A Genome-Wide Association Study for the Incidence of Persistent Bovine Viral Diarrhea Virus Infection in Cattle

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ABSTRACT: Bovine Viral Diarrhea Virus (BVDV) is a diverse group of viruses causing disease in ruminants. The objective was to determine genomic regions harboring single nucleotide polymorphisms (SNP) associated with presence or absence of persistent BVDV infections. A genome wide association approach based on 777,000 SNP markers was used. Samples of animals identified as positive for BVDV (n= 1,200), and animals negative for the presence of the BVDV (n= 1,200) were used. DNA samples were incorporated in 24 pools (100 animals per pool). A strong association ($P < 5x10^{-7}$) was detected on chromosome 14, located at 80,675,176 megabases (Mb). Fifteen SNP were moderately associated ($P < 1x10^{-5}$). These last SNP reside on chromosomes 1, 2, 6, 8, 10, 15, and 18. Results support the hypothesis that genomic regions identified in the present study are involved in suppression of the immune system.

Keywords: Bovine Viral Diarrhea; Cattle; Genome-Wide Association Study

Introduction

Infection with bovine viral diarrhea viruses (**BVDV**) causes economically important diseases in cattle. BVDV can establish lifelong persistent infection. Infection of the naive dam results in viremia and transmission of the virus across the placenta to the fetus. If the pregnancy survives, the calf will be born immunotolerant to the virus and will be a lifelong shedder.

Several genome-wide association studies have been conducted to establish the association of SNP with diseases in cattle (Kirkpatrick et al., 2010; Blaschek et al., 2011; Minozzi et al., 2012; Finlay et al., 2012). Neibergs et al. (2011) evaluated the association of single nucleotide polymorphisms (**SNP**) on chromosome 2 and 26, with bovine respiratory disease and persistently infected BVDV animals, and later refined the position of the genomic region (Zanella et al., 2011). Leach et al. (2012) established the association of genomic regions with vaccination response to Bovine Respiratory Syncytial Virus. The present study is the first genome-wide association study for persistently infected BVDV cattle. The objective was to determine genomic regions harboring SNP associated with presence or absence of persistent BVDV infections.

Materials and Methods

Animals. Cattle were sampled from a starter feedlot in southwestern Kansas. All animals used were crossbred with unknown pedigree. The average weight at the time of arrival to the feedlot was 233.2 ± 1.7 kg. A total of 2,400 ear notch samples were used in the study. All samples were collected from summer of 2004 to summer of 2008. Of these, 1,200 were from animals identified as positive for the presence of BVDV, while 1,200 were from negative animals.

Trait. Ear notches of cattle were tested for BVDV according to the procedure described by Fulton et al. (2006). A second sample was collected within 48 hours after the initial result for cattle with positive results for additional testing (Hessman et al., 2009). For analyses, calves diagnosed as positive were classified as "affected" by BVDV. Negative animals were classified as "unaffected".

Genomic Screening. Ear notches were stored at -20°C until DNA was extracted (Qiagen Dneasy Blood and Tissue kit). Twelve pools of DNA were generated for affected animals and twelve pools for unaffected animals. Samples were allocated randomly in pools for affected and unaffected groups. One hundred animals were combined in each pool. Pools were genotyped with the Illumina BovineHD chip (Illumina, San Diego, CA).

Statistical Analysis. Data was analyzed with the procedure described by MacGregor et al. (2006, 2008). Allele frequency of pools was calculated for each SNP within each pool. Allele frequency is an estimate of (or proxy for) the allele frequency that would be obtained by genotyping individually the population; however, the relationship between pooling allele frequency and allele frequency is not exact. Pooling allele frequency was adjusted for average intensity differences between red and green within a stripe (segment of the BovineHD array) across SNP.

Mixed model methods were used to estimate differences in allele frequency between affected and unaffected pools while accounting for variation specific to SNP array platform, binomial sampling and pool construction error. Pool effects were considered fixed when estimating bead variance components. Statistical analysis was performed using R (R Development Core Team, 2011). Statistical thresholds for moderate ($P< 1x10^{-5}$) and strong ($P< 5x10^{-07}$) associations were established according to the Welcome Trust Case Control Consortium (2007).

Based on the SNP association results, genes of interest in the genomic region were identified within 100

Table 1. Single nucleotide polymorphism identification (SNP), chromosome, position in the chromosome, allele frequency of "B" allele in the unaffected pools (unaffected), allele frequency of "B" allele in affected pools (affected), and significance for incidence of persistent bovine viral diarrhea virus.

