Rapid Communication: Genome-wide association analyses identify loci associated with colostrum production in Jersey cattle¹

Jennifer Nicole Kiser,[†] Macy A. Cornmesser,[†] Kevin Gavin,[‡] Alea Hoffman,^{||} Dale A. Moore,[‡] and Holly L. Neibergs^{†,2}

[†]Department of Animal Sciences, Washington State University, Pullman, WA 99164; and [‡]Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA 99164 ^{||}Sunrise Veterinary Service, Dalhart, TX 79022

ABSTRACT: Consumption of an adequate volume of high-quality colostrum soon after birth is critical for a calf's health. Few studies have focused on the genetics associated with colostrum production, even though several dairy herds in the United States have reported incidents of low to no colostrum production during the fall and winter seasons. The objectives of this study were to identify loci associated with quantity and quality of colostrum production in a herd of Jersey cattle ($n = 345$) and to identify potential positional candidate genes and/or transcription factor binding site motifs located near associated loci. Cattle that freshened between the months of October and December of 2016 at a single dairy were enrolled in the study and produced on average 3.03 kg of colostrum at their first milking. This study included 112 cattle genotyped with the GeneSeek GGP50k BeadChip and another 233 cattle previously genotyped with various other arrays. The 233 cattle genotyped at lower densities were imputed to the GGP50k BeadChip density using BEAGLE 4.1.1, and 2 genome-wide association analyses (GWAA) were conducted using an additive efficient mixed-model association expedited method with a genomic relationship matrix

(EMMAX-GRM). The first GWAA investigated loci associated with colostrum quantity and identified 7 loci: 6 that were moderately associated ($5 \times 10^{-07} > P < 1 \times 10^{-05}$) and 1 that was strongly associated ($P < 5 \times 10^{-07}$). The second GWAA investigated colostrum quality and identified 1 moderately ($5 \times 10^{-07} > P < 1 \times 10^{-05}$) associated locus. Five loci harbored positional candidate genes which had functional relevance to colostrum production, and 1 locus located on BTA10 contained a transcription factor binding site motif for *TFAP2A* which has previously been linked to mammary gland development. Pseudoheritability estimates were moderate for colostrum quality (0.19 ± 0.06) and high for colostrum quantity (0.76 ± 0.11), suggesting that genomic selection for these traits would be possible. Diminished colostrum quantity or quality can have a significant impact on herd health and herd economics. The identification of loci, positional candidate genes, and transcription factor binding site motifs associated with colostrum production could be used in genomic selection to allow producers to select for cattle with good colostrum production, improving calf health, and reducing economic losses to the herd.

Key words: colostrum quantity, colostrum quality, genome-wide association analysis, Jersey

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²Corresponding author: neibergs@wsu.edu

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INTRODUCTION

To produce healthy calves, dairies need to ensure that calves ingest an adequate amount of quality colostrum, critical to calf health, within 24 h of parturition (Godden, 2008). Although the amount of colostrum that calves need to consume varies, 3 to 4 liters of colostrum are sufficient for passive transfer of factors like immunoglobulins, growth factors, and cytokines that calves lack at birth (McGuirk and Collins, 2004). Inadequate colostrum intake can result in increased morbidity and mortality rates in calves (Donovan et al. 1998; McGuirk and Collins, 2004).

The molecular mechanisms that control colostrogenesis have not been well characterized (Dembinski and Shiu, 1987; Barrington et al., 2001). However, it is likely due to a combination of local factors within the mammary gland that affects the transcytosis of immunoglobulin G (IgG) into each quarter (Baumrucker et al., 2014) as well as complex endocrine factors associated with late pregnancy and impending parturition (Barrington et al., 2001; Gross et al., 2014).

