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Candidate gene association analyses for ketosis resistance in Holsteins

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ABSTRACT

High-yielding dairy cattle are susceptible to ketosis, a metabolic disease that negatively affects the health, fertility, and milk production of the cow. Interest in breeding for more robust dairy cattle with improved resistance to disease is global; however, genetic evaluations for ketosis would benefit from the additional information provided by genetic markers. Candidate genes that are proposed to have a biological role in the pathogenesis of ketosis were investigated in silico and a custom panel of 998 putative single nucleotide polymorphism (SNP) markers was developed. The objective of this study was to test the associations of these new markers with deregressed estimated breeding values (EBV) for ketosis. A sample of 653 Canadian Holstein cows that had been previously genotyped with a medium-density SNP chip were regenotyped with the custom panel. The EBV for ketosis in first and later lactations were obtained for each animal and deregressed for use as pseudo-phenotypes for association analyses. Results of the mixed inheritance model for single SNP association analyses suggested 15 markers in 6 unique candidate genes were associated with the studied trait. Genes encoding proteins involved in metabolic processes, including the synthesis and degradation of fatty acids and ketone bodies, gluconeogenesis, lipid mobilization, and the citric acid cycle, were identified to contain SNP associated with ketosis resistance. This work confirmed the presence of previously described quantitative trait loci for dairy cattle, suggested novel markers for ketosis-resistance, and provided insight into the underlying biology of this disease.
Key words: candidate gene, ketosis, single nucleotide polymorphisms

INTRODUCTION

All modern dairy cattle that have been selected for a high level of milk production undergo metabolic stress during the periparturient period. As the cow's demand for nutrients greatly increases after calving to sustain lactogenesis, she must alter energy metabolism in the liver and peripheral tissues (Bauman and Currie, 1980; Drackley et al., 2005). In response to negative energy balance, ruminants mobilize body stores of protein and fat to oxidize as body fuel so that carbohydrates may be conserved to support milk synthesis (Tamminga et al., 1997). Decreasing glucose and insulin concentrations mediate the major metabolic changes needed to stabilize blood glucose, which include increased rate of gluconeogenesis, reduced lipogenesis and increased release of nonesterified fatty acids from adipose, increased uptake and metabolism of fatty acids in the mitochondria of hepatocytes, and increased ketogenesis (Herdt, 2000). The fatty acids and ketone bodies are used as an alternate fuel source in the heart, kidney, skeletal muscle, and mammary gland to further conserve glucose and restore energy balance (Heitmann et al., 1987; Schäff et al., 2013); however, cows that do not make the necessary metabolic adaptations are susceptible to hyperketonemia. Differences in metabolite profiles, nutrient partitioning and hepatic regulation of metabolism of ketotic and nonketotic cows have been described, although the results of these studies have been inconclusive in explaining why some high-yielding cows develop ketosis whereas most do not (Loor et al., 2007; Moyes et al., 2013; van Dorland et al., 2014).

Interest in breeding more robust dairy cattle with improved resistance to disease is global (Miglior et al., 2005; Kargo et al., 2014; Parker Gaddis et al., 2014; Koeck et al., 2015). In 2016, Zoetis Genetics (Kalamazoo, MI) released a commercial genetic evaluation system for dairy wellness traits (Vukasinovic et al., 2017). The Canadian Dairy Network (Guelph, ON), the dairy genetic evaluation unit in Canada, has released a metabolic disease-resistance index (Koeck et al., 2014; Jamrozik et al., 2016a,b; Miglior et al., 2017). Ketosis is a multifactorial disease that is most likely influenced by multiple loci. Genetic evaluations of ketosis have produced low estimates of heritability (0.02–0.06) that limits the selection of cattle for ketosis resistance; milk BHB, an indicator trait for ketosis, has a relatively higher heritability (0.14–0.28; Zwald et al., 2004; van

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der Drift et al., 2012; Koeck et al., 2012; Koeck et al., 2014).

