Single Nucleotide Polymorphisms associated with growth and carcass traits located on QTL Regions previously associated with Bovine Respiratory Disease

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ABSTRACT. The objective of the current study was to evaluate single nucleotide polymorphisms (SNP) for potential growth and carcass trait associations located in two previously described quantitative trait loci (QTL) regions associated with bovine respiratory disease. A population of 323 crossbred steers sired by five purebred sire breeds between 2010-2013 (Angus, Braford, Braunvieh, Charolais, and Simmental) were evaluated from birth until harvest. Eighty-two SNP were evaluated in the current study for potential significant associations with growth and carcass traits (58 on BTA6 and 24 on BTA20). A total of nine unique SNP (rs41595713, rs42403565, rs42571566, rs42900130, rs41931108, rs42480445, rs43451134, rs42524450, rs41626155) were significantly associated ($P < 0.05$) with specific growth traits such as birth weight, weaning weight and hip height. Six of these significant SNP were located on BTA6 and three were located on BTA20. When evaluating the carcass traits hot carcass weight (HCW), yield grade (YG), marbling score (MARB), and rib eye area (REA) a total of nine unique SNP (rs42900130, rs42961882, rs43446022, rs41931108, rs41595713, rs41653357, rs43036576, rs42823614, rs42512588) were significantly associated ($P < 0.05$) with carcass traits. For both of these regions, animals inheriting differing genotypes from the previously described SNP, had significantly different levels of performance for specific

growth and carcass traits. Although multiple SNP were identified as significant with growth and carcass traits, these SNP identified herein must be validated in a larger more diverse population prior to implementation into marker assisted selection programs.

KEY WORDS: Carcass Composition, Carcass Quality, Growth, SNP

INTRODUCTION

The bovine genome has been extensively evaluated for regions that may contain genes and variants that contribute to the performance of economically important traits in beef cattle. Specifically, BTA6 and BTA20 have been hotspots for QTL associated with growth, performance, carcass quality and composition and bovine respiratory disease (BRD) (http://bovinegenome.org/bovineqtl_v2/findQTL.html). Previous studies evaluating disease susceptibility have identified QTL regions associated with BRD susceptibility and growth traits located on BTA6 and BTA20 (Li et al., 2004; Casas et al., 2010; Snelling et al., 2010). However, it has also been reported that BTA6 and BTA20 have been shown to harbor the majority of the significant single nucleotide polymorphisms (SNP) associated with growth (Snelling et al., 2010) as well as many carcass traits (Casas et al., 2003; Saatchi et al., 2014).

Previous work has also demonstrated the negative correlative effects that BRD can have on carcass traits such as hot carcass weight (HCW) and performance traits like average daily gain (Schneider et al., 2009). Additionally, it has been reported that selection for BRD resistance may have little effect on HCW, longissimus muscle area (LMA), and fat due to the low genetic correlation estimates. However, results indicated favorable genetic correlations existed for birth weight (BW) and marbling score (MS) with both affected and unaffected animals (Schneider et al., 2010). An additional study reported that steers with clinical signs of BRD had less internal fat, and lower MS compared to the steers with no clinical sign of BRD at time of slaughter (Gardner et at., 1999). Thus, the objective of the current study was to evaluate SNP located on previously described QTL regions of BTA6 and BTA20 that overlap with BRD for potential associations with growth and, carcass traits in a population of crossbred steers sent to the feedlot and harvested at a commercial packing facility.

MATERIALS AND METHODS

Experimental Animals

All animals were treated and maintained in accordance with the principles and guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching. The animals utilized in the current study were comprised of 323 crossbred steers born at the Louisiana State University Ag Center Central Research Station in Baton Rouge, LA and LSU Ag Center Hill Farm in Homer, LA from 2010 to 2013. Calves were born during the spring calving season and were managed until weaning, or approximately six to seven months of age. Calves were sired by Charolais, Braunvieh, Simmental, Angus or Braford bulls. The dam breeds at the LSU Ag Center utilized for this study have been previously described during the characterization of the Germplasm Evaluation VIII studies (Wheeler et al., 2011). The dams utilized by the LSU Ag Center Hill Farm in Homer, LA were comprised of various breed backgrounds (Table 1).

