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# Association of single nucleotide polymorphisms in the *ANKRA2* and *CD180* genes with bovine respiratory disease and presence of *Mycobacterium avium* subsp. *paratuberculosis*[1]

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**Summary**

The objective was to determine whether single nucleotide polymorphisms (SNPs) in the *ANKRA2* and *CD180* genes are associated with incidence of bovine respiratory disease (BRD) and presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in cattle. Two independent populations were used. The first population (BRD-affected; $N = 90$) was composed of 31 half-sib progeny, from a Brahman × Angus sire, that were treated for BRD. Untreated offspring from the sire were selected to serve as controls. The second population (MAP-infected) of 330 animals of unknown parentage was evaluated for the presence of MAP in ileocecal lymph node and classified as positive or negative. Markers in both genes were assessed for association in these two populations. In the BRD-affected population, five SNPs in the *ANKRA2* gene were significantly associated ($P < 0.05$), and two SNPs were highly associated ($P < 0.01$) with incidence of BRD. In addition, two SNPs in the *CD180* gene were found to be associated with this trait. In the MAP-infected population, one SNP in the *ANKRA2* gene was significantly associated ($P < 0.05$) with the presence or absence of MAP, and a SNP in the *CD180* gene was highly associated ($P < 0.01$) with the trait. Haplotypes, using significant markers, showed a positive association with both incidence of BRD ($P = 0.0001$) and with the presence of MAP ($P = 0.0032$). Markers in the *ANKRA2* and *CD180* genes are associated with the ability of the animal to cope with pathogens.

**Keywords** bovine chromosome 20, bovine respiratory disease, Johne's disease, single nucleotide polymorphism.

## Introduction

Bovine respiratory disease complex (BRD) is a major economic factor affecting beef cattle production. It has been estimated that mortality because of this complex is approximately 6% (NAHMS, 2000). The disease complex costs the U.S. beef industry from $500 to $750 million annually (Griffin 1997; Miles 2009). Viruses associated with BRD include the ones that produce bovine viral diarrhoea, infectious bovine rhinotracheitis, respiratory syncytial and parainfluenza. Bacterial causes of BRD are *Mannheimia haemolytica, Pasteuralla multocida* and *Haemophilus somnus.* Other microbial organisms that associated with BRD are Mycoplasmas (Ellis 2001). Miles (2009) suggests that 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 themselves.

The pathogen, *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is known to cause Johne's disease in ruminants, resulting in poor performance and extreme weight loss over time. It has been proposed that MAP is a potential food-borne pathogen for humans (Collins 1997), as in recent years the pathogen has been implicated as a possible cause of Crohn's disease because of its similar aetiology to the bovine disorder (Collins *et al.* 2000). It has been estimated that the prevalence of the pathogen is approximately 21.6% across all herds in the United States

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(NAHMS, 1997). This disease costs to the United States dairy industry between $200 and $250 million annually (Ott *et al.* 1999).

Additive genetic variance is known to exist in cattle for BRD and MAP. Snowder *et al.* (2006) estimated the heritability of BRD at between 0.00 and 0.08. The heritability estimated for MAP ranges from 0.09 to 0.18 (Gonda *et al.* 2006). Incidence of both conditions has genetic variability associated with it, and thus it is possible to identify genomic regions associated with this.

Multiple independent studies indicate that bovine chromosome 20 harbours genes influencing immunological defence mechanisms. Gonda *et al.* (2007) identified a region on the centromeric end of this chromosome that was associated with susceptibility to Johne's disease in Holstein cattle. The same region has been associated with somatic cell score in dairy cattle (Rodriguez- Zas *et al.* 2002; Ashwell *et al.* 2004) and incidence of pathogenic diseases including footrot, pinkeye and BRD complex (Casas & Snowder 2008). Taken together, these studies provide evidence that variation in genes along the centromeric region of chromosome 20 is associated with immune response in cattle.

