Genomics Of Disease In Beef Cattle

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Introduction

The current U.S. beef cattle inventory has decreased from 103.5 million in 1996 to 94.5 million in 2008 (Miles, 2009). This implies that a more efficient production system needs to be implemented to satisfy consumer needs. Infectious diseases are a major economical factor influencing productivity of beef cattle. As an example, it has been estimated that mortality due to bovine respiratory disease complex is approximately 6% (NAHMS, 2000) and it costs the U.S. beef industry from US$500 million to US$750 million per annum (Griffin, 1997; Miles, 2009). Miles (2009) suggests that perhaps it is time to look for ways to reduce losses by focusing on the animal's response to the pathogen, instead of continuing to focus on the pathogens.

Chromosomal regions with relatively large effects associated with cattle diseases have been previously identified (Gonda et al., 2007; Casas and Snowder, 2008; Ogorevc et al., 2009). Quantitative trait loci (QTL) for mastitis and somatic cell scores have been readily detected (Ogorevc et al., 2009), but few studies are available regarding the report of other diseases, especially in beef cattle. Limited attempts have been made to identify chromosomal regions associated with diseases in beef cattle (Casas and Stone, 2006; Casas and Snowder, 2008).

Insight into genomic variation affecting disease incidence may be revealed by genome-wide association studies. Availability of the bovine sequence (The Bovine Genome Sequencing and Analysis Consortium, 2009) has allowed the identification of thousands of single nucleotide polymorphisms (SNP; Van Tassell et al., 2008). These SNP provide suitable markers to be used in arrays to identify the underlying genetic basis of disease tolerance. Genome-wide associations are possible due to the availability of high-density array SNP chips. These arrays have been used to identify chromosomal regions associated with diseases in cattle (Settles et al., 2009).

Genetic variation of diseases. The condition most commonly evaluated in beef cattle to estimate its heritability is bovine respiratory disease complex. It has been estimated that the heritability ranges from 0.00 to 0.26 (Muggli-Cockett et al., 1992; Snowder et al., 2005b; Schneider et al., 2010). Using a crossbred population (n=2,700) from the Germplasm Evaluation Project at the U.S. Meat Animal Research Center, it has also been estimated that the heritability of bovine respiratory disease is 0.1. Genetic variation of bovine respiratory disease is considered low. Genetic variability of additional diseases has also been estimated. Snowder et al. (2005a) indicated that the heritability of infectious keratoconjunctivitis (pinkeye) ranged from 0.00 to 0.28. In a different study, General Disease Resistance (GDR) incidence was defined as frequency of treatment of animals for one or more of three diseases: bovine respiratory disease, infectious keratoconjunctivitis, and infectious pododermatitis. Heritability of GDR incidence was 0.07. Heritability of tolerance to diseases is considered low. The range of heritability estimates for

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these disease phenotypes may indicate that genetic variation is difficult to identify. One possible reason may relate to inconsistent levels of exposure to pathogens in different years and locations. Therefore, identifying the genes associated with tolerance to diseases is essential if the focus of study is to be the response of the animal to pathogens.

Quantitative trait loci harboring genes associated with diseases. Many studies have identified chromosomal regions associated with diseases in dairy cattle. The most common scans are focused on the identification of QTL associated with mastitis, somatic cell score, or somatic cell count (Ogorevc et al., 2009). Measurements associated with mastitis can be obtained in an efficient and economical fashion when compared to other diseases. Chromosomal regions associated with mastitis (or other related measurements) in dairy cattle have been detected in practically the entire bovine genome. These QTL can be efficiently compared from databases (Hu and Reecy, 2007).

There have been several studies with the objective of detecting QTL for additional diseases in dairy cattle. Hernandez-Sanchez et al. (2002) identified regions on chromosomes 5, 10 and 20, associated with Bovine Spongiform Encephalopathy (BSE) susceptibility/resistance alleles in Holstein. Gonda et al. (2007) reported that a region of chromosome 20 is associated with susceptibility to Johne's disease in Holstein cattle. Maltecca et al. (2008) identified regions on chromosome 11 and 20 associated with passive immune transfer in a Holstein and Jersey crossbred population.

