

EDITORIAL

TLRs, SNPs and VLBWs: Oh My!

Journal of Perinatology (2013) 33, 745–747; doi:10.1038/jp.2013.94

Elegant sensing mechanisms have evolved to facilitate detection of potentially pathogenic microorganisms. Since Charles Janeway predicted their existence,¹ multiple classes of pathogen recognition receptors (PRRs) have been discovered that serve as detectors of microbial products, including cell wall and membrane components, flagellum, nucleic acids and carbohydrates. Perhaps no class of PRRs is as well characterized and more widespread in publications across multiple scientific disciplines than the Toll-like receptors (TLRs). The tale of TLRs is an exciting story that has generated two Nobel Prizes in Physiology or Medicine (1995, 2011). It began with the discovery of Toll protein as an important regulator of *Drosophila* embryogenesis in 1985 by Christiane Nüsslein-Vollhard^{2,3} (Nobel Prize 1995). Then Bruno Lemaitre in the laboratory of Jules Hoffman discovered that flies deficient in Toll were more susceptible to fungal infection, thereby

establishing the critical connection of *Toll* and immune function.⁴ In 1997, a human variant of Toll ('Toll-like') was identified by Ruslan Medzhitov and Charles Janeway, Jr, as a type I transmembrane protein receptor with an extracellular leucine-rich repeat domain and a cytoplasmic domain homologous to the human interleukin (IL)-1 receptor.⁵ The subsequent cloning of TLR4 by Bruce Beutler⁶ (Nobel Prize 2011) and multiple other landmark studies that have identified TLRs as key innate immune system receptors established TLRs as a permanent and critical part of pathogen recognition.⁷ Ten TLRs have been described in humans, each for the most part with a specific agonist that triggers activation of second messenger signaling and ultimately cellular activation along with the production of inflammatory mediators (Figure 1a).

Single-nucleotide polymorphisms (SNPs, pronounced 'snips') are DNA sequence variations that occur when a single nucleotide in the genome sequence is altered (Figure 1b). Common SNPs occur in > 1% of the population, account for ~90% of all human genetic variation, and may happen every 100–300 bases anywhere

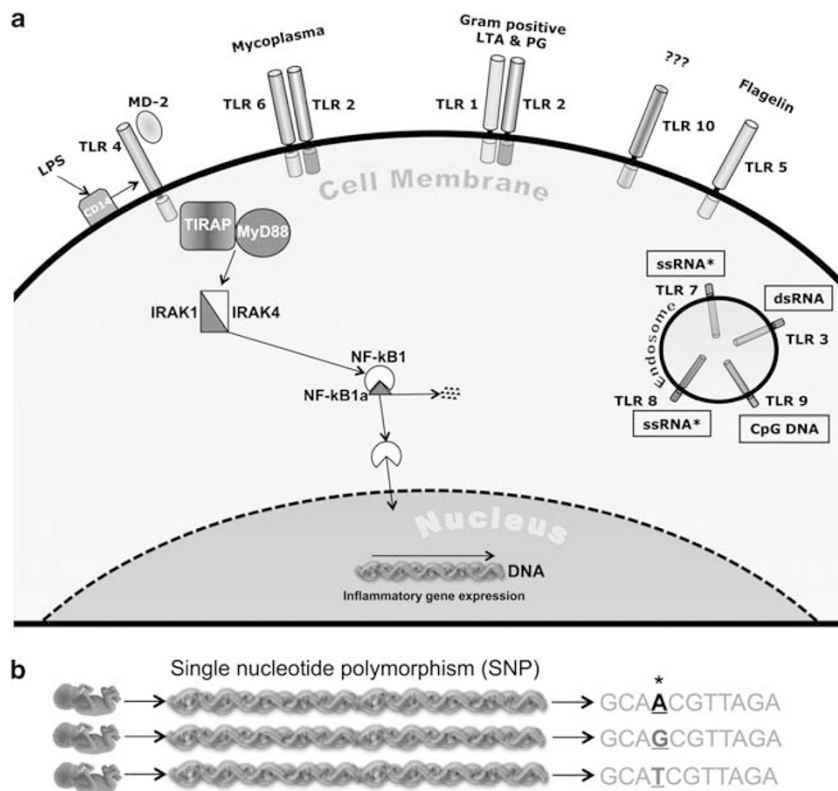


Figure 1. (a) Toll-like receptors are present on the cell membrane and within endosomes. Selected intracellular second messenger signaling molecules examined in the Sampath *et al.*²⁹ study are shown. Following stimulation of TLR4, a cascade of intracellular second messengers (TIRAP, MyD88, IRAK1, IRAK4) ultimately leads to destruction of the inhibitor NF-κB1a (blue triangle), which allows NF-κB1 to enter the nucleus and stimulate transcription of inflammatory gene products such as cytokines and chemokines. (b) Single-nucleotide polymorphisms (SNPs) are variations in the DNA sequence. For each infant, there is a single-nucleotide variation (represented by *) in the hypothetical gene sequence shown. DNA, deoxyribonucleic acid; IRAK1, interleukin-1 Receptor-Associated Kinase 1; IRAK4, interleukin-1 Receptor-Associated Kinase 4; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MD-2, myeloid differentiation factor 2; MyD88, myeloid differentiation factor 88; NF-κB1, nuclear factor of kappa light polypeptide gene enhancer in B cells 1; NF-κB1a, nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor alpha (also called IκBα); PG, peptidoglycan; ssRNA, single-stranded ribonucleic acid; TIRAP, toll-interleukin-1 receptor domain containing adaptor protein; TLR, Toll-like receptor. *These TLRs may also respond to synthetic agonists including imidazoquinolines and nucleoside analogs.