Steers that met shipping criteria were vaccinated and shipped to commercial feedlots in Texas and Oklahoma. When the finishing process was completed, animals were sent to a commercial packing plant where carcass quality and composition traits were recorded. These trait measurements included hot carcass weight (HCW), marbling score (MS), rib eye area (REA), back fat thickness (BF) and yield grade (YG).

Table 1. Total number of animals for each sire breed.

Sire Breed	Total Number of Animals
Angus	55
Braford	29
Braunvieh	46
Charolais	133
Simmental	60

DNA Extraction and Genotyping

Ear notches were collected from all calves at birth for future DNA extraction. Extraction of DNA was conducted using a saturated salt procedure previously described by Miller et al. (1998). DNA stock solutions were diluted to 25 ng/µl concentrations for future genotyping reactions. Fifty-eight SNP were selected from a previously described QTL region with SNP associated with incidence of BRD spanning between 40-80 Mbp on BTA6 (Li et al., 2004) (Miller et al., 2016). Twenty-four SNP were selected from a previously described QTL region with SNP associated with incidence of BRD spanning 0-30 Mbp on BTA20 (Casas et al., 2011; Miller et al., 2016). Single nucleotide polymorphisms were selected using the QTL database (http://www.animalgenome.org/cgi-bin/QTLdb/index). Single nucleotide polymorphisms, allele substitutions, and upstream and downstream genomic sequences are reported in Tables 2 and 3. Single nucleotide polymorphism genotyping was performed by Neogen, Inc. (Lincoln, Nebraska) via the Sequonom platform.

Statistical Analysis

The Mixed Model procedure of SAS (version 9.4, SAS Institute, Cary, NC) was utilized to evaluate potential SNP associations located on BTA 6 and BTA 20 with growth traits, carcass composition and quality traits. Only the SNPs with more than one genotype were included in the analysis. The LSMEANS function, along with the pre-planned pairwise comparisons procedure, was utilized to evaluate if significant differences existed between individuals inheriting differing genotypes for SNP identified as significant for specific traits. Dependent variables in the model included birth weight (BW), weaning weight (WW), hip height (HH), HCW, YG, MS, REA, and BF. Independent variables included sire breed, SNP genotype and birth year. Sire breed (year) was fit into the model as a random nested variable to account for confounding effects of sire breeds among the four years. Significance was set at $P < 0.05$.

Table 2. Single nucleotide polymorphisms ID, allele substitutions, and upstream and downstream genomic sequences utilized for amplification and visualization of genotypes for BTA20.

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs41595713	C/T	TCTCGGTTCCTAACACAGCCAAGAC	GTTGTCCCGAACGGGTGAGGAATGG
rs41931108	C/G	TTGGTGTGCCAAGCACATCCCCAGC	GAGGAAGGCAGGTTGTGCCCATATT
rs42476237	C/T	CCTGGCCCACCCTTCCTTCCTTCCC	ATTTGTGGAGAAGCACGTGGGGAAC
rs42476290	A/G	CTGAGGCCAGAATTCTTGAAAGAAT	TGTTTGCATGGTGACAGCAAAGCAT
rs42477340	C/T	CTCCCGCCTCCTTCTCTGCTCCCTC	GGCTCCCTCTCTGCTCCCTCCGGCT
rs42480445	C/T	TGGCCCCAAATGCCAAAAGGTTATC	TCATTTTTTTCCAAGCAATCCCACC
rs42481107	A/G	AACAACACCTTCCACCGCCCCATCC	GGTCTCAGCCTAAGCATCAGCTCTT
rs42512588	C/T	GAATGGGGAGTGACTGCTTCCTAGG	CTGGGGTTTCTGTTGGGTTGATGCT
rs42520493	A/C	CATGCTGTATATCGGAGGGTCTAGG	CTGTTAAGCAGGAAATGAGAACTCC
rs42524445	C/T	GGTTCTTAAAAGTGAAATGATAATG	AGAAGAAATAGAGTGATGTGATGTG
rs42524450	C/T	TCCTTGGAAGTGGGGTTGCTCCTTC	GGCCGCCACCCCTGGCCTCAGGCGT
rs42524466	C/G	ATTTTATGTCGCAGTTTTCTCTCAC	AATCTAAGTTTAAATCTCTCAGAGG
rs42524468	A/T	AATAGACCCACAGACATAGAAAACA	ATGTATGGTTACCAAAGGGGAAAGG
rs42524472	A/T	AAAATAAATAGTAAATCACAAACAC	AATCACAGATAGGAAGAAAATGCAA
rs42524503	A/T	GTTGTATAGACAGATATCTGTCACT	ATTCTTTCCAAATGCTCTGACAGAT
rs43036576	A/G	TAATCATGAAGCCATCCTGTAGGGT	GAGCTAGGGTTTATAGCGGCTGTGA
rs42524459	C/T	ATCCACACAGTCAAAGCCTTTGGCA	AGTCAATAAAGCAGAAATAGATGTT
rs42481060	C/T	ACTGCCTCAGGCCTGGCACACAGCC	GAGAGGCCATGGGGCCCTGTGGAGC
rs41931083	C/T	GACTTCATTTCTCTCCGTGATAATC	TGCGGGGCAGGTCCCCAGGTCTGGA
rs42524449	A/G	TCTGCCCCTGCTGACCTTCAACGTG	AATAGCTCCTCTAGGACCTCCTGCG
rs42524457	G/T	GTTTATTGTGATCCACACAGTCAAA	CCTTTGGCACAGTCAATAAAGCAGA
rs42476309	C/T	GATGGTTTAGTCACTAAGTCATGTC	GACTCTTGAAACCCCATGGACTGTA
rs42236701	A/C/G	TCCACTTGATTTCACATTCCAGGAT	TCTGGCTCTAGGTGAGTGATCACAC
rs41931859	C/T	GAGGAGCCTGGGCTACAGTTCATGG	GTCACAGAGAGTCGGACACAACTGA