There are a limited number of studies regarding the identification of QTL associated with diseases in beef cattle. Gasparin et al. (2007), identified 3 chromosomal regions (chromosomes 5, 7, and 14), associated with tick resistance in a population derived from Holstein and Gyr in Brazil. Casas and Stone (2006) indicated that regions on chromosomes 1 and 20 could be associated with tolerance to infectious bovine keratoconjunctivitis (pinkeye). An additional study by Casas and Snowder (2008) revealed that a region on chromosome 20 could be associated with general bacterial disease resistance (GDR). Leach and Glass (Roslin Institute; personal communication) have identified a region of chromosome 20 for levels of immunoglobulins associated with immunization to Foot-and-Mouth disease in a Charolais-Holstein F_2 population. Searches for QTL involved in the immune system seem to indicate that chromosome 20 harbors a gene or group of genes associated with the ability of the animal to respond when exposed to a pathogen.

Genome-wide association studies for diseases. High-density single nucleotide polymorphism (SNP) genotyping assays for cattle have become available (Matukumalli et al., 2009). This technology has allowed further identification of chromosomal regions associated with economically important traits in beef cattle. Sherman et al. (2010), using 464 steers from Angus, Charolais, and crossbred bulls, detected 150 SNP throughout the genome associated with residual feed intake and feed efficiency. The most significant SNP reside on 16 chromosomes. Snelling et al. (2010), using a population of approximately 2,700 steers, heifers and bulls, identified 231 SNP (meeting stringent significance criteria) associated with growth traits (birth, weaning and yearling weight, pre- and post-weaning growth gain). When the significance criterion was

relaxed, 12,425 SNP were significantly associated with growth traits. Chromosomes 6, 11, and 20 seem to be the chromosomes with the majority of the significant SNP associated with growth.

Diseases in cattle have also been studied using genome-wide associations. Settles et al. (2009) used 245 Holstein cows to identify chromosomal regions associated with Johne's disease. The pathogen responsible for this condition is *Mycobacterium avium paratuberculosis*. Regions on chromosomes 1, 5, 7, 8, 16, 21, and 23, had SNP significantly associated with the presence or absence of *M. paratuberculosis*. Pant et al. (2010), using 232 animals, identified chromosomes 1, 5, 6, 7, 10, 11, and 14, as harboring genes associated with the presence of the same pathogen using ELISA techniques.

Bovine respiratory disease is one of the most important diseases affecting the U.S. beef industry. At the U.S. Meat Animal Research Center, approximately 2,700 animals (resource population from Snelling et al., 2010) were used to assess the presence of SNP associated with incidence of bovine respiratory disease complex. When a stringent significance criterion was used, 30 SNP on 15 chromosomes were found to be associated with the disease. When significance stringency was relaxed, 550 SNP were found to be associated with the disease. Figure 1 shows the relative position of the SNP in the genome, their significance and effect. An additional evaluation of this population was pursued by generating a single trait that included treatment for one or more of the following diseases: bovine respiratory disease, infectious keratoconjunctivitis, and infectious pododermatitis. The trait was defined as GDR. Results of the genome-wide association study are shown on Figure 2. Using a relaxed significance criterion, 483 SNP were associated with GDR. When a stringent significance criterion was used, 27 SNP were still significant for the trait on 13 chromosomes. Seventy eight markers were common to both traits (Bovine Respiratory Disease Complex and General Disease Resistance).