Several studies have investigated colostrum production, with most focusing on factors influencing colostrum quality like parity, previous milk production, nutrition, and dry period length (Mann et al., 2016; Dunn et al., 2017). However, few studies have focused on the volume of colostrum produced (Maunsell et al., 1998; Mann et al., 2016), and in recent years, producers have been reporting incidents of reduced colostrum quantity in cattle that are freshening in the fall season (Litherland, 2009). Recently, colostrum yield was found to be associated with photoperiod, dry period length, and cow parity in Jersey cattle, and that colostrum quantity varied by sire line, suggesting that genetics plays some role in colostrum production (Gavin et al., 2018). Given this, the objectives of this study were to determine whether colostrum production is heritable and if so identify loci associated with quantity and quality of colostrum in a herd of U.S. Jersey cattle.

MATERIALS AND METHODS

Study Population

All animal care and sample collections were approved and performed in accordance with the Institutional Animal Care and Use Committee at Washington State University (05043). A Texas Jersey dairy with a milking capacity of 2,500 cows was the site of colostrum sampling from freshening

heifers and cows. The current study contains a subset of cattle that were part of a colostrum production study previously described by Gavin et al. (2018). An initial group of 100 cattle with extreme colostrum weights were selected and genotyped and included 50 animals that produced <0.45 kg of colostrum and 50 animals that produced >5.44 kg of colostrum. This initial population was then supplemented with genotypic data on 245 additional cattle previously genotyped by the dairy that also had colostrum data available. In total, 69 heifers, 62 primiparous, and 214 multiparous cows (up to 7 yr of age) were used in the analysis. Year of birth was found to be associated with colostrum quantity ($P = 5.3 \times 10^{-05}$) as well as colostrum quality ($P = 0.007$), and was therefore included as a covariate in the analyses. All 345 cattle in this study freshened between October and December of 2016 and produced an average of 3.03 kg (range = 0 to 14.47 kg) of colostrum with an average Brix refractometry value of 21.94 (range = 14.2 to 37.4) that served as the measure for colostrum quality.

Colostrum Harvesting

Colostrum collection procedures for this population have been previously described (Gavin et al., 2018). Briefly, immediately after calving cows were milked out into a stainless steel bucket and the colostrum weight was recorded using a WeiHeng 50 kg Mini Digital Hanging Scale (WeiHeng Digital Co., Ltd, Shenzhen, China). A 3- to 5-mL colostrum sample was removed and used for the Brix refractometry analysis to assess colostrum quality as it is a common method used to estimate the IgG concentration in colostrum samples (Quigley et al., 2013). Cattle that did not produce colostrum subsequently had no Brix data recorded.

Genotyping and Imputation

Bovine DNA was isolated from approximately 8 mL of whole blood for the 100 extreme colostrum quantity cattle collected in EDTA tubes using the Puregene DNA extraction kit following manufacturer's guidelines (Qiagen, Germantown, Maryland). Samples were then quantified using a NanoDrop 1000 spectrophotometer (Wilmington, Delaware) and genotyped using the GeneSeek BovineGGP50 BeadChip (Neogen, Lincoln Nebraska; $n = 48,268$ SNP). The positions of the SNP within the bovine reference genome and their alleles were assigned using the forward strand of the UMD 3.1.1 reference genome (ftp://ftp.cbcb.umd.edu/pub/data/Bos_taurus/).

Additional genotypic data from cattle previously genotyped by the dairy were available on 245 cattle with various genotyping arrays ([Supplementary Table 1](#)). Twelve animals had genotypes that exceeded the 48,268 SNP present in the GeneSeek BovineGGP50 BeadChip and were combined with the GGP50 genotypes from the 100 animals that had extreme colostrum weight values to form the imputation reference population. The average number of genotypes on the remaining 233 cattle to be imputed was 19,668 genotypes, with a range from 2,900 to 30,106 genotypes. Genotypes of the reference population that passed quality control filtering parameters (call rate >90% and minor allele frequency >1%) were phased in Beagle v4.1 ([Browning and Browning, 2007](#); [Browning and Browning, 2016](#)). Data from the 235 cattle genotyped at lower densities were quality control filtered using the same parameters, phased, and imputed to the BovineGGP50 density in a one-step imputation process using Beagle v4.1 ([Browning and Browning, 2007](#); [Browning and Browning, 2016](#)). Imputation accuracy was assessed to be 74% when 37 individuals from the reference population, which had previously been genotyped at lower levels ranging from 3,000 to 20,000 genotypes, were run through the imputation process. This level of accuracy is due to the limited density of SNP genotypes in the Jersey cattle from which genotypes were imputed and the limited number of cattle in the reference population, as accuracies for imputation from 50,000 to 778,000 genotypes within a breed have been >98% using this same process with a larger reference population. This low level of accuracy will reduce the power to detect associations with colostrum quantity and quality and may increase the possibility for a false positive association.

