A Genome-Wide Association Study for Immune Response Traits in Canadian Holsteins: An Update

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INTRODUCTION

The inclusion of immune response traits in breeding indices has been suggested to improve inherent disease resistance in dairy cattle (Abdel-Azim et al., 2005; Mallard et al., 2011). Using the High Immune Response (HIR) Technology developed at the University of Guelph, cows with superior cell-mediated (CMIR) and antibody-mediated immune responses (AMIR) can be identified (Wagter and Mallard, 2007). Holstein cows classified as high AMIR have been shown to have lower occurrence of mastitis in 2 out of 3 herds tested, improved response to commercial vaccine and increased milk and colostrum quality (Wagter et al., 2000). High immune response cows have also been shown to have decreased incidence of diseases such as mastitis, metritis, ketosis and retained placenta (DeLapaz, 2008; Thompson-Crispi et al., 2012a). These previous studies found many benefits to identifying HIR cows in a herd, however, they were performed on one or a few herds in a single region.

Subsequently, immune response profiles were measured on 680 Holsteins from 58 herds across Canada in collaboration with the Canadian Bovine Mastitis Research Network. Significant variation in immune response phenotypes between cows, herds and regions was found, indicating it is possible to classify cows as high, average or low immune responders on a national scale (Thompson-Crispi and Mallard, 2012). Genetic parameters of the immune response traits for these cows were estimated, and AMIR and CMIR were found to be moderately heritable, 0.29 and 0.19, respectively, demonstrating the feasibility of breeding for enhanced immune response (Thompson-Crispi et al., 2012b). Using estimated breeding values, these cows were classified as high, average or low for AMIR and CMIR. Associations with mastitis were investigated, and high AMIR cows were found to have significantly lower incidence rates of clinical mastitis compared to average and low AMIR cows (Thompson-Crispi et al., 2011). Also, the low AMIR cows tended to have the most severe mastitis. These previous studies demonstrate breeding cattle for enhanced immune response, on a national scale, may decrease the incidence and severity of disease in the dairy industry.

The objectives of this study were to identify SNPs associated with AMIR using a genome-wide association approach and to determine potential genes and biological pathways associated with AMIR. Results of this work are expected to demonstrate the potential to include immune response traits in genomic selection indices to improve animal health and decrease the occurrence and severity of disease in the dairy industry.
MATERIALS AND METHODS

Animals

Immune responses of 680 lactating Holsteins, outside the peripartum period, from 58 herds across Canada were evaluated (Thompson-Crispi and Mallard, 2012) in collaboration with the Canadian Bovine Mastitis Research Network. All experimental procedures were approved by the Animal Care Committee of the University of Guelph under guidelines of the Canadian Council of Animal Care.

Genetic parameters and breeding values of the adaptive immune response traits AMIR and CMIR in these herds have been estimated and reported previously (Thompson-Crispi et al., 2012b). For use in the genome-wide association study, cows were ranked based on AMIR. Cows with an EBV > +1 or < -1 standard deviation from the mean were considered high immune responders or low immune responders, respectively.

Genotyping and Quality Control

A total of 163 cows (81 high AMIR and 82 low AMIR) were selectively genotyped using the Bovine SNP50 BeadChip (Illumina, San Diego, CA). DNA was extracted by Maxxam Analytics (Guelph, Ontario, Canada) and genotyping performed by DNA Landmarks (Saint-Jean-sur-Richelieu, Quebec, Canada). Quality control measures included the exclusion of SNP markers with a minor allele frequency of less than 0.05 and individuals with call rates < 85%.

Candidate Gene Discovery and Pathway Analysis

Significant SNPs were mapped to corresponding or nearby genes using NGS-SNP scripts (Grant et al., 2011). Genes 250,000 bp up or downstream of significant SNPs were obtained for pathway analysis. The identified genes were submitted to database for annotation, visualization and integrated discovery (DAVID) bioinformatics resource 6.7 to perform enrichment analysis to determine biological pathways associated with AMIR (Huang et al., 2009).

Statistical Analysis

A generalized quasi-likelihood score method (Feng et al., 2011) was used to determine SNPs significantly associated with AMIR. This method accounts for the genetic relationship among animals and is not biased by selective genotyping, since it is based on a logistic regression approach. In this model, the allele frequencies are treated as the response and the trait (continuous or categorical) is treated as a covariate which allows the distribution of the trait values to be unspecified. A chromosomal False Discovery Rate (FDR) of 5% was used to account for multiple comparisons.