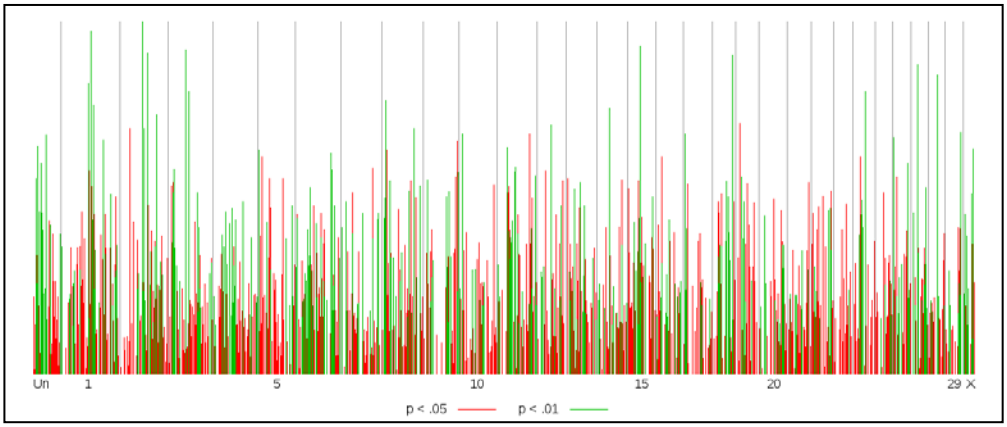

Figure 1: Genomic map of SNP associated with bovine respiratory disease (BRD). Un= mapping to unassigned scaffolds, autosomes (1 to 29) and X is based on *Bos taurus* 4.0 draft genome assembly. Chromosome boundaries are indicated by dashed vertical lines. Height indicates relative magnitude of estimated effect.

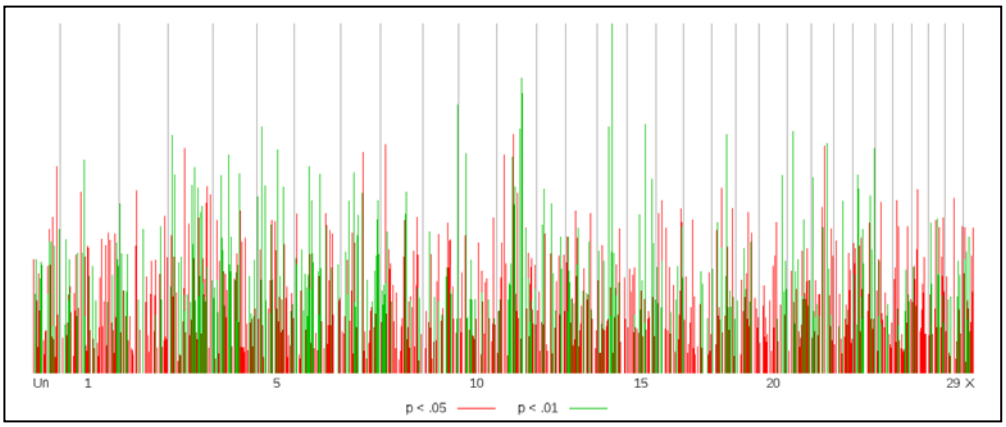

Figure 2: Genomic map of SNP associated with general disease resistance (GDR). Un= mapping to unassigned scaffolds, autosomes (1 to 29) and X is based on *Bos taurus* 4.0 draft genome assembly. Chromosome boundaries are indicated by dashed vertical lines. Height indicates relative magnitude of estimated effect.

Results from genome-wide association studies could be used as a starting point to identify genes underlying genetic variation for tolerance to pathogens. However, results from genome-wide association studies seem to yield unclear chromosomal regions associated with immunity-related traits. This can be due to several reasons. One of these reasons is that genome-wide association studies do not account for gene interactions. This will be discussed further, but additional reasons will not be discussed here (limited genetic variability of the trait, population size, collected phenotypes, informativity of SNP, etc.).

