have been linked to human diseases [44]: first, a C>T transition in nucleotide 2029 (rs121917864), which replaces Arg677 with Trp (Arg677Trp), is common in African and Asian populations, but appears to be absent among white populations. *In vitro*, this SNP has been shown to inhibit both *Mycobacterium leprae*- and *M. tuberculosis*-mediated NF-κB activation and production [45]. In a Korean and a Tunisian population, this SNP was associated, respectively, with leprosy [46] and susceptibility to tuberculosis [47]; this agrees with data that patients carrying this allele show reduced basal and *Mycobacterium*-stimulated serum IL-12 levels, which is required for activation of the IFN-γ pathway and the induction of the T helper type 1 (Th1) response against intramacrophagic pathogens.

Another functional *TLR-2* variant (rs5743708) consists of a G>A substitution at nucleotide 2251, which replaces Arg753 by Gln (Arg753Gln). This SNP maps in a region of highly conserved amino acids in the C-terminal end of TLR-2 (Fig. 2), is present in 3% of healthy white blood donor control subjects, is identified in patients with staphylococcal septic shock [48], is associated with increased risk of developing tuberculosis in a Turkish population [49] and has been shown to be associated with a significantly increased risk for development of infective endocarditis [50]. Conversely, Arg753Gln was shown to occur at a significantly lower frequency in Lyme disease (LD) patients (especially late-stage disease) compared with matched controls [51], due possibly to a reduced signalling via TLR-2/TLR-1 [51].

An immune response is elicited through TLR-2 by products of the symbiotic bacterium *Wolbachia* [52], which is present in filarial parasites *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus* (the agents for lymphatic filariasis). A −196 to −173 deletion (del) polymorphism in the 5′ untranslated region of *TLR-2* and two synonymous SNPs, 597C>T (rs3804099, Asn199Asn) and 1350C>T (rs3804100, Ser450Ser) in exon 3, are associated with asymptomatic bancroftian filariasis in Thailand by *W. bancrofti* [53], with the molecular mechanisms possibly involving changes in protein levels, mRNA structure, stability, kinetics of translation and alternative splicing [54].

Alternatively, synonymous SNPs might be proxies for non-examined polymorphisms. The 597C>T has been reported previously in connection with increased susceptibility to tuberculous meningitis [55].

TLR-3

TLR-3 recognizes dsRNA from viruses and also synthetic oligonoucleotides such as a synthetic dsRNA analogue, poly(I : C) (polyinosine : polycytidyllic acid). Analysis of a number of SNPs occurring on *TLR-3* on 57 Japanese patients with Stevens–Johnson syndrome or toxic epidermal necrolysis with ocular surface complications revealed a strong association between two SNPs: 299698T>G (rs3775296, 5′UTR) and 293248A/G (rs3775290, exon 4, silent SNP Phe459Phe) of *TLR-3* [56]. Two genetic variations in *TLR-3* were shown to affect host susceptibility to enteroviral cardiomyopathies; first, the rare non-synonymous substitution TLR-3 Pro554Ser (rs121434431, 1660C>T), in patients with Coxsackievirus B3 myocarditis, and secondly, the common single nucleotide polymorphism, TLR-3 Leu412Phe, (rs3775291, 1235C>T), which

Table 3. Single nucleotide polymorphisms in Toll-like receptor (TLR)-3 and association with human diseases.

TLR	SNP id	SNP	Amino acid change	Association studied	Frequency*	Reference
TLR-3	rs3775296	G/T	mRNA pos 95 (5′-UTR of exon 2)	Association with Stevens–Johnson syndrome or toxic epidermal necrolysis	0·1828	
TLR-3	rs3775290	C1377T	Phe459Phe		0·2663	[56]
TLR-3	rs121434431	C1660T	Pro554Ser	Susceptibility to Coxsackievirus B3	n.a.†	[57]
	rs3775291	C1235T	Leu412Phe	myocarditis	0·2273	
TLR-3	rs121434431	C1660T	Pro554Ser	Resistance to HIV-1 infection	n.a.†	[59]
TLR-3	rs3775291	C1235T	Leu412Phe	Susceptibility to Herpes simplex virus encephalitis (HSV-1)	0·2273	[58]