along the length of the human genome.⁸ Because only ~5% of human DNA represents protein-coding genes, most SNPs naturally occur in noncoding regions of the genome. Of note, with the exceptions of the amino acids methionine and tryptophan that have a single codon, all other amino acids manifest codon redundancy (multiple codons lead to translation of the same amino acid). Thus, a SNP may produce no overt effects even if it occurs within a gene. However, a SNP may result in a clinical phenotype (for example, altered disease risk or response to medication) if it occurs within a specific gene, including key regulatory elements (for example, transcription factor). Because TLRs and the downstream molecules that follow TLR stimulation have an essential role in pathogen recognition and response, SNP-induced alterations in their structure may adversely affect function and the capability of the host to respond to infectious challenge.

This month in the Journal, Dr Sampath *et al.*⁹ report the impact of nine functional TLR SNPs (TLRs 2, 4, 5, 9, and downstream signaling genes including Interleukin-1 Receptor-Associated Kinase 1 (IRAK1), toll-interleukin-1 receptor domain containing adaptor protein (TIRAP), and nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NF- κ B1), and its inhibitor nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NF- κ B1a), also called I κ B α) on bacterial infections in a cohort of 408 VLBW infants. Importantly, the authors performed an *a priori* power calculation to determine the sample size (400 infants) required to detect a 12 to 15% difference in SNP prevalence between groups. Ninety infants had culture-positive infections (40 with bacteremia, 26 tracheal infections, 3 urinary tract infections and 21 multi-site infections). After controlling for confounders, the presence of a TLR4 SNP was associated with increased risk of Gram-negative infections (odds ratio=3.1). Interestingly, the presence of the TLR5 variant was associated with increased circulating white blood cell counts.

Several recent neonatal studies have demonstrated an association between small variations in DNA, specifically SNPs, and infection development and outcomes.^{10–14} In the Abu-Maziad *et al.*¹⁰ study, several SNPs were identified as potentially important risk factors for the development of infection in preterm (<37 weeks completed gestation) infants. These included mediators of pathogen recognition (TLR2, TLR5), mechanisms of host defense against bacteria (phospholipase A2) and a critical anti-inflammatory cytokine (IL-10). SNPs are associated with abnormal host responses to infectious challenges, risk of the development of infection and outcomes following infection in other neonatal populations.^{11–13,15–17} A recent twin study that assessed frequency of infections among mono- and di-zygotic prematurely born twins identified that 49.0% ($P=0.002$) of the variance in susceptibility to late-onset sepsis was due to genetic factors alone, and 51.0% ($P=0.001$) was the result of residual environmental factors to variation in infection risk attributable to inherited factors.¹⁸

SNPs in TLRs are associated with increased risk for infection in adults^{19–22} and in children^{23–25} but have been less well characterized in neonates. Specific SNPs are associated with hearing loss after meningitis,²⁶ disease progression in HIV-infected children,²⁷ the effects of vitamin A supplementation,²⁸ and atopy.²⁹ In addition to their present report, Dr Sampath *et al.*⁹ have also recently reported that a TLR5 SNP was associated with severe bronchopulmonary dysplasia.³⁰ The frequency of TLR4 SNPs differs among African, Asian and European populations that may become important for stratifying disease risk.³¹ Coding modifications for upstream and downstream TLR signaling molecules may also impact risk of infection. Genetic variations in the lipopolysaccharide (LPS) coreceptor CD14, LPS binding protein (which binds to intravascular LPS and facilitates its attachment to CD14), and myeloid differentiation-2 (a small protein that associates with TLR4 to enhance LPS signaling) are associated with increased risk for sepsis in adults.^{32–35} Polymorphisms in

downstream (post-TLR activation) intracellular signaling molecules including myeloid differentiation factor 88,³⁶ IL-1-receptor-associated kinase 4 (IRAK4)³⁷ and NF- κ B essential modulator (NEMO)³⁸ are associated with invasive bacterial infection in older populations.

As with any diagnostic or prognostic test, studies that have identified SNPs associated with clinical conditions have limitations and important issues for consideration.³⁹ Studies must be powered appropriately and employ rigorous statistical approaches to avoid type I and II errors. The results from well-designed and appropriately powered genome-wide association studies that identify SNPs could have a significant impact in neonatology. The presence of SNPs may inform us which women may have an increased risk of preterm birth, chorioamnionitis, pregnancy-induced hypertension and so on before they become pregnant and/or deliver preterm. After birth, specific SNPs may help us identify neonates at highest risk for the development of devastating complications of prematurity including sepsis, bronchopulmonary dysplasia, retinopathy or prematurity, white matter damage, necrotizing enterocolitis and poor neurodevelopmental outcomes. SNPs may help to stratify patients, so risk may be evenly matched in future interventional randomized clinical trials. The impact of SNPs in the field of pharmacogenetics is an area of intense research and may lead to more effective and personalized treatment strategies and a reduction in drug-related complications of treatment. The discovery of genetic variations associated with infection and other adverse outcomes in neonatology is in its early infancy. Dr Sampath *et al.*⁹ have illustrated how these personal molecular data may be translated from the bench to bedside to help us improve care for our smallest infants.

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