Table 3. Single nucleotide polymorphisms ID, allele substitutions, and upstream and downstream genomic sequences utilized for amplification and visualization of genotypes for BTA6.

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs29025265	C/T	CAGTTAGAGTTCAAAGGGACTTTTG	GTCAAACTGAGTACAAAATCTTTTC
rs41626155	C/T	TCCTGCCCTGCCTTCTTTAACTTCT	TCCCCAATCTCTGGTTGCCATTCAT
rs41653357	A/C	TGGAGAATCCTTTAGACAATAGGAG	TTGGTGGGCTATAGTCCATGGGGTT
rs42402825	A/G	GAGAATCCAAAGACAATACCAAAAT	AAGTCTATTGAAAGCCCACTCCTTG
rs42403565	C/T	ATTTCTATTACCCTATGTGTCAGAT	TCTGATTCACTCTTCTGCCTCCTCT
rs42571566	A/G	GCCGTCTATGGGGTCGCACAGAGTC	GACACGACTGAAGCAACTTAGCAGC
rs42579150	C/T	ATATGCCAATGATCTTAAAATTACT	GGTAAATATTTGAACATTTTTCTGC
rs42579164	A/C	CTCTATTTTTACAACATGGATGGAC	TAGAGATGATTATACTAAGTGAAGA
rs42725112	C/T	TTCATTAAAACACAAAAATCACAAC	AACTGCTGAACAACCACCAGCAAAA
rs42823614	A/C	AGGCAAATTCTTCACCAGCTGAACC	CAGGGAAAGCCTAATTCCCACCTTC
rs42824344	C/T	GTAGCATCATTGCCCTTTAATTATC	AAACTAGAAGCAAACTGAATGTCCA
rs42880470	A/G	TCTGGAGTAGGTACTGTGGGAGCAA	CTCAATCAGAGTTGTGAATAGCCTC
rs42880522	A/G	CTGAGGCTGGCCCTGACCTGAGATA	CCACCCTTTCTTACTCTCTTTCTTC
rs42900120	G/T	GGGGAAGGGGAGGAAGGATAAATTG	GAGATTGGGACTGACATATACACAC
rs42900130	A/G	CACAGGAGATAATCCTCTGCCTCCA	TTATGGTCTTCTGTGAAAAGTACTG
rs42900481	A/G	ACTTTAGATTCAATTCTTCTTGGCT	GGGATGGAGAATCTTTGAATTTCTC
rs42961863	C/T	GGACCAGAAGTCCCTTTCCCTTGCT	ATGTGTATTTTTAATGGTGATGACA
rs42961866	G/T	TGTTGTTTCCAGCTCTCCAATCTAG	TATTGTCCATTACTATTAAACATTC
rs42961882	A/T	CTTCTTTTTTGGTATGATTTTGGTC	CTGTCTCCTATACACTATTACAGAT
rs42968197	C/T	TGACAAGTAGATGCTTTTTATTAAA	TCATTCTATGTAAGAGACAGCTGAG
rs42968891	A/G	TTCATACCTAGATAATTGCAATTTC	TACCTAGCCTTTCCAGTCCTTTGGA
rs42968895	C/T	TCATATTCAGAGGTGGGATGTCATT	TTAAGGCTTTCAAGGCACTAATCCT
rs43089863	C/G	CATCTCTCTGAGTTGTCCTCTATTG	AGTCAGGGAGCAGGGCCTTTTTACC
rs43138398	C/T	TTGCAAGATAATTACAGTCACTTCC	TTTTCATGATCATTGGCCTTGAGCT
rs43194943	A/G	ATATCTTCTTAATATCTTCTTTTTT	TTAGGTCTGCACCATTTCTGTCCTT
rs43446022	G/C	TATGTTCAGAGGAATTAAGTCTTGA	CTTGTCATAAATACAACAAAATGAG
rs43446601	G/T	GTTTCCTGGAATTTGGATGAAAATT	CCTTCAATGTTTATATCTGAATCTT