*Frequencies given are taken from the National Center for Biotechnology Information (NCBI) database, unless a comment is made. †n.a. = not available in NCBI database; SNP = single nucleotide polymorphism.

was detected more frequently as homozygous for phenylalanine in the patient population by comparison with controls [57]. The 554Ser variant was also related to HSV-1 encephalitis (HSV-1), which blunted TLR-3 signalling in response to infection with the virus and acted in a dominant-negative manner, suggesting that its presence could contribute to the host susceptibility to infection and possibly determine clinical outcome [58]. The 412Phe mutation may confer resistance to HIV-1 infection [59]. Leucine 412 is next to an asparagine, whose glycan moiety contacts the dsRNA [60] (Fig. 2). As the Asp413 mutation to alanine significantly reduces TLR-3 signalling [61], it is possible that Leu412Phe could either affect the glycosylation of asparagine 413 or hinder its glycan moiety interaction with dsRNA, thus explaining the reduced signalling activity of Phe-412 TLR-3 [57]. The crystal structure of TLR-3 identifies histidine 539 and asparagine 541 as critical for ds RNA binding [11] and this may justify the importance of Proline 554, which is very close to the RNA binding site; the change into a serine could either prohibit ligand-induced dimerization or prevent conformational changes necessary for downstream signalling.

The aforementioned SNPs in relation to respective infectious diseases are summarized in Table 3.

TLR-4

The fact that TLR-4 is the receptor recognizing LPS has stimulated studies on its role in human disease. Two polymorphisms in *TLR-4* exist with population frequencies >5% and are reported to be up to 18% [42] (Table 4). These are a 1063A>G transition, resulting in an aspartic acid sub-

Table 4. Single nucleotide polymorphisms in Toll-like receptor (TLR)-4 and association with human diseases.

TLR	SNP id	SNP	Amino acid change	Association studied	Frequency*	Reference
TLR-4	rs4986790†	A1063G†	Asp299Gly†	Blunted response to inhaled LPS in humans	0·0445	[62]
	rs4986791†	C1363T†	Thr399Ile†		0·0530	
TLR-4	rs4986790†	A1063G†	Asp299Gly†	Increased susceptibility to respiratory syncytial virus	0·0445	[71]
	rs4986791†	C1363T†	Thr399Ile†	infection	0·0530	
TLR-4	rs4986790†	A1063G†	Asp299Gly†	Increased risk for severe malaria	0·0445	[70]
	rs4986791†	C1363T†	Thr399Ile†		0·0530	
TLR-4	rs4986790	A1063G	Asp299Gly	Protection from mortality due to cerebral malaria	0·0445	[64]
TLR-4	rs4986790†	A1063G†	Asp299Gly†	Invasive aspergillosis in recipients of haematopoietic cell	0·0445	[72]
	rs4986791†	C1363T†	Thr399Ile†	transplants	0·0530	
TLR-4	rs4986790	A1063G	Asp299Gly	Chronic cavitary pulmonary aspergillosis	0·0445	[73]
TLR-4	rs4986790†	A1063G†	Asp299Gly†	Elevated viral load in HIV-infected individuals	0·0445	[76]
	rs4986791†	C1363T†	Thr399Ile†		0·0530	
TLR-4	rs4986790†	A1063G†	Asp299Gly†	Increased risk for septic shock from infection by	0·0445	[65]
	rs4986791†	C1363T†	Thr399Ile†	Gram-negatives	0·0530	
TLR-4	rs4986790†	A1063G†	Asp299Gly†	Higher susceptibility to infection by Gram-negatives	0·0445	[66]
	rs4986791†	C1363T†	Thr399Ile†		0·0530	
TLR-4	rs4986790	A1063G	Asp299Gly	Increased mortality in children with invasive meningococcal disease	0·0445	[75]
TLR-4	rs4986790	A1063G	Asp299Gly	Susceptibility to systemic inflammatory response syndrome	0·0445	[68]
TLR-4	rs4986790†	A1063G†‡	Asp299Gly†‡	Resistance to infection by *Legionella pneumophila*	0·0445	[78]
	rs4986791†	C1363T†‡	Thr399Ile†‡		0·0530	