The *ankyrin repeat, family A (RFXANK-like), 2* (*ANKRA2*) gene is located in the centromeric region of chromosome 20 and is thought to play a major role in the regulation of the major histocompatibility complex class II (MHC-II) genes (Krawczyk *et al.* 2005; Mckinsey *et al.* 2006). The MHC-II plays a role in the immunological response in livestock. The *ANKRA2* gene is 96% paralogous to *RFXANK* (Long & Boss 2005), mutations in which cause immunodeficiency by producing the bare lymphocyte syndrome (Masternak *et al.* 1998). In addition, increasing the expression (Krawczyk *et al.* 2005) of *ANKRA2* activates MHC-II expression, demonstrating that both genes (*ANKRA2* and *RFXANK*) have a similar capacity to activate transcription of the MHC-II genes.

The gene encoding the radio-protective protein of 105 kD (*CD180*) also resides in the centromeric portion of bovine chromosome 20. This is a gene from the family of the Toll-like receptors (Yazawa *et al.* 2003; Divanovic *et al.* 2007) known to function in the regulation of signalling in B cells of the innate immune system when lipopolysaccharides from Gram-negative bacteria are present (Yazawa *et al.* 2003; Divanovic *et al.* 2007).

The location of the *ANKRA2* and *CD180* genes in the genome and their role in immunological processes make them suitable candidates to assess their relevance in bovine infectious disease pathogenesis and control. Therefore, the objective of the present study was to determine whether SNPs in the *ANKRA2* and *CD180* genes are associated with BRD and/or presence of MAP in cattle.

## Materials and methods

Experimental procedures were approved and performed in accordance with U.S. Meat Animal Research Center Animal Care Guidelines and the Guide for Care and Use of Agricultural Animals Research and Teachings (FASS 1999).

### Animals

Two independent populations were used for the two traits. Table 1 shows the number of individuals used in each population. The first group of animals was defined as the BRD-affected population, and the second group was defined as the MAP-infected population.

The BRD-affected population was developed at the U.S. Meat Animal Research Center as a large half-sib family from a single Brahman × Angus sire to detect quantitative trait loci affecting economically important traits in beef cattle (Casas *et al.* 2004; Casas & Snowder 2008). The sire was mated to Hereford, Angus, MARC III (¼ Hereford, ¼ Angus, ¼ Red Poll, and ¼ Pinzgauer) and $F_1$ cows from the Germplasm Evaluation project Cycle IV to produce 259 offspring in 1995, and mated to MARCIII cows to produce 361 offspring in 1996 (620 total progeny). Breeds of sires for the $F_1$ cows were Hereford, Angus, Shorthorn, Charolais, Gelbvieh, Pinzgauer, Galloway, Longhorn, Nellore, Piedmontese or Salers. Breeds of dams for the $F_1$ cows were Hereford and Angus. Calves were weaned at an average age of 205 days. The average age of slaughter was 467 days. Of the total 620 progeny from this population, 31 were selected because they were treated for BRD and were used for the present study as cases. Cohorts of these 31 animals were selected to serve as controls. A total of 59 animals were selected as controls for this study.

The MAP-infected population consisted of culled cows. Ileocecal lymph nodes were removed on the line from caecum–small intestine tissues and bagged separately at a commercial slaughter facility. All ileocecal lymph node samples (Table 1) were included in the analysis. No additional information pertaining to cattle history or breed composition was available for these samples.

**Table 1** Number of animals treated and not treated for bovine respiratory disease (BRD) in the BRD-affected population, and number of cows in which *Mycobacterium paratuberculosis* was present or absent in the MAP-infected population.

| Population[1] | 1[2] | 0[3] | Total |
|---|---|---|---|
| BRD-affected | 31 | 59 | 90 |
| MAP-infected | 113 | 217 | 330 |

[1]BRD-affected = Offspring derived from a Hereford × Brahman sire treated and not treated for bovine respiratory disease. MAP-infected = Commercial culled cows sampled at a slaughter facility, in which *Mycobacterium paratuberculosis* was present in lymph nodes.
[2]Animals treated for bovine respiratory disease in the BRD-affected population. Cows with presence of *M. paratuberculosis* in lymph nodes in the MAP-infected population.
[3]Untreated animals for bovine respiratory disease in the BRD-affected population. Cows with absence of *M. paratuberculosis* in lymph nodes in the MAP-infected population.

