

Genomic variation in innate immunity and susceptibility to uterine diseases in Holstein cows

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Introduction

Fertility is crucial for efficient dairy production, and adequate uterine health is a key element that allows for rapid resumption of reproductive function after calving. The challenge of uterine contamination with microorganisms at parturition is unavoidable. Some cows respond to this challenge successfully; however, approximately 20% of cows will develop metritis or clinical endometritis. Subsequently, the prevalence of subclinical endometritis will range from 25% to 75%. Innate immunity provides the first line of defense against infection by eliciting host responses to invading pathogens without requiring prior exposure. This response depends mainly on the action of neutrophils (PMNs) and macrophages with pattern recognition receptors that recognize molecular ligands from microbial species, which then initiate signaling pathways that result in the release of pro-inflammatory cytokines. One important group of receptors is the toll-like receptors (TLR), which regulate the activation of both innate and adaptive immunity. Likewise, the initial defense of the endometrium against invading microbes is dependent on the innate immune system, including initial recognition by host TLR proteins. Genetic selection for increased disease resistance in cattle has been proposed, and current molecular technologies provide the opportunity for exploring genomic variation related to health. We hypothesized that naturally occurring variation within the bovine *TLR* genes may be associated with differential susceptibility to reproductive diseases in Holstein cattle. The objective of this study was to test for associations between bovine single nucleotide polymorphisms (SNPs) and insertion-deletion (indel) mutations occurring in 7 bovine *TLR* genes (*TLRs*1, 2, 4, 5, 6, 9, and 10) that are known to recognize bacterial ligands and uterine diseases in dairy cows including metritis (MET), clinical endometritis (CE), and cytologic endometritis (CYE).

Materials and Methods

The study was conducted within the University of Florida Dairy Unit (Gainesville, FL) with a cohort

that consisted of 358 Holstein cows (164 primiparae; 194 multiparae). Metritis was defined as an abnormally enlarged uterus with a reddish-brown, fetid discharge with or without a fever within 21 days-in-milk (DIM). Presence of purulent or mucopurulent vaginal discharge at 35 DIM was considered CE, and a proportion of 10% or more of PMN cells in endometrial cytology samples collected using the cytobrush technique was considered CYE. DNA was isolated from blood. Custom allele-specific genotyping assays derived from multiple bovine *TLR* sequencing studies were used. Genotypes for 110 loci (SNPs and indels) that are known to be variable in cattle were determined, resulting in 46 monomorphic loci and 35 loci that did not meet our inclusion criterion for minor allele frequency (> 0.1). The association between specific *TLR* genotypes and each of the uterine diseases was evaluated by logistic regression with correction for confounding variables including season of calving (CS); parity; body condition score at calving, at enrollment (BCSEn), and at 35 DIM; dystocia; ketosis before and after 17 DIM; retained fetal membranes; hypocalcemia; twins; calf dead on arrival; abortion; sire; and maternal grand sire.

Results

Logistic regression models were constructed for each of 29 variable sites. Four, 2, and 1 SNPs (*TLRs* 2, 4, 6, and 9) produced uncorrected *P*-values < 0.05 with respect to MET, CE, and CYE, respectively, but none of the SNP associations endured correction for multiple testing. Covariates included in the final model for MET included CS, parity, dystocia, BCSEn, and ketosis <17 DIM. Covariates for CE were CS, dystocia, ketosis <17 DIM, and hypocalcemia. No covariates were retained for CYE. The analysis suggested that some *TLR* SNPs (*TLRs* 2, 4, 6, and 9) may potentially elicit relatively small effects on uterine health in Holstein cows, and that some confounding variables are actually more predictive for the incidence of disease than any genetic markers evaluated herein.

Significance

Uterine health is influenced by a number of environmental variables, which make it difficult to accurately estimate the precise role of host genetic components. Our results indicated that known variation within 7 bovine *TLR* genes does not modulate large effects on risk for uterine disease. Weak associations were observed

between SNPs occurring in 4 bovine innate immune genes and uterine health in Holstein cows, thereby suggesting that variation in innate immune genes may potentially modulate small effects on the incidence of uterine disease. Future studies employing whole-genome approaches are needed to help elucidate unknown genetic risk factors for uterine diseases in Holstein cows.