*Frequencies given are taken from the National Center for Biotechnology Information (NCBI) database, unless a comment is made. †Presence of two single nucleotide polymorphisms (SNPs) in the same cell means that both were identified in the subjects' sequence and therefore their co-existence was linked to the association studied. ‡Synonymous to A896G and C1196T, as given in the original paper. LPS = lipopolysaccharide.

stitution by glycine at amino acid location 299, Asp299Gly (rs4986790), and a 1363C>T transition conferring a threonine substitution by isoleucine at amino acid location 399, Thr399Ile (rs4986791). Individuals with Asp299Gly and/or Thr399Ile polymorphisms had a blunted response to inhaled LPS in humans [62], and this early finding triggered a number of studies in search of associations between these two polymorphisms and infectious diseases. Today, there are contradictory conclusions on the role of Asp299Gly on susceptibility to Gram-negative bacterial infections [63], due possibly to the fact that many studies looked only at the effect of either the Asp299Gly or Thr399Ile polymorphisms separately, neglecting the fact that these SNPs exist in a co-segregated (299Gly/399Ile) manner, which implies that a total of four haplotypes (wild-type/wild-type, 299Gly/wild-type, 399Ile/wild-type and 299Gly/399Ile) are represented in the population [64]. Additionally, the discrepancies may be attributed to the use of small sample sizes, different stimulatory methods or the use of *in-vitro* systems which may not mimic primary cells conditions [36]. Different studies have investigated the link between Asp299Gly SNP and sepsis. Two studies have demonstrated a link between this SNP and increased risk of septic shock due to infection by Gram-negatives [65,66]; the haplotype Asp299Gly/Thr399Ile had a higher prevalence of Gram-negative infections only in the latter study, but had little, if any, effect on susceptibility to septic shock [66]. The single TLR-4 Asp299Gly haplotype has been shown to alter the cytokine response to LPS, thus possibly affecting susceptibility to Gram-negative infection [67], and has also been associated with an increased incidence of systemic inflammatory response syndrome [68]. Taken together, these results suggest that the Asp299Gly haplotype may predispose to septic shock, but this impact may be restricted to Gram-negative infections, because it has been shown not to impact polymicrobial sepsis [69]. The effect of the rare Thr399Ile haplotype on function and susceptibility remains unclear due to its scarcity in the population [64].

Both the TLR-4-Asp299Gly and the TLR-4-Thr399Ile variants confer increased risk of severe malaria, respectively, in Ghanaian children, linking these SNPs to disease manifestation [70], while TLR-4 299Gly granted a protective effect against mortality due to *P. falciparum* cerebral malaria in Africa [42], where the TLR-4 299Gly allele is highly prevalent: in some populations, up to 15%. Despite this protective effect, its fixation (an increase in allele frequency of 100%) in Africa has been hindered by the deleterious effect of its protein product on the severity of Gram-negative infection, which may also be the cause of the near-complete elimination of 299Gly polymorphism from Europe and Asia [42].

The strongest association between TLR-4 polymorphisms and disease susceptibility has been reported in respiratory syncytial virus (RSV) infection, where infants heterozygous for Asp299Gly and Thr399Ile showed increased susceptibility to infection [71].

The presence of TLR-4-Asp299Ile and TLR-4-Thr399Ile in unrelated donors has been associated with an increased risk of invasive aspergillosis (IA) among recipients of haematopoietic cell transplants [72], and Asp299Gly has been associated significantly with chronic cavitary pulmonary aspergillosis [73].

