Genome-Wide Association of Immunoglobulin G Concentration in Colostrum and Milk of Holstein Cattle

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Summary

Dairy cows classified as high immune responders, have inherently enhanced concentrations of total immunoglobulin G (IgG), and antibodies (antigen-specific immunoglobulin) in their colostrum and milk compared to average and low immune responders. FcRn (Fc receptor neonatal), a specialized receptor in the mammary tissue aids in the transport of circulating IgG from the blood of the dam into colostrum and the subsequent uptake of IgG from the digestive tract of calves into the systemic circulation. The present study was conducted to identify autosomal genomic regions associated with total IgG in colostrum and milk using a 50K SNP panel with imputation to the high-density panel (777K) and 556,279 markers were included in a genome wide association study (GWAS). SNP markers on chromosomes 12 (at ~60-65 Mb) and 15 (at ~44-46 Mb), were found to be significantly associated with total IgG concentration in milk at a 5% false discovery rate (FDR) level. No significant associations in colostrum or milk were found between total IgG and SNPs in the gene region encoding FcRn. However, raw p-value (without FDR adjustment), for 2 SNPs located within the *FCGRT* gene region on chromosome 18 were shown to be significant at the 5% level for colostrum. One limitation of this study was the small number of animals tested (n=57), which will affect the power of the GWAS. Further evaluation of these SNPs with a larger sample size, and of their associated candidate genes and their biological pathways, is required to understand the genetics of development of high IgG concentrations in colostrum and milk.

Keywords: immunoglobulin G, colostrum, milk, antibody-mediated immune response, GWAS

Introduction

Dairy cows classified as high immune responders (HIR) have enhanced concentrations of serum antibodies, and higher concentrations of total immunoglobulin G (IgG) and specific antibodies (antigen-specific IgG) in their colostrum and milk (Fleming et al., 2016, Wagter et al., 2000) compared to average and low responders. Up to 6 weeks postpartum, these cows have higher concentrations of antibodies in milk than the colostrum from average or low immune response cows at calving (Wagter et al., 2000). Therefore, the more specific antibodies a dam can produce in response to a prevalent environmental pathogen, the better her calf can resist disease after consumption of her colostrum. This is known as passive immunity. Cows with a low or average adaptive immune response phenotype are not capable of producing high levels of specific antibodies and thus cannot provide superior passive immunity. In a recent study, calves given colostrum with high concentrations of antibodies from their dams also had high

concentrations of specific antibodies in their blood at two days of age (Wagter-Lesperance, PhD Thesis 2017). Taken together, this provides evidence that HIR cows provide enhanced passive immunity to their calves and are more able to protect their calves from disease than animals that have a low or average immune response.

Calves require an adequate amount of colostrum from their dams within 6-24 hours after calving. Colostrum contains antibodies and cells transferred from the blood of the dam that have been produced following natural exposure or immunization to microorganisms. In order to acquire adequate passive immunity, however, calves must receive colostrum from their dam soon after calving. Failure of passive transfer (FPT) results when newborn calves do not attain adequate blood concentrations of IgG, and this is associated with increased calf morbidity and mortality. While the quality and quantity of colostrum fed in the first hours of life are critical to calf health, so too are the genetics of the dam and calf. Various receptors with known single nucleotide polymorphism (SNP) variance, including the Fc neonatal receptor (FcRn), play a role in colostrum uptake. The FcRn is a heterodimer of a class 1 major histocompatibility (MHC) chain homologue (*FCGRT)* located on chromosome 18 and Beta-2 microglobulin (*B2M)* located on chromosome 10. FcRn aids in the transport of circulating IgG from the dam into colostrum and the subsequent uptake of IgG from the digestive tract of their calves into the systemic circulation. Therefore, the objectives of this study were to: 1) conduct a genome-wide association study (GWAS) for IgG in colostrum and milk on High (H), Average (A) and Low (L) AMIR responders based on a high-density SNP panel, and 2) evaluate the associations between colostrum and milk IgG and variations in SNPs for the genes associated with FcRn.

Materials and methods

Animals and Immune Response classification. Fifty-seven cows previously phenotyped for antibody-mediated immune response (AMIR) were classified by their estimated breeding value (EBV) into High, Average, and Low groups based on a procedure described by Thompson-Crispi et al. (2013). Cows with an EBV of above +1 or below −1 standard deviation from the mean were considered high immune responders or low immune responders respectively.

Colostrum and Milk Collection. Colostrum samples were collected on day 0 (at calving) and milk samples at day 5 (post-calving) as described by Fleming et al., 2016.

Radial Immunodiffusion Assay. A total of 57 colostrum samples and 55 day-5 milk samples were evaluated for total IgG using a radial immunodiffusion (RID) kit and an IgG standard (Triple J farms, Bellingham, WA) as described by Fleming et al., 2016.

Genotypes. Cattle were genotyped using the Bovine SNP50 BeadChip (Illumina, San Diego, CA). DNA was extracted from hair follicle samples and genotyping was performed by Zoetis Canada (Calgary, AB, Canada). Quality control on SNPs was performed according to Wiggans et al. (2009). After QC and removal of sex chromosomes, 44,546 SNPs remained for the analysis.