Investigation of candidate genes for ketosis and related traits could contribute to our understanding of the genetic architecture of this trait. Candidate gene association analyses, which are often limited by scope, are commonly conducted by estimating the association of polymorphisms in a few genes expressed in key tissue sites with the incidence of a disease. The construction of a custom array of markers for candidate genes specific to the trait of interest would allow for the investigation of many key genes simultaneously, as well as identifying probable quantitative trait nucleotides with biological significance. The objective of our study was to test the association of a custom panel of candidate gene SNP markers with a ketosis-resistance trait.

MATERIALS AND METHODS

Candidate Gene Markers

A custom genetic marker panel of SNP was developed by investigating candidate genes related to ketosis. Candidate genes were proposed based on their biological role in metabolic pathways related to the development of ketosis. Enzymes and regulatory factors known to be involved in gluconeogenesis, fatty acid synthesis and degradation, the citric acid/tricarboxylic acid cycle, and ketone synthesis and degradation were recognized by searching the species-specific Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg) pathway database. Candidate genes for ketosis that have been suggested in the scientific literature were also considered; those studies have identified key genes by comparing the transcriptome, proteome and metabolome of transitioning cows (Loor et al., 2005, 2007; van Dorland et al., 2009, 2014; Li et al., 2012; Moyes et al., 2013; Akbar et al., 2015a,b). Additionally, genes suggested by studies that have mapped QTL to important candidate genes for metabolic traits were also chosen (Clempson et al., 2012; Buitenhuis et al., 2013; Tetens et al., 2013). More than 100 genes were investigated in silico to categorize reported SNP from dbSNP (www.ncbi.nlm.nih.gov/snp) and to detect novel SNP. For each gene, the species-specific GenBank, UniGene, and dbSNP databases that are maintained by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) were mined for gene reference sequences, expressed sequence tags, and SNP data, respectively. The downloaded sequence and SNP data for each gene was compiled and aligned by the variant calling software Sequencher v. 4.9 (Gene Codes Corp., Ann Arbor, MI), using the UMD 3.1.1 (www.bovinegenome.org) for *Bos taurus*. The SNP located in coding regions were prioritized for inclusion based on their predicted effect on gene products; the Sorting Intolerant From Tolerant (SIFT; www.sift.jcvi.org) algorithm was used to determine if an allele substitution would change the encoded AA, and if this change would alter protein function (Sim et al., 2012). A total of 998 SNP were classified and incorporated onto an Illumina (Illumina Inc., San Diego, CA) 8K microarray to create a custom panel of markers. These putative SNP are located in 120 candidate genes spanning 28 chromosomes. With the exception of 14 SNP, the panel contains markers that were not previously included on a commercial bovine SNP chip. The SNP are a combination of novel and reported SNP that are located within or near candidate genes related to ketosis. The position of the 998 markers included on the panel are presented in Supplemental Table S1 (https://doi.org/10.3168/jds.2017-13374).

Animals and Data

Genomic DNA samples were obtained through coordination with previous Canadian Dairy Network (Guelph, ON) initiatives. All samples were from cows that had previously been genotyped with the BovineSNP50 BeadChip (Illumina Inc.), and these 50K genotypes were also provided by Canadian Dairy Network. A sample of 653 Canadian Holstein cows were regenotyped with the low-density array containing the 998 putative ketosis markers. Quality control removed animals with genotyping call rate less than 90% and SNP with minor allele frequency less than 1% and excess of heterozygosity greater than 15%. A pedigree file was derived for these cows, consisting of 24,260 individuals traced back 7 generations.

The cows came from 5 large herds of at least 100 recorded cows located within Quebec and Ontario with an annual ketosis disease-reporting frequency greater than 1% for each year of recording to ensure herds with inconsistent recording were avoided. Selective genotyping was implemented to increase the frequency of recorded cases of clinical ketosis in the small sample and consequently increase the power of the association analyses. Therefore, cow selection for genotyping was not randomized, but based roughly on creating a sample with close to equal numbers of cases and controls for ketosis based on the producer-recorded health records (322 cases and 331 controls). A case animal was defined as a cow with at least 1 reported case of ketosis within the first 100 d postpartum, which is the risk period for ketosis, and a control was defined as a cow with no reported case of ketosis in any period of her recorded