Read *et al.* supported that there was no association between the Asp299Gly polymorphism and the susceptibility or severity of meningococcal infection, as the allele frequency of the Asp299Gly polymorphism was 5·9, 6·5 and 4·1% among blood donors, patients with microbiologically proven meningococcal disease and patients who died of meningococcal disease [74]. More recently, the results of another study suggested that the heterozygous TLR-Asp299Gly genotype is linked to an increased mortality in children with invasive meningococcal disease [75].

The TLR-4 polymorphisms Asp299Gly and Thr399Ile were shown to be associated with elevated viral load in HIV-infected individuals [76], possibly through a more central role of TLR-4 on the modification of the innate response at the colonized mucosal surfaces and the host's ability to fight invasive pathogens, and not due to an interaction of TLR-4 with the pathogen [77]. The TLR-4 polymorphisms Asp299Gly and Thr399Ile have also been shown to be associated with resistance to infection by *Legionella pneumophila*, an intracellular Gram-negative bacterium with an unusual LPS structure that is recognized primarily by TLR-2 rather than TLR-4, suggesting that *Legionella* stimulates TLR-4 in an unusual fashion that differs from other Gram-negative bacteria and may be cell-specific [78].

The crystal structure of the TLR-4 Asp299Gly/Thr399Ile has been solved as a complex with MD-2 and LPS [79], and its comparison to the wild-type TLR-4/MD-2/LPS complex structure demonstrated that the overall arrangements of the two complexes were similar and that topical differences were present only around the Asp299Gly SNP site, which induces a structural change modulating the surface properties of TLR-4. This effect may be more apparent upon stimulation of TLR-4 by ligands with weak agonistic activity. The impact of the Thr399Ile change was minor, as almost no structural differences were observed [79].

TLR-5

A cytosine–thymidine transition polymorphism at base pair 1174 in *TLR-5* (rs5744168) changes the arginine at amino acid 392 to a stop codon and results in a truncated TLR-5, which lacks the whole transmembrane domain as well as the 198 amino acid-long signalling cytoplasmic tail. This polymorphism acts in a dominant fashion with respect to the wild-type allele and is associated with susceptibility to infection with a flagellated organism, *L. pneumophila*, in

humans [80] without, however, rendering human carriers universally susceptible to infection with flagellated bacteria. The frequency of this SNP was not significantly different in patients with typhoid and matched control subjects and did not have any measurable effect on clinical parameters associated with typhoid fever [81]. Interestingly, the TLR-5_{392}STOP, a null variant, is present at frequencies ranging from 10% in Europeans to up to 23% in some populations, which suggests the TLR-5 function being partially compensated by other genes and to TLR-5 being functionally redundant [82]. Alternatively, pathogen recognition may occur on many levels and this is a plausible, previously unappreciated feature of the innate immune system. The identification of a second flagellin receptor, Ipaf (presently called NLCR4) [83,84], is consistent with this idea, while the fact that these two genes follow different downstream paths and sense different types of bacteria may indicate co-operation between them in the recognition of flagellated bacteria, rather than a complete functional redundancy.

TLR-6

TLR-6 forms heterodimers with TLR-2 (like TLR-1) to mediate host responses against lipopeptides from different organisms (Table 1). There is little information on functional studies on TLR-6 polymorphisms and association studies with infections. In one study, Kesh *et al.* [85] examined the association between SNPs in TLR-1, TLR-4 and TLR-6 genes and the risk of developing IA in 127 allogeneic haematopoietic stem cell transplant recipients consisting of 22 patients with IA and 105 unaffected control subjects. Ser249Pro polymorphism was found to potentiate IFN-γ release following injection of the bacillus Calmette–Guérin (BCG) vaccine [86] TLR-6 SNPs have been also associated with non-infectious diseases, but these are outside the scope of this review.