rs43446955	C/T	TGCTTGTTTATATCACTTTGATATA	ACTATATTAAATTATAATGCTCTTT
rs43447179	A/G	TTTCTTTTTTCCCACCAGGAAATAC	CATTTCCTGGCCTCATAAAGACCAT
rs43448463	A/G	AGAATGCAAAGAGGAACTAAAGAGC	TCTTGATGAGGTTGAAGGAGAAGAA
rs43448512	A/C	AGATAAACTGAGACTTTCATGACGG	AGGCTCTTGAAGGAGAAGTTCTTTG
rs43449040	A/G	CACATTGATCGCTCTAATCTTAGAG	AAAAGTGCTTAAAAACTTAGACACT
rs43449194	C/T	TGAAAATGTTTCTTGCATTATTTTA	TATCAATTTCTTCATTTTGCTGTTA
rs43449209	A/G	AGTTGCTCAAGATCACACAGCATGT	TGCTGGAGCTAGGATTGAAAGCTCA
rs43449835	C/T	TAGTATCCTTTGCTAAATTTATCAT	AGTAGGTTAAAGAAGCCTTCAGGAT
rs43449896	A/C	TCCACTGGATGATCCACTGGATCAT	GAAAAAGCAAGAGAGTTCAAGAAAA
rs43451134	A/T	CATACTATATAGCACAGGAAACTAT	TTCAATATCCTGGGATAAATCATAA
rs42403543	C/T	AAGGAAATGCTTTCAATTTTTCACT	TTTATTATGATGCAAGCTGAAGGTT
rs42481129	A/G	TTCTCCCACACCACAGTTTAAAAGC	TCAATTCTTCGGCACTCTGCCTTCT
rs42579148	G/T	TATGACTTACCTACTGCTTTTCTTT	TATCTATGATGTCATAGAATGTAAG
rs42823610	C/T	GCCATCCAGCCATCTCATCCTCTGT	GTCCCCTTCTCCTCCTGCCCCCAAT
rs42824331	A/G	CATGGGGTCGCTGAGGGTCAGACAC	ACTGAGTGACTTCACTTTCACTTTT
rs42725042	G/T	AGGGGAGAAGGGGACGATAGAGGAT	AGATGGCTGGATGGCATCACTGACT
rs43080446	G/T	TTAAAGGAAAGATTACTTTATACAA	TATAAAGTATTGAAACAATAGTCTA
rs43185776	C/G	TCCTATGTCATCCCCTTCTCCTCCT	CCCTCAATCCCTCCCAGCATCAGAG
rs43178720	A/T	TGTATGTCTGTATGTACAGACATAC	GTGAAATATGTATATATGTACAGAC
rs43449906	G/T	TATATAAAATTGCATTTTAGAAAAC	TAAAGGTGATTAATGCTTTTTAATT
rs43449868	A/G	CCTAGAGCCAGACATCCTGGAATGC	AAGTCAAGTGGGCCTTAGGAAGCAT
rs43448433	A/G	ATTGAAGAATCTCTTTCTATATTCT	AATATTCTTAGTTTTCACATCCCCC
rs42940872	C/G	ATACAGCCAAAGGCTTTAGCAAAGT	ATGAAGCAGAAGTGTATGATTTTCT
rs43130086	A/G	AACTTAGGTGAGCTGAGGGGGCTGA	GGAAATCCACACAAGTCGCCCATGA
rs43444877	A/G	TCTGAAGAGTTCTTATCCCAAGAAA	AAAATTTTTTTTCTATTTCTTTAAT
rs43445941	G/T	AAACTCCAATACTTTGACCACCTGA	GCAAAGAACTGACTCATTAGAAAAA
rs43445971	A/G	TACATTTAGAACTGCTTACTTTCAT	TAAGTTCTTATGTAACACATAGATT
rs42900433	G/T	CTGACTCTTGGCGATCCCATGGACT	TAGCATACCAGGCTCCTCTGTCCAT
rs42961881	C/T	TGAATGCAACACTTTAACAGCATCA	CTTTAGTATTTGAAATAGCTCAGCT
rs42725037	A/G	ATATATGTTCCTTAAGAAACAAAAA	TAGACCTACCATATGTAATCTTGCA
rs43452444	C/T	AAGAAAAGGCAGTGTGCAAACAGGG	GTGAGCCACGTGAGAGAGAAGGTCG