## Traits

For the BRD-affected population, BRD was observed from birth to slaughter. Calves were monitored daily by the staff veterinarian, the beef cattle research technicians, or both. An animal was considered affected with respiratory disease when it displayed one or more of the following symptoms: fever, rapid breathing, coughing, discharge from the nose, eyes or both, diarrhoea, dehydration, and depression of appetite. When observed with clinical symptoms, calves were administered an appropriate treatment. Respiratory disease was treated with one or more medications (oxytetracycline, ceftiofur, flunixin meglumine, florfenicol, tylosin, enrofloxacin and sulfadimethoxine) as described by Snowder et al. (2006). The binary classification for BRD was defined as treated (1) or non-treated (0). Of the total 620 progeny from this population, 31 were selected because they were treated for BRD and were used for the present study as cases. Non-treated animals were selected based on having being born the same year, being the same sex, the same dam breed, and being born on approximately the same date as treated animals. A total of 59 animals were used as control for this study.

For the MAP-infected population, the presence or absence of MAP was determined in ileocecal lymph nodes as described by Wells *et al.* (2009). Briefly, presence of MAP was established by extracting DNA from lymph nodes using the DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Extracted DNA was subject to amplification targeting the MAP-specific IS900 repeat genomic sequence (Ellingson *et al.* 2005). Amplified products were separated by electrophoresis in 2% agarose gels. Confirmation of MAP was done by amplifying the MAP-specific IS-MAP02 repeat genomic sequence (Stabel and Bannantine 2005). Stabel and Bannantine (2005) indicate the possibility of other pathogens sharing similar sequences to the IS900 region; furthermore, amplification of the MAP-specific IS-MAP02 repeat genomic sequence was conducted to confirm the presence of MAP in lymph nodes. Amplified products were visualized as previously described. The binary classification for MAP infection was defined as infected (1) or non-infected (0). A total of 330 samples were used.

## Single nucleotide polymorphisms

Sequences of the SNPs used were downloaded from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Tables 2 and 3 show the SNPs used, and their relative positions on bovine chromosome 20. There were 35 SNPs in or near the *ANKRA2* gene, and 5 SNPs in the *CD180* gene. There were 35 SNPs included in the analysis.

## Genotyping

For the BRD-affected population, a saturated salt extraction procedure (Miller *et al.* 1988) was used to obtain DNA from white blood cells. Blood samples were collected in 60-ml syringes with 4% EDTA. Blood was centrifuged at 1300 ***g*** for 25 min, and buffy coats were aspirated, cleaned and frozen until DNA was extracted. For the MAP-infected population, DNA was extracted using the DNA Mini Kit.

Genotyping was performed using a primer extension method with mass spectrometry-based analysis of the extension products on a MassArray system as suggested by the manufacturer (Sequenom, Inc.), and as described by

**Table 2** Single nucleotide polymorphisms (SNPs) in the *ANKRA2* and *CD180* genes used in the BRD-affected population, their location on bovine chromosome 20, their minor allele frequency (MAF), and their association (*P*-value) with bovine respiratory disease (BRD).