TLR-7/TLR-8

TLR-7 and TLR-8 recognize viral single-stranded RNA. Multiple uridine-rich oligoribonucleotides derived from HIV-1 virus have been demonstrated to activate human TLR-7 and TLR-8, and the TLR-7/TLR-8 ligation to the viral RNA is suggested to affect susceptibility to or course of the infectious disease [63]. Data on the impact of TLR-7/8 SNPs on HIV infection remain scarce. The presence of the most frequent TLR-7 polymorphism, TLR-7 Gln11Leu (rs179008), was associated with higher viral loads and accelerated progression to advanced immune suppression in HIV patients [87]. Conversely, presence of the most frequent TLR-8 polymorphism, TLR-8 1A>G, Met1Val (rs3764880) was shown to confer a significantly protective effect regarding progression of the disease [88]. These polymorphisms are also related to chronic hepatitis C virus (HCV) infection, with TLR-7 Gln11Leu SNP not affecting HCV viral load, but decreasing IL-29/IFN-λ expression and being associated with disease-induced portal lymphoid aggregates [89]. Wang and co-workers demonstrated that the TLR-8 129G>C (rs3764879, 5′ near gene) and TLR-8 1G>A variants were in complete linkage disequilibrium, and that the frequency of TLR-8-129C/+1A was significantly higher in male patients with HCV infection compared with healthy controls, as well as that variations in TLR-7 and TLR-8 genes might impair immune responses during HCV infection [90]. The same group reported recently that these variations affect NF-kB signalling and cytokine production upon stimulation of human monocytes with specific TLR-7/TLR-8 agonists, thus modulating immune responses during HCV infection [91]. Finally, the TLR-8 SNPs Met1Val and −129G/C polymorphisms render individuals more susceptible to Crimean–Congo haemorrhagic fever [92] and tuberculosis [93]. The association of TLR-7/TLR-8 SNPs and infection are presented in Table 5.

Table 5. Single nucleotide polymorphisms in Toll-like receptor (TLR)-7 and TLR-8 and association with human diseases.

TLR	SNP id	SNP	Amino acid change	Association studied	Frequency*	Reference
TLR-7	rs179008	A32C	Gln11Leu	Higher viral load and fast progression to immune suppression in HIV patients	0·152	[87]
TLR-7	rs179008	A32C	Gln11Leu	Chronic hepatitis C virus infection (HCV)-induced portal lymphoid aggregates	0·152	[89]
TLR-8	rs3764880	A1G	Met1Val	Protection from progression of disease in HIV patients	0·439	[88]
TLR-8	rs3764879†	G129C†	5′-near gene†	Susceptibility to pulmonary tuberculosis (Indonesian population)	0·152	[93]
	rs3788935†		5′-near gene†		0·434	
	rs3764880†		5′-near gene†		0·439	
	rs3761624†	A1G†	Met1Val†		0·434	
TLR-8	rs3764879†	G129C†	5′-near gene†	Susceptibility to HCV infection	0·152	[90]
	rs3764880†	A1G†	Met1Val†		0·439	
TLR-8	rs3764879†	G129C†	5′-near gene†	Susceptibility to Crimean–Congo haemorrhagic fever		[92]
	rs3764880†	A1G†	Met1Val†		0·439	

*Frequencies given are taken from the National Center for Biotechnology Information (NCBI) database, unless a remark is made. †Presence of two or more single nucleotide polymorphisms (SNPs) in the same cell means that all were identified in the subjects' sequence and therefore their co-presence was linked to the association studied.

Table 6. Single nucleotide polymorphisms in Toll-like receptor (TLR)-9 and association with human diseases.

TLR	SNP id	SNP	Amino acid change	Association studied	Frequency*	Reference
TLR-9	rs352140	A1635G	Pro545Pro	Rapid progression of HIV type 1 infection†	0·4579	[95]
	rs352139	G1174A	intronic		0·4642	
TLR-9	rs352140	A1635G	Pro545Pro	Lower viral set-point and slower progression of HIV type 1 infection†	0·4579	[76,96]
TLR-9	rs5743836	T1237C	– (promoter region)	Allergic bronchopulmonary aspergillosis Lack of mediating role in the natural history of the HPV infection	0·1781	[73,97]
TLR-9	rs187084	C->T	5′-near gene	Development of sepsis and multiple organ dysfunction	0·3967	[94]
	rs352162	T>G	3′-flanking region		0·4726	

*Frequencies given are taken from the National Center for Biotechnology Information (NCBI) database, unless a comment is made. †This discrepancy may be due to the fact that the Swiss 2007 cohort included subjects enrolled before the application of anti-retroviral therapy, and rapid progressors were not included as a consequence of higher mortality rates. HPV = human papilloma virus; SNP = single nucleotide polymorphism.