RESULTS

Analyses of SNPs revealed significant genotypic effects for growth traits, and carcass traits in both QTL regions. When evaluating growth traits, multiple SNP were significantly associated with BW, WW and HH as shown in Table 4. Specifically, four SNP (rs41595713, rs42403565, rs42571566, rs42900130) located on BTA6 and two on BTA20 (rs41931108, rs42480445) were significantly associated (P <0 .05) with BW (Table 4). Animals inheriting the heterozygous (TC, AG) and minor homozygous (CC, GG) allele genotypes from SNP rs41595713, rs42480445, and rs42900130 had significantly ($P < 0.05$) heavier BW than those inheriting the major homozygous allele genotype (Table 5). Animals inheriting the heterozygous (CG, CT, GA) allele genotype from SNP rs41931108, rs42403565 and rs42571566 had significantly heavier BW than those inheriting the major or minor

homozygous allele genotypes (Table 5). Breed was also a significant (P < 0.0001) contributing factor for BW effects with regards to SNP rs42571566 (Table 4).

When evaluating WW, two SNP located on BTA20 (rs41931108, rs42524450) and one SNP located on BTA6 (rs43451134) were identified as significant (P < 0.05) (Table 4). Animals inheriting the minor homozygous (GG) and major homozygous (CC) allele genotypes from SNP rs41931108 had significantly (P < 0.05) heavier WW than those inheriting the heterozygous allele genotype for this marker (Table 5). Animals inheriting the heterozygous (CT) and minor homozygous (TT) allele genotypes from SNP rs42524450 had significantly (P < 0.05) heavier WW than animals inheriting the major homozygous allele genotype (Table 4). Animals inheriting the major homozygous (TT) allele genotype from SNP rs43451134 had significantly (P < 0.05) heavier WW than animals inheriting the heterozygous and minor homozygous allele genotypes (Table 5). A single SNP marker on BTA6 was identified as being significantly (P < 0.05) associated with HH (Table 4). Animals inheriting the minor (TT) and major (CC) homozygous allele genotypes from SNP rs41626155 had higher (P < 0.05) HH than those inheriting the heterozygous allele genotype (Table 5).

Table 4. Level of significance and frequency of animals from each genotype associated with birth weight, weaning weight and hip height.

