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Innate immune gene variation and differential susceptibility to uterine diseases in Holstein cows

P.J. Pinedo [a,b], K.N. Galvão [c], C.M. Seabury [b,*]

[a] Texas A&M AgriLife Research and Extension Center, Amarillo, Texas, USA
[b] Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University System, College Station, Texas, USA
[c] Department of Large Animal Clinical Sciences and D. H. Barron Reproductive and Perinatal Biology Research Program, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

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ABSTRACT

An immune response is mounted after binding of Toll-like receptors (TLRs) to pathogen-associated molecular patterns. The primary objective of this study was to test for the associations between bovine single-nucleotide polymorphisms (SNPs) and insertion-deletion (indel) mutations occurring in seven bovine *TLR* genes (*TLR*s 1, 2, 4, 5, 6, 9, and 10) that are known to recognize bacterial ligands and the most significant uterine diseases in dairy cows, including metritis (MET), clinical endometritis (CE), and cytologic endometritis (CYE). Custom allele-specific genotyping assays derived from multiple bovine *TLR* sequencing studies were utilized. Genotypes for 110 loci (SNPs and indels) that are known to be variable in domestic cattle were determined, resulting in 46 monomorphic loci, 64 loci with two alleles, and 35 loci that did not meet our inclusion criterion for minor allele frequency ($\geq$0.10). The association between specific *TLR* genotypes and each of the uterine diseases (MET, CE, CYE) was evaluated by logistic regression with correction for confounding variables. Collectively, seven SNPs produced uncorrected P values $\leq$0.05 with respect to three different uterine diseases investigated, but none of the SNP associations endured correction for multiple testing (P values $\geq$ 0.05). Several confounding variables, including parity, dystocia, and ketosis before 17 DIM, remained significant after correction for multiple testing. Our analysis of these data suggest that some bovine *TLR* SNPs (i.e., *TLR*s 2, 4, 6, 9) may potentially elicit relatively small effects on uterine health in Holstein dairy cows and that some confounding variables are actually more predictive for the incidence of disease than any genetic markers evaluated herein.

1. Introduction

A key component for bovine reproductive efficiency is the maintenance of adequate uterine health that allows for rapid resumption of reproductive function after calving. However, the challenge of uterine contamination with microorganisms at parturition is unavoidable with bacteria present in the uterus of 80% to 100% of cows within the first 2 weeks after calving [1,2]. Notably, some cows respond to this challenge successfully, but about 20% of the cows will subsequently develop metritis (MET) within 3 weeks postpartum [2,3]. After 3 weeks, a similar proportion of cows will develop clinical endometritis (CE) [4,5], and the prevalence of the subclinical condition will range from 26% to 74% between 40 and 60 DIM [5–7]. Importantly, both metritis and endometritis contribute to increased days to first breeding, decreased conception and pregnancy rate, and increased culling [5–9]. In addition to tangible economic losses consisting of ~$285 per lactation [6], animal welfare is also compromised by uterine diseases; affected cows suffer from loss of appetite, often become dehydrated, and may also show signs of pain [1].

* Corresponding author. Tel.: +1 979 845 2720; fax: +1 979 845 9972.
E-mail address: cseabury@cvm.tamu.edu (C.M. Seabury).

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Genetic selection for increased disease resistance in cattle has been proposed [10,11], and a recent study reported that dairy cattle identified as high immune responders (i.e., individuals mounting a significant general antibody and cell-mediated immune response) were at lower risk of developing disorders such as mastitis, metritis, and retained placenta [12]. At the molecular level, genes that represent one of the first lines of host defense by modulating innate immunity responses against a variety of invading pathogens have also been considered putative candidate loci for improving host resistance to disease in agricultural species [13]. Innate immune receptors recognize a variety of molecular ligands derived from diverse microbial species, thereafter eliciting host responses to invading pathogens without requiring prior exposure [14–16]. The totality of this response depends on the action of neutrophils (polymorphonuclear cells (PMNs)), monocytes, and macrophages, with pattern recognition receptors that initiate signaling pathways leading to the release of proinflammatory cytokines [17]. One important group of pattern recognition receptors are the Toll-like receptors (TLRs), which recognize a variety of microbial components (pathogen-associated molecular patterns), and subsequently regulate the activation of both innate and adaptive immunity [14,15,18]. At least 10 members of the *TLR* gene family are known to exist in mammals, with all members encoding type I transmembrane proteins of the interleukin-1 receptor family that consists of N-terminal leucine-rich repeats involved in ligand recognition, a transmembrane domain, and a C-terminal intracellular Toll/IL-1 receptor homologous domain for signal transduction [10,14,15]. Four *TLR*s have been associated with the recognition of viral constituents (*TLR*s 3, 7, 8, 9), whereas six (*TLR*s 1, 2, 4, 5, 6, 9) are known to recognize microbial and/or synthetic ligands [18–20]. Still, the ligand specificity of TLR10 remains elusive [21].