Imputation. 50k genotypes of the 57 animals were imputed to a high density (HD) panel using 3,080 reference animals by FImpute (Sargolzaei et al., 2014). There were 734,077 autosomal SNPs on the HD panel. After imputation, SNPs with minor allele frequency <0.05 (n=173,466) or having excess of heterozygosity >0.15 (n= 4,332) were excluded. In total, 556,279 autosomal

SNPs were used for GWAS.

Statistical Methods for GWAS. GWAS for total IgG expression data was carried out using mixed linear models by snp1101 software (Sargolzaei, 2014) with the following models:

$Y_{IgGC=} \mu + \beta_1$Milk Weight + AMIR group + parity + a + β_2SNP + error (1)
$Y_{IgGM} = \mu + \beta_1$Milk Weight + AMIR group + parity + a + β_2SNP + error (2)

where Y is $\log_{10}$ of the concentration of total IgG in mg/dL, Milk Weight is the weight of colostrum or milk collected at the time of IgG evaluation and β_1 is the corresponding regression coefficient, AMIR group is the classification of cows into high, average, or low antibody response groups, parity is 1,2,>=3, a is random additive polygenic effect, and SNP is the vector of genotypes and β_2 is the allele substitution effect.

Results

GWAS. Imputation of SNPs from the 50k density chip to high density allowed for the identification of markers that were significantly associated with **milk total IgG** mainly on chromosomes 12 and 15 at 5% FDR level (**Figure 2**). There were suggestive SNPs (at 10% FDR level) on chromosome 1 and 9. For **colostrum total IgG**, only one SNP marker approached an FDR of 10% on chromosome 12 but this was not in the same chromosome region identified for milk total IgG (**Figure 1).**

FcRn. No significant SNPs were identified on chromosome 18 and chromosome 10, for total IgG in colostrum or milk, however, raw p-value (without FDR adjustment) for two SNPs at the *FCGRT* gene region on chromosome 18 was shown to be significant ($P < 0.05$) for colostrum.

Discussion

This was the first GWAS investigation of colostrum and milk in the context of the adaptive immune response trait AMIR. In this study, significant SNPs on chromosome 12 and 15 were associated with total IgG in milk (**Figure 2**). Future biological pathway analysis of the genes located near these SNPs using tools like DAVID (Database for Annotation, Visualization and Integrated Discovery) and Innate DB, is required and may reveal the importance of these SNPs in the regulation of immunoglobulin and antibody concentrations in colostrum and milk.

The present study also sought to investigate the role that FcRn plays in the variation in total IgG among high average and low AMIR phenotypes. An evaluation of the SNPs indicated that there was a degree of heterozygosity in the regions where the *FCGRT* (56,415,700 to 56,420,861 bp) and *B2M* genes (104,139,090 to 104,145,312 bp) are located (results not shown). Examination of raw p-values for SNPs within *FCGRT* gene region on chromosome 18 highlighted two significant SNPs at the 5% level for colostrum, which may indicate differences in allele expression in this region. Differences in *FCGRT* genotype in beef cattle have been reported previously in a haplotype study by Laegreid et al., 2002, where the authors defined 5 haplotypes of *FCGRT* based on the identification of 5 SNPs, and associated these with FPT or high passive transfer (HPT). Dams with haplotype 3 were found to have a

greater risk of FPT in their calves (Odds ratio [OR] = 3.8, $p < 0.035$), and, calves with haplotype 2 were less likely to have HPT (Odd ratio 0.18, $p < 0.011$). In addition, a study by Clawson et al., 2004, further defined 8 haplotypes based on 12 identified SNPs for *B2M* and calves homozygous for one of the eight haplotypes (*B2M* 2,2) were at increased risk for FPT (OR = 10.6, $p<0.0005$).

The present study explored the GWAS for total IgG in colostrum and day 5 milk post-calving. Future studies are required to explore the association of dam genotypes and the concentration of total IgG and antigen-specific antibodies in the sera of their calves after colostrum consumption. One of the limitations of this study was the sample size which can affect the power of the GWAS. The accuracy of results from this study could be improved with the addition of more dams, but more importantly more dam-calf pairs, to fully elucidate SNP variance associated with the concentration of IgG in colostrum and milk, and the mechanism for efficient transfer from the dam's circulation into colostrum and from the gut into the circulation of the calf.