| Gene | dbSNP | Position | SNP | MAF | *P*-value[1] |
|---|---|---|---|---|---|
| *ANKRA2* | rs41933871 | 6585172 | G/T | 0.328 (T) | 0.091 |
| | rs41933870 | 6586364 | A/C | 0.430 (A) | 0.025 |
| | rs41933869 | 6586389 | A/C | 0.331 (A) | 0.063 |
| | rs41933867 | 6586995 | T | 1.000 | N/A |
| | rs41933866 | 6587090 | C | 1.000 | N/A |
| | rs41933865 | 6587110 | G | 1.000 | N/A |
| | rs17870711 | 6587863 | C/T | 0.324 (C) | 0.007 |
| | rs17870348 | 6588030 | C/T | 0.329 (C) | 0.057 |
| | rs17870347 | 6588043 | A/G | 0.323 (G) | 0.022 |
| | rs17870709 | 6588694 | A/G | 0.006 (A) | 1.000 |
| | rs17870710 | 6588785 | A/G | 0.058 (A) | 0.099 |
| | rs17871543 | 6589429 | C/T | 0.076 (T) | 0.390 |
| | rs17871544 | 6589440 | C/T | 0.322 (T) | 0.087 |
| | rs17871567 | 6589625 | C/T | 0.283 (T) | 0.331 |
| | rs17871560 | 6589693 | A/G | 0.245 (G) | 0.001 |
| | rs41933863 | 6589763 | A/G | 0.250 (G) | 0.035 |
| | rs41933862 | 6590128 | C/T | 0.329 (C) | 0.06 |
| | rs41933861 | 6590285 | A/G | 0.125 (A) | 1.000 |
| | rs41933901 | 6592101 | A/G | 0.407 (A) | 0.122 |
| | rs41933902 | 6592258 | C/G | 0.320 (G) | 0.166 |
| | rs41933903 | 6592366 | C/T | 0.327 (C) | 0.018 |
| | rs41933904 | 6592806 | C/G | 0.007 (C) | 0.339 |
| | rs41933906 | 6592928 | C/T | 0.320 (T) | 0.121 |
| | rs41933907 | 6595021 | C/T | 0.329 (C) | 0.071 |
| | rs41933908 | 6596287 | G | 1.000 | N/A |
| | rs41933909 | 6596327 | C/G | 0.319 (C) | 0.146 |
| | rs41933910 | 6596394 | A/G | 0.011 (A) | 0.119 |
| | rs41933911 | 6596489 | A/C | 0.324 (C) | 0.127 |
| | rs41933912 | 6596508 | A/G | 0.304 (C) | 0.143 |
| | rs41933913 | 6596660 | A/G | 0.331 (A) | 0.078 |
| | rs41933914 | 6596884 | C/G | 0.277 (C) | 0.042 |
| | rs41933915 | 6597070 | A/G | 0.326 (G) | 0.088 |
| | rs41933916 | 6597216 | C/G | 0.326 (G) | 0.088 |
| | rs41933917 | 6597292 | A/G | 0.316 (A) | 0.223 |
| | rs41933918 | 6597721 | A/G | 0.012 (G) | 0.114 |
| *CD180* | rs42819483 | 11002170 | G/T | 0.094 (G) | 0.014 |
| | rs42819484 | 11002328 | A/G | 0.063 (G) | 0.011 |
| | rs42819485 | 11004065 | A/G | 0.182 (A) | 1.000 |
| | rs42819487 | 11011680 | A | 1.000 | N/A |
| | rs42819488 | 11015190 | C/T | 0.045 (T) | 0.447 |

[1]N/A = Not available because marker was monomorphic.

**Table 3** Single nucleotide polymorphisms (SNPs) in the *ANKRA2* and *CD180* genes used in the MAP-infected population, their location on bovine chromosome 20, their minor allele frequency (MAF), and their association (*P*-value) with the presence or absence of *Mycobacterium paratuberculosis* in lymph nodes.