Quality Control

Prior to running the genome-wide association analysis (GWAA), sample and SNP data were run through an additional series of quality control filters after imputation. Samples were filtered for individual call rate (<90%) and no cattle were removed from the study. SNP were removed if the minor allele frequency was <1% ($n = 1,382$), or if they deviated from Hardy Weinberg Equilibrium ($P < 10^{-20}$; $n = 38$). After quality control filtering, 345 samples and 38,475 SNP remained for the colostrum quantity GWAA. For the colostrum quality analysis, any cattle that lacked Brix data were removed ($n = 66$), leaving 279 cattle and 38,475 SNP for the GWAA.

Statistical Analysis

The two GWAA in this study were performed using an additive efficient mixed-model association expedited with a genomic relationship matrix (EMMAX-GRM) model as part of the [Golden Helix SNP and Variation Suite v8.3](#) (SVS; Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com). The general mixed model can be defined as follows: $y = X\beta + Zu + \epsilon$, where y is an $n \times 1$ vector of the observed phenotypes, X is an $n \times q$ incidence matrix relating observations to levels of fixed effects, β is a $q \times 1$ vector representing the levels of the fixed effects, and Z is an $n \times t$ matrix relating the instances of the random effect to the observations. Residuals were estimated based on maximum likelihood parameters and are assumed to be independent and identically distributed ([Kang et al., 2010](#)). In this study, it was assumed that $\text{Var}(u) = \sigma^2 K$ and $\text{Var}(\epsilon) = \sigma_e^2 I$, such that $\text{Var}(\gamma) = \sigma_g^2 ZKZ' + \sigma_e^2 I$, where K is the matrix of pair-wise genomic relationships and Z is the identity matrix I . Using SVS, pseudoheritability was estimated using the equation, $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$, where σ_g^2 is the genetic variance and σ_e^2 is the environmental variance ([Kang et al., 2010](#)). The [Wellcome Trust Consortium \(2007\)](#) thresholds for unadjusted P values were used to determine whether there was evidence of moderate ($5 \times 10^{-7} > P < 1 \times 10^{-5}$) or strong ($P < 5 \times 10^{-7}$) association between the phenotypes and each SNP. Any SNP in linkage disequilibrium ($D' > 0.9$) with the lead-SNP was considered to constitute the same locus as the lead SNP. Using SVS, the average haplotype block size (53 kb) was estimated following the method described by [Gabriel et al. \(2002\)](#) using a SNP allele correlation of >0.9. The DNA sequence surrounding the locus associated with colostrum quality and quantity was investigated within 1 average haplotype block before and after the associated SNP (106 kb region) to identify positional candidate genes.

Transcription factor binding site (TFBS) motifs were identified that spanned the loci associated with colostrum quality or quantity and were altered by the allele present at the SNP. Identification of TFBS was done using PROMO ([Messeguer et al., 2002](#); [Farré et al., 2003](#)), a virtual laboratory that utilizes the TRANSFAC transcription factor database ([Matys et al., 2006](#)) to query putative TFBS motifs. PROMO calculates specific binding site weight matrices to predict potential TFBS within a specific DNA sequence that the user inputs ([Messeguer et al., 2002](#); [Farré et al., 2003](#)). In addition to the TFBS motifs, PROMO outputs a significance value called random

expectation (**RE**) query which is defined as the likelihood of finding each specific motif by chance using a dummy sequence with the same nucleotide frequency as the query sequence. For this study, a 31 base-pair sequence (15 bp 5' and 3' of the SNP) was downloaded from UMD 3.1.1 for each SNP associated with colostrum quality or quantity and queried through PROMO twice: once with the reference allele and once with the alternate allele for the SNP. In order for TFBS motifs to be considered significant, the motif site had to change given the allele present at the SNP and the RE query value of <0.05.