TLR-9

Little is known about the clinical relevance of TLR-9 gene polymorphisms in critical illness. It was concluded that the TLR-9 polymorphisms rs187084 C>T transition (occurring in the 5′ near gene of *TLR-9*) and rs352162 T>G (located in the 3-flanking region of *TLR-9*) were useful to provide relevant risk estimates for the development of sepsis and multiple organ dysfunction in patients with major blunt trauma [94]. Data from a Swiss HIV cohort in 2007 implicated two TLR-9 polymorphisms, A1635G (rs352140, Pro545Pro) and G1174A (rs352139, intron near 5′ UTR) with the rapid progression of HIV type 1 infection, with patients displaying a rapid CD4 cell decline [95]. SNP A1635G occurs in exon2 of the *TLR-9* gene but does not lead to any amino acid change (Pro545Pro), while G1174A is located in intron 1. Two more recent studies, however, showed that the A1635G allele was associated with a lower viral load set-point and slower progression [76,96]. This discrepancy may be attributed to the fact that the Swiss 2007 cohort included subjects enrolled before the application of highly active anti-retroviral therapy, therefore not including rapid progressors who may have deceased and thus posing a bias in the study.

Finally, a link between allergic bronchopulmonary aspergillosis (ABPA) was demonstrated by Carvalho and co-workers [73], where patients with ABPA had a significantly higher frequency of allele C for the T-1237C SNP in *TLR-9* (rs5743836) than control patients (20·5 *versus* 9·4%). This polymorphism is located within the putative promoter of the TLR-9 gene and has been implicated in chronic inflammatory diseases, including asthma. The same SNP was tested for a possible role in infection risk by human papilloma virus (HPV), but found not to be associated significantly with either HPV clearance or persistence [97]. Table 6 presents TLR-9 SNPs and their association with human infectious diseases.

TLR-10

No ligand has been described for TLR-10. Due to its phylogenetic and physical proximity of TLR-10 to TLR-1 and TLR-6, several groups have looked for associations between this receptor and human diseases, with indications that TLR-10 SNPs may be linked to increased risk of certain cancers (to be described elsewhere) [36].

Rare polymorphisms of Toll-like receptors

An increased frequency of rare mutations at the TLR-4 locus was clearly observed among UK patients with systemic meningococcal disease. Moreover, only rare mutations of TLR-4, and only missense mutations, appeared to have been concentrated within the meningococcal population [98]. These were 4350 G>A (Gly9Glu), 8457A>G (Tyr46Cys, rs78848399), 12293T>C (no protein change), 12413C>A (no protein change), 12874 A>G and 13174 C>T (Asp299Gly and Thr399Ile), 13040 A>G (no protein change), 13174C>T (Thr399Ile), 13398 G>A (Glu474Lys, rs5030718) 13937G>A (no protein change-No rs), 13982 T>G (no protein change), 14059 A>G (Lys694Arg, rs5030722) and 14266 G>A (Arg763His, rs5030723). However, in this study, the relatively common variant, containing Asp299Gly, Thr399Ile and Arg763His was present in both the patient group and the control group, and is thus absolved of suspicion as a causative factor in meningococcal sepsis.

TLR-9 SNPs were characterized *in vitro* in human embryonic kidney (HEK)293 cells and two relatively rare variants, Pro99Leu (rs5743844) and Met400Ile (rs41308230), were associated with altered receptor function regarding NF-κB activation and cytokine induction. In the most impaired variant, P99L, the ability to respond to physiological and therapeutic TLR-9 ligands was severely compromised, while binding to CpG-containing oligonucleotides (CpG-ODN)

remained normal, implying that their recognition by TLR-9 may involve two separate events, CpG-ODN binding and sensing and that residue Pro-99 is important for the latter process [99]. However, further research is needed into their relevance for infectious disease susceptibility or responsiveness to CpG-ODN-based therapies.