| Gene | dbSNP | Position | SNP | MAF | *P*-value[1] |
|---|---|---|---|---|---|
| *ANKRA2* | rs41933871 | 6585172 | G/T | 0.076 (T) | 0.732 |
| | rs41933870 | 6586364 | A/C | 0.226 (A) | 0.517 |
| | rs41933869 | 6586389 | A/C | 0.063 (A) | 0.867 |
| | rs41933867 | 6586995 | T | 1.000 | N/A |
| | rs41933866 | 6587090 | C | 1.000 | N/A |
| | rs41933865 | 6587110 | A/G | 0.006 (A) | N/A |
| | rs17870711 | 6587863 | C/T | 0.050 (C) | 0.657 |
| | rs17870348 | 6588030 | C/T | 0.063 (C) | 1.000 |
| | rs17870347 | 6588043 | A/G | 0.060 (G) | 0.851 |
| | rs17870709 | 6588694 | G | 1.000 | N/A |
| | rs17870710 | 6588785 | A/G | 0.075 (A) | 0.638 |
| | rs17871543 | 6589429 | C/T | 0.208 (T) | 0.049 |
| | rs17871544 | 6589440 | C/T | 0.055 (T) | 0.707 |
| | rs17871567 | 6589625 | C/T | 0.038 (T) | 0.644 |
| | rs17871560 | 6589693 | A/G | 0.030 (G) | 0.790 |
| | rs41933863 | 6589763 | A/G | 0.192 (G) | 0.349 |
| | rs41933862 | 6590128 | C/T | 0.061 (C) | 0.719 |
| | rs41933861 | 6590285 | A/G | 0.055 (A) | 0.445 |
| | rs41933901 | 6592101 | A/G | 0.142 (A) | 0.791 |
| | rs41933902 | 6592258 | C/G | 0.064 (G) | 0.851 |
| | rs41933903 | 6592366 | C/T | 0.067 (C) | 0.437 |
| | rs41933904 | 6592806 | C/G | 0.118 (C) | 0.540 |
| | rs41933906 | 6592928 | C/T | 0.058 (T) | 0.570 |
| | rs41933907 | 6595021 | C/T | 0.058 (C) | 0.572 |
| | rs41933908 | 6596287 | G | 1.000 | N/A |
| | rs41933909 | 6596327 | C/G | 0.063 (C) | 0.721 |
| | rs41933910 | 6596394 | A/G | 0.057 (A) | 1.000 |
| | rs41933911 | 6596489 | A/C | 0.102 (C) | 0.881 |
| | rs41933912 | 6596508 | A/G | 0.060 (C) | 1.000 |
| | rs41933913 | 6596660 | A/G | 0.082 (A) | 0.625 |
| | rs41933914 | 6596884 | C/G | 0.054 (C) | 0.551 |
| | rs41933915 | 6597070 | A/G | 0.062 (G) | 0.851 |
| | rs41933916 | 6597216 | C/G | 0.056 (G) | 0.575 |
| | rs41933917 | 6597292 | A/G | 0.062 (A) | 0.728 |
| | rs41933918 | 6597721 | A/G | 0.042 (G) | 0.669 |
| *CD180* | rs42819483 | 11002170 | G/T | 0.239 (G) | 0.005 |
| | rs42819484 | 11002328 | A/G | 0.112 (G) | 0.484 |
| | rs42819485 | 11004065 | A/G | 0.214 (A) | 0.128 |
| | rs42819487 | 11011680 | A | 1.000 | N/A |
| | rs42819488 | 11015190 | C/T | 0.027 (T) | 0.610 |

[1]N/A = Not available because marker was monomorphic.

Stone *et al.* (2002). A universal mass tag sequence was added to the 5′ end of each amplification primer sequence as recommended by the manufacturer. Genotypes for each animal were collected, and the automated calls were checked by visualization of the spectrographs to minimize errors. When necessary, genotype assays were performed a second time to increase the number of successful genotypes, but samples were not tried a third time.

### Analysis

Each population was analysed separately. Allele frequency for each SNP was obtained using the procedure ALLELE from SAS (SAS Inst., Inc.). The association of each marker with BRD and with MAP infection was tested using a chi-square process for association studies (Devlin & Roeder 1999) using the procedure CASECONTROL from SAS. This procedure is similar to a transmission disequilibrium test (Lynch & Walsh 1997).

The most significantly associated SNPs were incorporated in haplotypes. A transmission disequilibrium test (Lynch & Walsh 1997) was used to establish the association of haplotypes with the traits in each population using the procedure HAPLOTYPE from SAS. Haplotypes with a frequency of <0.05 were excluded from the study. Probability values were obtained by generating 10 000 permutations (Westfall & Young 1993).

## Results

### Allele frequencies

The genotypes, the minor allele frequencies, and the minor alleles for each marker in the BRD-affected and MAP-infected populations are presented in Tables 2 and 3, respectively. The minor allele was the same for all the SNPs in both populations, with two differences. Marker rs41933865 was monomorphic in the BRD-affected population, where only the 'G' allele was observed. In the MAP-infected population, the allele frequency of the 'A' allele was 0.006. Marker rs17870709 was monomorphic in the MAP-infected population, where only the 'G allele' was observed. In the BRD-affected population, the allele frequency of the 'A' allele was 0.006.

Allelic frequencies were different for each SNP when comparing both populations. For SNPs in the *ANKRA2* gene, the average frequency of the minor allele in the BRD-affected population was 0.259, while in the MAP-infected population it was 0.077. For SNPs in the *CD180* gene, the average frequency of the minor allele in the BRD-affected population was 0.096, while in the MAP-infected population it was 0.148. Population structure contributed to these differences. The BRD-affected population was part of a half-sib family, while no information was known for the MAP-infected population, although it is unlikely to have been a half-sib family.