RESULTS AND DISCUSSION

Eight SNP tagging 7 loci, 6 moderately ($5 \times 10^{-07} > P < 1 \times 10^{-05}$) and 1 strongly ($P < 5 \times 10^{-07}$), were associated with colostrum quantity, whereas one locus was moderately associated with colostrum quality (Table 1; Figure 1). Pseudoheritability for colostrum production was 0.76 ± 0.11 and 0.19 ± 0.06 for colostrum quality. These heritability estimates for colostrum traits suggest that selection for colostrum production would be possible. However, given the small sample size of the current study, additional larger scale studies will need to be conducted to obtain more precise estimates of heritability for colostrum production.

Two (BTA10 and BTA17) of the loci associated with colostrum quantity were located in intergenic regions without putative positional candidate genes in the surrounding regions. However, the locus located on BTA10 (*rs134301532*) harbored a TFBS motif for transcription factor AP-2 alpha (*TFAP2A*; RE query = 0.0002). The AP-2 transcription factor

family is highly conserved across species (Pfisterer et al., 2002; Eckert et al., 2005) and is known to interfere with signal transduction pathways often altering cellular proliferation and differentiation. *TFAP2A* has been linked to mammary gland development and lactation. For example, the overexpression of Tfp2a during late gestation has been linked to lactation failure in mice, due to its interference with the differentiation of secretory mammary epithelial cells (Zhang et al., 2003). Although lactogenesis and colostrogenesis differ between rodents and ruminants, the high conservation of *TFAP2A* across species suggests that it may have a similar functional role in ruminants. Further research is needed to elucidate the role of *TFAP2A* in colostrogenesis in cattle to determine whether it is contributing to reduced colostrum production.

Five loci associated with the quantity of colostrum GWAA harbored 16 positional candidate genes (Table 1). Most of the positional candidate genes identified in this study have not been characterized in cattle, and thus little is known about how their functions might be influencing colostrum production. However, there were 3 genes (*BRI3BP*, *HCK*, and *VASH1*) that have previously been linked to bovine milk or have functions that could affect colostrum production. Of these, brain protein I3 binding protein (*BRI3BP*) located on BTA17 has been associated with bovine milk composition. A study by Govignon-Gion et al. (2015) found several SNP located within introns of *BRI3BP* to be associated with short-chain saturated fatty acids in milk. However, there is little information about how this gene might be influencing the colostrum production in cattle.

Table 1. Loci associated with colostrum production in Jersey cattle

	Chromosome*	SNP ID [†]	P-value	Positional candidate genes [‡]
Colostrum quantity				
	2 (119)	<i>rs109132347</i>	4.72×10^{-06}	<i>SP140</i> , <i>LOC787234</i> , <i>SP110</i>
	10 (89)	<i>rs42341516</i>	5.76×10^{-06}	<i>ANGEL1</i> , <i>LOC104973252</i> , <i>VASH1</i>
	10 (96)	<i>rs134301532</i>	1.42×10^{-06}	—
	13 (62)	<i>rs43406561</i>	8.13×10^{-06}	<i>HCK</i> , <i>CCM2L</i> , <i>TM9SF4</i>
	17 (51)	<i>rs110033106</i>	4.58×10^{-08}	—
	17 (53)	<i>rs110145575</i>	5.73×10^{-06}	<i>BRI3BP</i> , <i>DHX37</i>
	18 (58)	<i>rs210108864</i>	1.84×10^{-06}	<i>ZNF614</i> , <i>LOC787309</i> , <i>ZNF350</i> , <i>ZNF432</i> , <i>ZNF613</i>
Colostrum quality				
	3 (37)	<i>rs41567949</i>	4.77×10^{-06}	—

*Chromosome location of the loci followed by the location of SNP in megabases (Mb), in parentheses, as measured by numbered nucleotides in the UMD3.1 reference genome assembly.