Genes of the immune system. Immunological defense mechanisms are highly complex and not limited to a single genomic region. The Bovine Leukocyte Antigen (BoLA) is the term by which the Major Histocompatibility Complex is referred to in the bovine. This complex contributes to the immune response and it determines the response of the host to the pathogen. This complex resides on bovine chromosome 23 (Brinkmeyer-Langford et al. 2009). Other components of the immune system reside in other chromosomes. The constant region of the heavy chain of bovine immunoglobulins resides on chromosome 21 (Zhao et al., 2003), while the lambda light chain is located on chromosome 17 (Chen et al., 2008). Differences in sequence have been observed in genes comprising the immune system.

The Toll-like receptors (TLR) are a family of conserved glycoproteins that play a key role in the innate immune system. The function is to sense the presence of a pathogen and initiate the immune response (Turin and Riva, 2008). The genes that generate these proteins have been located to several chromosomes throughout the genome. Seabury et al. (2010) characterized the variation and haplotype structure of 11 TLR, to facilitate whole-genome-assisted methods for animal selection with increased tolerance to diseases. Several TLR genes reside on chromosomes 6, 8, 16, 17, 18, 22, 27, and X (Seabury et al., 2010). It is possible that results from QTL scans and

genome-wide association studies could be due to differences in sequence in one or more of these genes.

Gene interactions. It is possible that despite all methodologies used to identify genomic regions associated with genes influencing immune response, there will be the need to further evaluate the genome. Interactions between and within genes will need to be evaluated to ascertain the role of each gene in the immune system response to pathogens. The following results may illustrate the significance of interactions.

Bovine chromosome 20 shows evidence of harboring genes associated with disease resistance. Two genes located on chromosome 20 were identified as being involved with immune response in cattle. One gene is the Ankyrin-repeat protein 2 (*ANKRA2*). This gene may play a major role in the regulation of the MHC complex (Krawczyk et al., 2005; McKinsey et al., 2006). The *ANKRA2* gene is 96% similar paralogue to *RFX-B* (Long and Boss, 2005). It has been observed that mutations in the *RFX-B* gene cause immunodeficiency by producing a bare lymphocyte syndrome (Masternak et al., 1998). Krawczyk et al. (2005) indicated that increasing the expression of *ANKRA2* activates expression of the MHC, demonstrating that both genes (*ANKRA2* and *RFX-B*) have similar capacity to activate this complex. The other gene located on the centromeric region of chromosome 20 is *RP105*. Ogata et al. (2000) demonstrated that the response of B cells to membrane lipopolysacharides from bacteria is also regulated by the *RP105* gene. The *RP105* gene is member of the Toll-like receptor family. Both genes are located in the centromeric region, between megabases 6 million and 12 million according to the *Bos taurus* 4.0 draft genome assembly (The Bovine Genome Sequencing and Analysis Consortium. 2009). Sequence information was obtained from 38 SNP from the *ANKRA2* gene and 5 SNP from the *RP105* gene (http://www.ncbi.nlm.nih.gov/projects/SNP/). The objective of the study was to ascertain the association of SNP markers in the *ANKRA2* and *RP105* genes with diseases.

Two independent populations were developed to assess the association of SNP on the *ANKRA2* and *RP105* genes. In the first population, the incidence of BRD was established and in the second population, the presence of *Mycobacterium avium paratuberculosis* was determined. The first population was a half-sib family derived from a Hereford by Brahman sire. Calves were monitored daily by the veterinarian. Bovine respiratory disease was diagnosed by physical examination and the calf was treated accordingly (Snowder et al., 2005b). All calves from this sire that were treated for BRD were included in the study. For every treated animal, non-treated animals were selected by being of the same dam breed and sex as the treated animal. They were also selected when possible by being born in the same pasture and as close to the date of birth as the treated animal. There were 90 animals included in this study. This was referred to as the BRD population. The second population comprised 330 unrelated culled cows. Ileocecal lymph nodes were collected at the slaughterhouse. The presence of MAP was established for each lymph node. The presence of *M. paratuberculosis* was no indication of development of Johne's disease. Development of the disease was not assessed in the population. No additional information was available for this population (Wells et al., 2009). This was referred to as the MAP population.