Three markers were not identified as SNPs in the populations used in this study. Markers rs1933867 and rs1933866 in the *ANKRA2* gene, and marker rs42819487 in the *CD180* gene were monomorphic in both populations.

### Single nucleotide polymorphism associations

The strength of association of each SNP with BRD in the BRD-affected population is shown in Table 2. Five SNPs in

the *ANKRA2* gene were nominally associated ($P < 0.05$) with incidence of BRD. Two markers were highly associated ($P < 0.01$) with incidence of BRD. In the *CD180* gene, two SNPs were associated ($P < 0.02$) with incidence of BRD.

The association of each SNP of the *ANKRA2* and *CD180* genes with the presence or absence of MAP in the MAP-infected population is shown in Table 3. One single nucleotide in the *ANKRA2* gene was significantly associated ($P = 0.049$) with the presence or absence of MAP in lymph nodes in the MAP-infected population. One marker in the *CD180* gene was associated ($P = 0.005$) with MAP infection in lymph nodes.

### Haplotype associations

Four SNPs were used to generate haplotypes in the BRD-affected population. Two markers (rs17870711 and rs17871560) resided in the *ANKRA2* gene. The other two markers (rs42819483 and rs42819484) were located in the *CD180* gene. In the MAP-infected population, two SNPs were used to generate haplotypes. Marker rs17871543 resided in the *ANKRA2* gene, and marker rs42819483 resided in the *CD180* gene.

Table 4 shows the haplotype frequencies for each haplotype observed in the BRD-affected population and their association with incidence of BRD. Two haplotypes with a frequency >5% were observed in this population. One haplotype in this population was highly associated ($P = 0.0006$) with incidence of BRD. This haplotype had the highest frequency observed in the population (0.619). The frequency of this haplotype in untreated animals was higher than in animals treated for BRD. The other haplotype was not associated with BRD complex.

Haplotype frequencies and their association with the presence or absence of MAP in lymph nodes in the MAP-infected population are shown on Table 5. Four haplotypes were observed in this population. Haplotype frequencies ranged from 0.063 to 0.617. Haplotypes in this population were associated ($P = 0.0032$) with the presence or absence of the pathogen in lymph nodes. The most significantly associated haplotype ($P = 0.0001$) with the trait of interest in this population was CT, with a frequency of 0.617 in the population. The frequency of this haplotype in lymph nodes where MAP was present was higher than in lymph nodes where the bacterium was absent.

**Table 4** Frequency and levels of significance of haplotypes that include markers rs17870711 and rs17871560 in the *ANKRA2* gene, and markers rs42819483 and rs42819484 in the *CD180* gene, which are associated with bovine respiratory disease (BRD) in the BRD-affected population.

| | Frequency | | | Haplotype | Overall |
|---|---|---|---|---|---|
| Haplotypes | 0[1] | 1[2] | Combined | *P*-value | *P*-value |
| CGTA | 0.246 | 0.346 | 0.273 | 0.1645 | 0.0001 |
| TATA | 0.718 | 0.433 | 0.619 | 0.0006 | |

[1]Untreated animals for bovine respiratory disease.
[2]Animals treated for bovine respiratory disease.

**Table 5** Frequency and levels of significance of haplotypes that include marker rs17871543 in the *ANKRA2* gene, and marker rs42819483 in the *CD180* gene, which are associated with the presence or absence of *Mycobacterium paratuberculosis* in the MAP-infected population.

| | Frequency | | | Haplotype | Overall |
|---|---|---|---|---|---|
| Haplotypes | 0[1] | 1[2] | Combined | *P*-value | *P*-value |
| CG | 0.205 | 0.116 | 0.175 | 0.0067 | 0.0032 |
| CT | 0.563 | 0.722 | 0.617 | 0.0001 | |
| TG | 0.070 | 0.049 | 0.063 | 0.3147 | |
| TT | 0.162 | 0.112 | 0.145 | 0.0959 | |

[1]Cows with absence of *M. paratuberculosis* in lymph nodes.
[2]Cows with presence of *M. paratuberculosis* in lymph nodes.