Importantly, previous studies reported that the mammalian *TLR* genes are primarily expressed by antigen-presenting cells, including macrophages, natural killer cells, and dendritic cells [19,22], with several studies also reporting that some naturally occurring *TLR* variants increased the risk of severe infections in humans, mice, and domestic cattle [23–25]. Relevant to bovine reproduction, the initial defense of the endometrium against invading microbes is dependent on the innate immune system, including initial recognition by host TLR proteins and secretion of antimicrobial peptides, cytokines, and acute phase proteins [3,26,27]. Moreover, neutrophils provide the initial cellular defense against bacterial colonization within the uterus [26,28–30], and cows diagnosed with puerperal metritis and subclinical endometritis are known to have significantly reduced blood PMN functions during the periparturient period, when compared with cows with normal uterine health [30,31]. Moreover, the ability of neutrophils to perform multiple antimicrobial effector functions as well as the induction of cytokines and chemokines that ultimately serve to recruit other immune cells is largely dependent on recognition and signaling by host *TLR*s [18]. Therefore, we hypothesized that some naturally occurring variation within the bovine *TLR* genes may be associated with differential susceptibility to several economically important reproductive diseases in Holstein dairy cattle. Moreover, the primary objective of this study was to test for associations between bovine single-nucleotide polymorphisms (SNPs) and indels within seven bovine *TLR* genes (*TLR* 1, 2, 4, 5, 6, 9, and 10) that are known to recognize microbial ligands and the most economically significant uterine diseases affecting dairy cows. Herein, we provide evidence that some bovine *TLR* variants may potentially elicit small effects related to risk for metritis, clinical endometritis, and cytologic endometritis.

2. Materials and methods

2.1. Study population, general management, and disease monitoring

The study was conducted for 1 year, spanning from October 2010 to October 2011 within the University of Florida Dairy Unit (Gainesville, FL, USA). The study cohort consisted of 358 Holstein cows (164 primiparae and 194 multiparae) that were genotyped. Diagnosis of CE and CYE was not available for a total of 6 and 25 cows, respectively. The research herd consisted of 550 lactating cows, with a yearly rolling herd average for milk production of 9910 kg. The herd was milked twice daily and was a member of the Dairy Herd Improvement Association (Raleigh, NC, USA), with an on-farm computer-based record system (AfiFarm, SAE Afikim Kibbutz Afikim, Israel). Prepartum transition cows that were within 2 weeks of calving were maintained in a maternity barn, fed a low DCAD diet, and monitored for signs of calving by farm employees trained to assist with parturition. Calving events, such as dystocia, twins, and retained fetal membranes (RFMs), were recorded by the farm personnel. Cows that did not expel the fetal membranes within 24 hours after calving were considered to have RFM. After parturition, cows were sent for 2 days to an open hospital facility with shade and a sand covered floor. At 3 days postpartum, healthy cows were moved to the lactating herd and kept in a barn consisting on freestall facilities with a concrete floor. Cows were fed a totally mixed ration formulated to meet or exceed the requirements of lactating dairy cows weighing ~680 kg and producing 45 kg of 3.5% FCM, as recommended by the National Research Council [32]. All cows went through a routine postpartum health monitoring protocol that consisted of an evaluation on Days 4, 7, and 12 after calving, as performed by trained farm personnel or veterinarians from the University of Florida. The protocol included the assessment of attitude, rectal temperature, rectal palpation, and examination of vaginal discharge, udder inspection, assessment of urine ketone bodies (Ketostix, Bayer Corporation, Elkhart, IN, USA) and investigation of abomasum displacement. In addition, automatic health reports were created for every milking event based on individual milk production and milk component levels provided by the AfiMilk meters (SAE Afikim Kibbutz Afikim, Israel). Cows with deviations from pre-established ranges on at least two parameters (milk and milk components) within two consecutive milkings were automatically sorted for a complete health check.