SNP	BTA	Position (bases)	Allele frequency (Unaffected)	Allele frequency (Affected)	Significance[a]
Rs43226513	1	32,138,151	0.243	0.159	3.32×10^{-6}
Rs136101709	1	154,175,502	0.197	0.281	4.92×10^{-6}
Rs43287585	1	156,065,876	0.325	0.216	1.19×10^{-6}
Rs133794462	2	105,626,910	0.771	0.878	6.38×10^{-6}
Rs135524200	2	107,608,745	0.383	0.232	2.93×10^{-6}
Rs109923296	2	107,747,873	0.254	0.106	7.16×10^{-7}
Rs135783786	6	79,369,511	0.220	0.354	9.32×10^{-6}
Rs134088824	6	79,385,615	0.658	0.500	7.08×10^{-7}
Rs135088957	6	79,389,382	0.610	0.474	5.67×10^{-6}
Rs42255579	6	99,531,916	0.646	0.498	3.84×10^{-6}
Rs109480556	8	99,789,997	0.304	0.410	5.95×10^{-6}
Rs135138396	10	65,281,936	0.312	0.220	3.47×10^{-6}
Rs136716397	14	80,675,176	0.853	0.735	9.41×10^{-8}
Rs110434337	15	1,385,504	0.472	0.388	7.93×10^{-6}
Rs109671280	15	35,368,078	0.063	0.138	8.13×10^{-7}
Rs110572575	18	44,427,437	0.246	0.161	1.35×10^{-6}

[a] Strong association threshold, $P < 5 \times 10^{-7}$; Moderate association threshold, $P < 1 \times 10^{-5}$

kilobases from the position of the significant SNP using the NCBI Map Viewer (www.ncbi.nlm.nih.gov/genome). Genes of interest were queried in the GWASdb (http://jjwanglab.org:8080/gwasdb/) to establish their potential role with diseases in human (Li et al., 2011).

Results and Discussion

Results of the genome-wide association study are presented in Table 1. Genomic regions on chromosomes 1, 2, 6, 8, 10, 14, 15, and 18, were associated with persistent BVDV infection. All genomic regions had a moderate association ($P < 1 \times 10^{-5}$), except an SNP which had a strong association ($P < 5 \times 10^{-7}$) on chromosome 14.

BVDV reduces immune cell populations, and it can be assumed that it reduces the functional output of the immune system. In a study conducted at the National Animal Disease Center, in Ames, Iowa, it was observed that cattle experimentally infected with BVDV had lower White Blood Cell (**WBC**) counts when compared to control animals (Drs. S. Falkenberg and J. Ridpath, unpublished data). Markers rs42255579, on chromosome 6, and rs110572575, on chromosome 18, could be associated with WBC count in cattle affected with BVDV. These markers are in orthologous regions to human chromosomes 4 and 16, respectively. Reiner et al. (2011) identified genomic regions of these human chromosomes as harboring genes associated with differing levels of WBC in a large African American population. Two markers on chromosome 1 (rs136101709 and rs43287585), reside in an orthologous region of human chromosome 21 known to harbor SNP associated with AIDS (Limou et al., 2009). Genes on genomic regions of chromosome 1, 6, and 18, are likely to be involved in suppression of the immune system by BVDV.

Marker rs109480556, on chromosome 8, neighbors the Toll-like Receptor 4 (*TLR4*). This gene is part of the Toll-like receptor family. The Toll-like receptors are a family of conserved glycoproteins that play a key role in the innate immune system. Their function is to sense the presence of a pathogen and initiate the immune response (Turin and Riva, 2008). It is possible the association observed in the present study is related to suppression of this gene.

Neibergs et al. (2011) identified a region of chromosome 2 associated with persistent BVDV in cattle. Neibergs et al. (2011) used microsatellite markers to identify the region that spanned from 126.7 megabases to 135.9 megabases. This region was of interest because of the identification of a Quantitative Trait Locus (**QTL**) for bovine respiratory disease (Neibergs et al., 2011). The support interval for this QTL spanned the telomeric end of chromosome 2. Zanella et al. (2011), using SNP, refined the location of the genomic region associated with persistent BVDV. It is possible that by using microsatellite markers by Neibergs et al. (2011) biased the search for the genomic region associated with BVDV. However, results from the present study support the finding by Neibergs et al. (2011) of a genomic region on chromosome 2 as harboring a gene or group of genes associated with persistent BVDV.

A marker on chromosome 14 presented a strong association ($P < 5 \times 10^{-07}$) with persistent BVDV. The gene RNA-binding Raly-like protein (*RALYL*) was identified in this genomic region. The genomic region where this gene resides has not been associated with diseases in human. A

single report of a QTL for mastitis was reported by Schulman et al. (2004) in dairy cattle. The association of the SNP on chromosome 14 needs to be evaluated further to establish its association with persistent BVDV.

Genomic regions on chromosomes 10 and 15, where SNP with a moderate association ($P< 1x10^{-5}$) with persistent BVDV reside, have not been reported on being associated with diseases in humans. Quantitative trait Loci for somatic cell score have been reported on chromosome 10 (Tal-Stein et al., 2010), and chromosome 15 (Boichard et al., 2003). Additional studies are needed to establish the association of these genomic regions with persistent BVDV.

Allelic frequencies of the B allele were different for each SNP (Table 1). Estimated allele frequencies for all significant markers were greater than 5%. Pool size of 100 animals minimizes cost while obtaining 80% statistical power to detect allele frequency differences of at least 5% (McDaneld et al., 2012). McDaneld et al. (2012) indicate that allele frequency of pools for affected and unaffected are biased relative to the actual allele frequencies, but the differences between allele frequencies are unbiased. Therefore, pooling of samples is an efficient approach on a genome-wide association study.

Conclusions

Results suggest there is genetic variability associated with the maintenance and/or survival of persistent BVDV infection. Results support the hypothesis that at least four genomic regions identified in the present study are involved in suppression of the immune system. This is the initial step for identifying the genes involved in the immune response of cattle affected with persistent BVDV.

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