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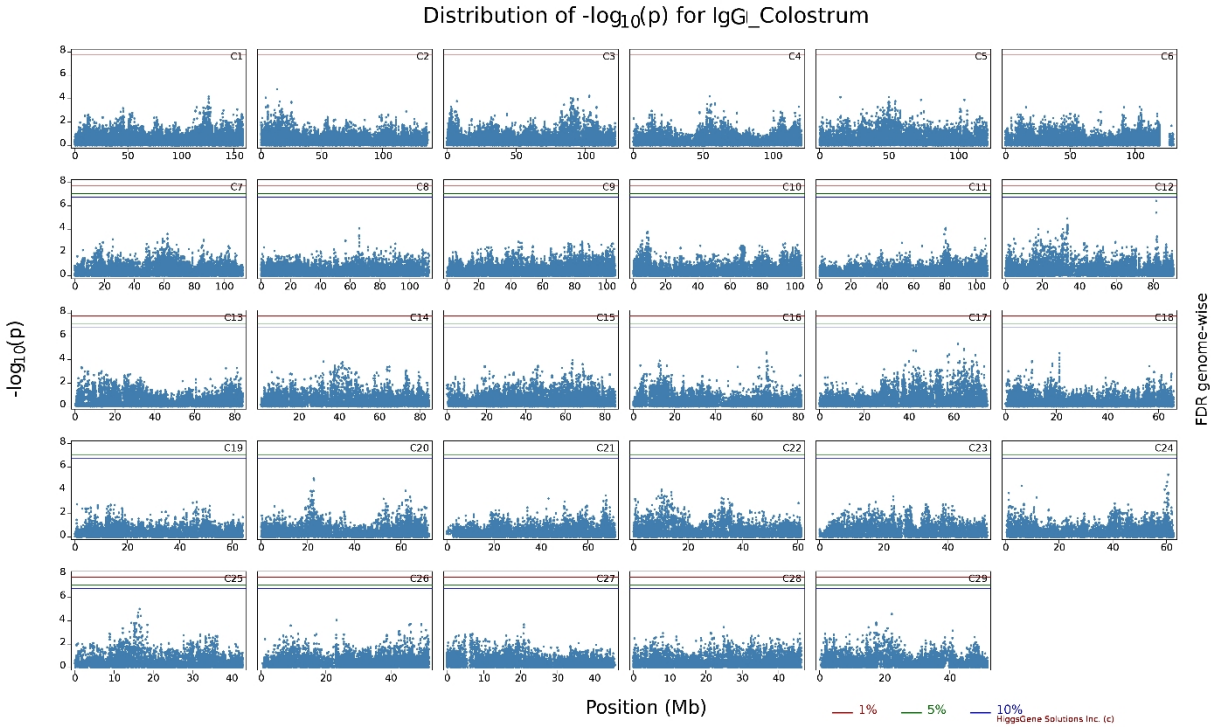

Figure 1. Genome-wide distribution of p-values for total IgG in colostrum.

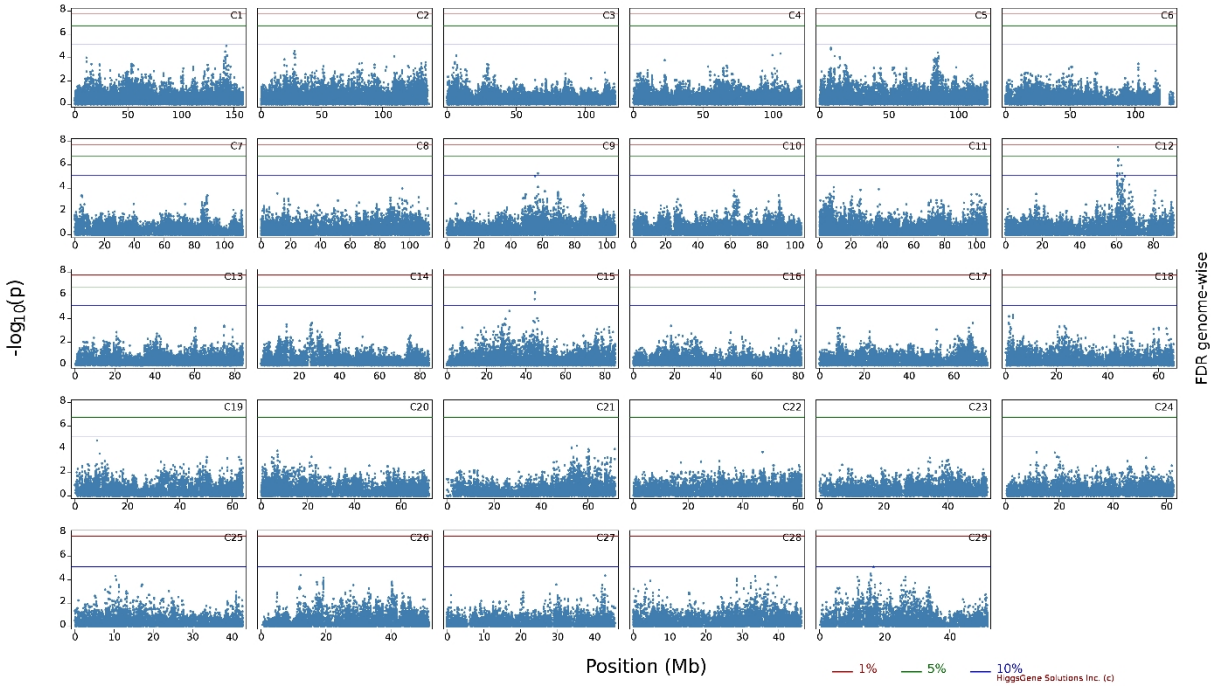

Figure 2. Genome-wide distribution of p-values for total IgG in milk.