Perspectives and conclusions

The initial recognition of pathogens induces inflammatory reactions at the infected site and triggers adaptive immunity against the pathogens, but different people exhibit different susceptibility to infections and different immune responses against the same pathogen. Besides the obvious role of HLA antigens for mounting an adaptive immune response, innate immunity responses can also be different from person to person. In this regard, polymorphisms in pattern recognition receptors and downstream signalling molecules have been associated with increased or decreased susceptibility to infections, suggesting that their detection may have an increasing impact on the treatment and prevention of infectious diseases in the coming years. SNPs in TLRs may indeed point to the susceptibility of a certain individual towards an infectious disease. Infectious risk stratification may be particularly relevant for a palette of patients, because of the high prevalence and severity of infections. As such, functional studies of allelic variants may provide a better understanding of pathophysiological processes. Recently, Hold and co-workers [100] demonstrated that transfection of TLR-4D299G, TLR-4T399I or TLR-4D299G/T399I into HEK cells results in constitutive activation of an NF-κB reporter gene and blunting of the LPS-induced reporter activation compared to wild-type TLR-4. Additionally, they showed that many genes, particularly the TRAM/TRIF signalling pathway constituents, are down-regulated in unstimulated human monocyte/macrophages from patients with the D299G and T399I SNPs, while upon LPS stimulation these cells display lower NF-κB levels, higher IFN-β gene expression levels and overall altered cytokine profiles, compared to the wild-type receptor. Taken together, these data support that these common TLR-4 SNPs affect constitutive receptor activity, thus impacting that ability of the host to respond to LPS challenge and a suboptimal immune response to infection [100].

In addition to SNPs, given that TLRs are associated with other gene products for proper function, deficiencies in either the tlr-coding genes or the genes encoding proteins associated with TLRs, constitute yet another domain of required study [101]. Examples include the association between a deficiency in the intracellular protein UNC-93B, which is involved in TLR-3 signalling, or a dominant-negative TLR-3 allele with HSV-1 encephalitis (HSE) [58,102].

Genetic association studies have indeed contributed to the understanding/unravelling of the genetic variations and their involvement in a range of diseases. The manifestation of a disease, however, does not result simply from the presence/absence of a particular SNP, but from the additive interaction between various genetic, epigenetic and environmental factors. Therefore, despite the contribution of genetic association studies, studies should also shift towards including the mapping of additional regions, such as intron sequences, regulatory elements, RNA molecules and unravelling the contribution of epigenetic factors, methylation patterns and post-translational modifications. Such data should then be considered under different micro- or macro-environments, as all genetic and epigenetic factors may alter predisposition to the environment. Infectious diseases are also determined by exposure, microbe diversity and microbe genetic heterogeneity, and therefore the precise understanding of the role of virulence and pathogenesis is of paramount importance.

Understanding of the magnitude of both the genetic/epigenetic variations, but also environmental factors, will catalyse the development of a therapeutic intervention.

As the idea that all diseases and traits can be explained by single mutations was proved wrong long ago, the effect of SNPs in complex traits and diseases must be assessed more specifically in the context of all the above parameters and through the replication of data indicating any genetic associations in order to establish the certainty for such findings. One way of achieving this goal would be through the use of large-scale cohorts, the increase in genomewide approaches/studies and a shift towards whole-genome (rather than exon-centric-only) studies. As TLRs are coupled with other receptors and molecules, precise understanding of the mechanisms involved in immune response and adaptive response is a prerequisite for the effort towards developing new therapeutic strategies and therapeutics to regulate the immune system efficiently and, eventually, advance quality of human life.

Disclosure

The authors declare that there are no financial or commercial conflicts of interest.

Author contributions

C. L. S. and J. R. designed the outline; K. K., C. L. S. and M. P. wrote the paper; and J. R. and A. T. reviewed the paper.

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