[†]The SNP identified by the *rs* number which is a reference number assigned to markers submitted to the National Center for Biotechnology Information SNP database.

[‡]Positional candidate genes were defined as genes located within 53 kb on either side of the associated SNP(s). Bolded gene names represent genes where SNP within the loci are located within the gene.

Figure 1. Manhattan plots identifying loci associated colostrum quantity (A) and colostrum quality (B) in the genome-wide association analyses. Single nucleotide polymorphisms (SNP) are represented by a single dot. Bovine chromosomes are listed on the x-axis. SNP located between 5.0 (black line) and 6.3 (red line) provide evidence of moderate association and SNP above 6.3 (red line) provide evidence of a strong association based on the [Wellcome Trust Case Control Consortium \(2007\)](#) guidelines.

Both the HCK proto-oncogene (*HCK*) and vasohibin 1 (*VASH1*) have functions that could affect colostrum production. For example, *HCK*, which is a member of the Src family of proteins, is involved in phagocytic function in humans ([Berton et al., 2005](#)) and has a role in the release of inflammatory molecules like IgG ([Berton et al., 2005](#)). The *HCK* protein enables the release of IgG through its functions related to signal transduction of immunoglobulins Fc receptors ([Durden et al., 1995](#)). In cattle, IgG is the most abundant immunoglobulin present in colostrum and has been shown to bind a specific Fc-receptor during colostrogenesis but not lactogenesis ([Kemler et al., 1975](#)). Additionally, in mice it has been reported that a knockdown of the Src family results in impaired secretory activation in the mammary gland ([Watkin et al., 2008](#)). However, this study was unable to determine which Src family member was directly involved in this function as they were unable to knockdown specific family members ([Watkin et al., 2008](#)). It is unknown if this impaired function of mammary secretory cells is due to a mutation in *HCK* or a different Src family member. Mutations that affect the function or receptivity of the IgG Fc receptor have the potential to alter the ability of IgG to migrate from the bloodstream into the mammary gland potentially inhibiting normal colostrogenesis. Further research

is needed to determine the specific role *HCK* plays in colostrogenesis.

Similar to *HCK*, *VASH1* has the potential to alter colostrum production through its function in angiogenesis. In mammals, *VASH1* negatively regulates angiogenesis through the inhibition of vascular endothelial growth factor A (VEGFA) function ([Watanabe et al., 2004](#); [Shirasuna et al., 2012](#)). In humans during gestation and lactation, the mammary gland undergoes extensive vascular remodeling ([Djonov et al., 2001](#)). In contrast to humans, there has been limited focus on the importance of angiogenesis during pregnancy and lactation in dairy cattle ([Akers, 2002](#)). However, impaired or dysregulated angiogenesis may have a deleterious impact on the mammary gland. For example, mice lacking functional VEGF exhibited signs of impaired lactation (e.g., smaller pups and decreased milk production) compared with controls; this impairment is likely due to reduced angiogenesis resulting in fewer, more unorganized blood vessels in the mammary tissue of the experimental group compared with controls ([Rossiter et al., 2007](#)). Additional research is needed to determine whether mutations affecting *VASH1* impact colostrogenesis in cattle. However, given that *VASH1* can act as an antiangiogenic factor, mutations that affect its function could potentially

modify the regulation of angiogenesis in the mammary gland and alter colostrum production.

The results of this prospective study suggest that colostrum production is heritable, and that selection for improved colostrum quality and quantity is possible. The results presented here represent the first loci identified to be associated with colostrum production in Jersey cattle. Although this study utilized a smaller initial population size, previous GWAA investigating complex traits such as fertility and disease have been conducted with similar or smaller populations (Lee et al., 2015; Lipkin et al., 2016; Neupane et al., 2017). To validate these newly identified loci, additional research is needed in larger, independent herds and across breeds. The validation of loci associated with colostrum production could be added to commercially available genotyping arrays to allow producers to select for cattle with sufficient colostrum production to aid in reducing calf morbidity and mortality and reduce economic losses associated with sick calves.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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