All markers were genotyped in both populations. In the BRD population, 8 SNP from *ANKRA2* and 2 SNP from *RP105* were significantly associated with bovine respiratory disease (P<0.05). In the MAP population, 1 SNP from *ANKRA2* and 1 SNP from *RP105* were associated with presence of *M. paratuberculosis* in lymph nodes (P<0.05). For the BRD population, the 2 most significant SNP in the *ANKRA2* gene (rs17870711, rs17871560) and the 2 significant SNP in the *RP105* gene (rs42819483 and rs42819484) that where associated with bovine respiratory disease, were selected for further evaluation. In the MAP population, the SNP in the *ANKRA2* gene (rs17871543) and the SNP in the *RP105* gene (rs42819483) that were associated with the presence of *M. paratuberculosis*, were also selected for further evaluation. Table 1 shows the SNP used, their relative position in the chromosome (in megabases), and the significance on both populations. Allele combinations using SNP in both genes were produced and were evaluated in the population. Allele combinations in the BRD population were derived from 4 SNP, and the allele combinations for the MAP population were derived from 2 SNP. Two allele combinations with more than 5% frequency were observed in the BRD population (Allele combinations A and B, in Table 2). Four allele combinations were observed in the MAP population (Allele combinations C, D, E, and F, in Table 2). Table 2 shows the association of allele combinations in the *ANKRA2* and *RP105* genes with bovine respiratory disease in the BRD population, and with the presence of *M. paratuberculosis*, in the MAP population. The association of allele combinations was stronger than when individual SNP were evaluated. The most significant allele combination in both populations had similar frequency (approximately 62%).

Table 1: Evidence of association of single nucleotide polymorphisms in the *ANKRA2* and *RP105* genes on bovine chromosome 20, with bovine respiratory disease (BRD) and presence of *Mycobacterium avium paratuberculosis* (MAP) in two independent populations

Gene	dbSNP	MB	BRD p-value	MAP p-value
ANKRA2	Rs17870711	6,587,863	0.007	0.657
	Rs17871543	6,589,429	0.390	0.049
	Rs17871560	6,589,693	0.001	0.791
RP105	Rs42819483	11,002,170	0.014	0.005
	Rs42819484	11,002,328	0.011	0.485

Table 2: Allele combination (AC) associations of single nucleotide polymorphisms of *ANKRA2* and *RP105* genes with bovine respiratory disease (BRD) and Johne's disease in two independent populations

Condition	AC	Frequency, %	AC p-value	Overall p-value
BRD	A	61.9	0.0006	<0.0001
	B	27.3	0.1645	
MAP	C	61.7	0.0001	0.0032
	D	17.5	0.0067	
	E	14.5	0.0959	
	F	6.3	0.3147	

Association of allele combinations were observed in 2 independent populations with 2 different conditions. Association was greater when allele combinations were used, compared to associations with independent SNP. This suggests these 2 genes (*ANKRA2* and *RP105*) may be complementary in the expression of the immune system, regardless of the condition affecting the cattle.

Several QTL studies have indicated that chromosome 20 harbors genes associated with the immune system. However, results from genome-wide association studies show limited association of SNP in this chromosome with bovine respiratory disease or with general disease resistance. Results from the study show that associations are observed when allele combinations are used. Individual SNP have not identified these associations. Further evaluation of the genome is needed to identify genes and gene interactions associated with expression of the immune system when exposed to pathogens. Interactions play a key role in expression of immunity and they need to be identified if successful selection is to be pursued.

Final considerations

Diseases continue to have a considerable effect in the productivity of the beef cattle industry. Given the low heritability associated with response of the immune system, it will be necessary to clearly identify the genes involved in the immune response of the animal to exposure to pathogens. Initial efforts to identify genomic regions associated with these characteristics in beef cattle were limited. Genome-wide association studies will be able to identify additional regions where genes involved in the immune system are located and affect the response of the animal to pathogens. These genome-wide association studies will need to account for interactions between and within genes to more completely explain effects on immunity.

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