## Discussion

The goal of the study was to examine two genes with known roles in immune function as promising candidate genes underlying reported QTL for respiratory disease on chromosome 20. The presence of a QTL using treatment records as the phenotype (Casas & Snowder 2008) was established using 547 half-sib animals from a Brahman × Hereford cross. In the present study, a case–control analysis was used on animals from a Brahman × Angus cross population to detect association of haplotypes in the *ANKRA2*/*CD180* gene region with the same phenotype, and which supported the earlier QTL results. While the QTL results from our earlier study reflect a difference between *Bos indicus*-derived vs. *Bos taurus*-derived alleles, this is unlikely in the present study, because two key markers of the four SNPs forming the haplotypes are homozygous in the sire (data not shown). This suggests that the variation being monitored is entering from the dam side in this population and supports the hypothesis that variation in or near the *ANKRA2* and *CD180* genes is associated with treatment for respiratory disease in a way that is not confined to *Bos indicus* x *Bos taurus* cattle.

Further support for the hypothesis that one or both of these genes are involved in immune function variability with regard to inhaled pathogens comes from the association of SNPs in these same genes with the presence of MAP in a completely independent population. This population was essentially randomly selected from a commercial processing facility, and is likely to be of very different breed composition. In contrast to the BRD-affected population, there was no diagnosis of disease in the MAP-infected population. Instead, the presence of the pathogen was used to infer the potential for disease. This strategy has been employed before using ELISA or positive faecal culture to detect the pathogen, and was the basis of the study (Gonda

*et al.* 2007) that originally detected a QTL for infection on chromosome 20. In the present study, despite no knowledge of variation in environmental factors, breed composition or age of the animals at slaughter, significant association of haplotypes across the *ANKRA2*/*CD180* region with the presence or absence of pathogen were detected.

Association in the MAP-infected population could be attributed to population stratification. No additional information was used besides presence or absence of the pathogen. Therefore, the significance observed could be a function of categorization within the population. However, previous evidence (Gonda *et al.* 2007) supports findings from the present study.

Taken together, the findings of association in different populations with different SNPs support a conclusion that variation close to the *ANKRA2*/*CD180* locus analysed in our study contributes to differential response to pathogen exposure. Both of the candidate genes have known functions in immunological processes, suggesting that one or both of the genes could be involved. Alternatively, the markers may be in linkage disequilibrium with variation in nearby, less obvious candidate genes. Complete examination of the gene sequences and markers in other nearby genes, in addition to genotyping of larger affected populations and additional, higher resolution phenotypes, will be required to identify putative causative variation and to determine the extent to which it modulates the immune response and the breadth of pathogen exposures that may be affected.

The most significantly associated haplotypes in both populations had similar frequencies (around 0.62). These haplotypes are most likely in linkage disequilibrium with the causative mutation. Given that the association was observed with haplotypes with similar frequency, it is possible that the causative mutation is the same in both populations.

Several chromosomal regions in cattle have been associated with Johne's disease. Gonda *et al.* (2007) identified a region on chromosome 20. Settles *et al.* (2009) identified SNPs on chromosomes 1, 3, 5, 7, 8, 9, 16, 21 and 23 that were associated with infection status of the disease. Zanella *et al.* (2011), using the same population used by Settles et al. (2009), indicated that SNPs on chromosomes 1, 2, 6 and 15 were associated with tolerance to Johne's disease. Koetz *et al.* (2010) found association of SNPs of the *toll-like receptor 2* gene that were associated with susceptibility to Johne's disease; this gene resides on chromosome 17 (Koetz *et al.* 2010). Results from the present study indicate that chromosome 20 in fact harbours a gene or group of genes influencing immune response in cattle.

The genes *ANKRA2* and *CD180* were selected for study to ascertain their association with incidence of diseases. These genes reside in a region of bovine chromosome 20 that has been associated with incidence of disease in cattle. SNPs in these genes were evaluated in two independent populations. One population was affected with BRD. The second population was infected with MAP. Markers on both genes were associated with both pathogenic conditions in these populations. Further studies are needed to ascertain the causative mutation.

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