RESULTS

The dataset contained 54,609 SNPs and the number of markers with a minor allele frequency (MAF) > 0.05 was 41,819. Figure 1 shows the Manhattan plot for all markers after applying quality control measures. A total of 2580 SNPs were significantly (comparison-wise $P < 0.05$) associated with AMIR. After accounting for multiple comparisons by applying an FDR of 0.05,
198 SNPs remained significant. The majority (84%) of the SNP markers associated with AMIR were on chromosome 23 (167/198). Chromosome 23 lost 37% of the significant SNPs after applying the FDR, versus the other chromosomes that lost > 90%. A total of 520 genes were found within 250,000 bp up or downstream of the 198 significant SNP markers. All genes were submitted to DAVID and 172 genes were mapped to 11 biological pathways through Kyoto Encyclopedia of Genes and Genomes (KEGG). The antigen processing and presentation pathway was significantly associated with AMIR, mainly due to the BoLA genes found on chromosome 23 (Figure 2).

DISCUSSION

This study was the first genome-wide association study for antibody-mediated immune responses in dairy cattle. Previous GWAS have evaluated differences in resistance or susceptibility to certain diseases (Pant et al., 2010; Minozzi et al., 2010), or somatic cell score as an indicator of udder health (Meredith et al., 2012); however the approach proposed here may identify SNP profiles associated with general disease resistance, since cows with superior immune responses are known to have a lower occurrence of disease (Thompson-Crispi et al. 2012a). The current study found significant variation in SNP profiles between cows classified as High or Low for AMIR, indicating that it may, one day, be possible to identify animals with superior AMIR and disease resistance based on genetic profiles.

The bovine major histocompatibility complex (MHC), known as BoLA, is located on chromosome 23 and is well known as a location of major genes associated with immune response and disease resistance (Stear et al., 2001). A relationship exists between BoLA class II and resistance or susceptibility to mastitis which has been known for over 20 years (Lunden et al., 1990; Sharif et al., 1998). The high antibody responding cows used in this study have previously been demonstrated to have a lower incidence rate of clinical mastitis compared to the low antibody responding counterparts that were selected for genotyping (Thompson-Crispi et al., 2011). Therefore, associations with BoLA, a highly polymorphic and complex gene region, are to be expected.

AMIR evaluated in the current study gives an indication of the cows’ ability to mount type 2 like responses, which generally predominate in control against extracellular pathogens (Estes and Brown, 2002). Since mastitis causing pathogens tend to be extracellular in nature, the associations of AMIR with mastitis, as reported previously (Thompson-Crispi et al., 2011), and MHC demonstrated in the current study, are to be expected. However, in order for cows to have protection to a variety of pathogens that have the potential to cause disease, variation within the MHC gene cluster is ideal, and an optimal balance between the two branches of the adaptive immune system, AMIR and CMIR, are required. The CMIR tends to predominate in control against intracellular pathogens, and cows with superior CMIR have been found to be less likely to be seropositive for Mycobacterium avium subspecies paratuberculosis (Pinedo et al., 2009). In the future, genome-wide association studies should be performed based on the CMIR trait to determine any potentially overlapping or novel genes and pathways associated with this branch of the immune response.
CONCLUSIONS

Results of the study found significant SNP markers associated with high and low antibody responses of dairy cattle, suggesting it may be possible to calculate genomic breeding values for this trait. The immune system provides the main defense against pathogenic micro-organisms and as such has the ability to vary the response in accordance with the nature of the invading pathogen or immunizing agent. This system is therefore under complex genetic regulation and individuals differ in their immune response profiles with protective responses not necessarily identical between individuals. The immune system is also dynamic in its capacity to deal with the variation found within and across various pathogens. Therefore it is unknown whether similar or predictable genome-wide SNP profiles can be identified across and within populations. If so, it may be possible to include immune response traits in genomic breeding indices to decrease the incidence and severity of disease in the dairy industry. This is the first genome-wide association study for general antibody immune responses in cattle, and provides a strong starting point for future studies with more animals to validate the current findings. Future studies aimed at evaluating differences in the cell-mediated immune response would provide further insight into the complex nature of adaptive immune responses of dairy cows and would complement the findings of this study.

ACKNOWLEDGEMENTS

Figure 1. Manhattan plot for antibody-mediated immune response in Holstein dairy cattle. The x-axis is the position of each SNP on the bovine chromosome and the y-axis is the $-\log_{10} P$.

Figure 2. Antigen processing and presentation pathway from KEGG significantly ($P = 1.25E-05$) associated with antibody-mediated immune response (AMIR). * represents candidate genes associated with AMIR.
REFERENCES