Traits	BTA	SNP ID	Allele[4]	Minor Genotype Frequency	Het Genotype Frequency	Major Genotype Frequency	SNP P-value	Breed P-value
BW[1]	6	rs41595713	T/C	28	170	78	0.0128	0.1473
BW	6	rs42403565	C/T	39	131	110	0.0379	0.2833
BW	6	rs42571566	G/A	28	98	124	0.0414	<.0001
BW	6	rs42900130	A/G	5	82	211	0.0438	0.2468
BW	20	rs41931108	C/G	60	122	86	0.0166	0.1875
BW	20	rs42480445	T/C	9	119	178	0.0360	0.1861
WW[2]	6	rs43451134	T/A	38	14	44	0.0471	0.3190
WW	20	rs41931108	C/G	60	122	86	0.0138	0.7168
WW	20	rs42524450	C/T	38	132	74	0.0187	0.5005
HH[3]	6	rs41626155	C/T	15	112	151	0.0033	0.3672

Note: [1]BW=Birth weight; [2]WW=Weaning weight; [3]HH=Hip Height[4]Representation of the major allele is located on the left.

Table 5. Single nucleotide polymorphisms associated with growth traits and least square means estimate comparisons between reported genotypes for birth weight, weaning weight and hip height.

Traits	BTA	SNP ID	Allele[4]	Major Genotype Mean	Het Genotype Mean	Minor Genotype Mean
BW[1]	6	rs41595713	T/C	40.07±0.83^{a}	38.54±0.67^{a}	35.87±1.37^{b}
BW	6	rs42403565	C/T	38.00±1.00^{a}	38.91±0.92^{ac}	41.12±1.23^{bc}
BW	6	rs42571566	G/A	39.99±0.68^{a}	38.92±0.70^{ab}	36.52±1.32^{b}
BW	6	rs42900130	A/G	38.99±0.85^{a}	38.78±1.05^{a}	46.02±2.89^{b}
BW	20	rs41931108	C/G	37.56±0.92^{a}	39.05±0.80^{ab}	40.62±0.98^{b}
BW	20	rs42480445	T/C	39.12±0.72^{a}	38.56±0.99^{a}	44.00±2.20^{b}
WW[2]	6	rs43451134	T/A	258.87±5.73^{a}	290.86±14.04^{b}	272.50±7.04^{ab}
WW	20	rs41931108	C/G	272.57±12.23^{a}	258.11±11.90^{b}	271.42±12.41^{a}
WW	20	rs42524450	C/T	260.12±8.92^{a}	263.40±8.43^{a}	281.29±9.88^{b}
HH[3]	6	rs41626155	C/T	113.86±0.78^{a}	112.33±0.80^{b}	116.36±1.44^{a}

Note: ^{a,b} Differing superscripts indicate a difference of means at P < 0.05 within rows; [1]BW=Birth weight; [2]WW=Weaning weight; [3]HH=Hip Height; [4]Representation of the major allele is located on the left.

When evaluating carcass traits, multiple SNP were significantly associated with HCW, YG, MS and REA as shown in Table 6. A total of four SNP, three located on BTA6 (rs42900130, rs42961882 and rs43446022) and one located on BTA 20 (rs41931108), were significantly associated with HCW (Table 4.5). Animals inheriting the major homozygous (AA, TT, GG) allele genotype from SNP rs42900130 rs42961882 and rs43446022 had significantly (P < 0.05) heavier HCW than those inheriting the heterozygous and minor homozygous allele genotypes (Table 7) Animals inheriting the minor homozygous (GG) allele genotype from rs41931108 had significantly (P < 0.05) heavier HCW than those inheriting the heterozygous and major homozygous allele genotypes (Table 7). A single SNP located on BTA20 was significantly (P < 0.05) associated with YG (Table 4.5). Animals inheriting the heterozygous (TC) and minor homozygous (CC) allele genotypes from SNP rs41595713 had a significantly (P < 0.05) higher YG than animals inheriting the major homozygous allele genotype (Table 7).

A single unique SNP located on BTA6 (rs41653357) and another unique SNP located on BTA 20 (rs43036576) were significantly (P < 0.05) associated with MS (Table 6). Animals inheriting the heterozygous (AC) and major homozygous (AA) allele genotypes from SNP rs41653357 had significantly (P < 0.05) greater MS than animals inheriting the minor homozygous allele genotype (Table 7). Animals inheriting the major homozygous (AA) allele genotype from SNP rs43036576 had significantly (P < 0.05) greater MS than animals inheriting the heterozygous and minor allele genotypes (Table 7). A single SNP marker located on both BTA6 (rs42823614) and BTA20 (rs42512588) was significantly (P < 0.05) associated with REA (Table 6). Animals inheriting the major homozygous (CC, AA) allele genotype from SNP rs42512588 and rs42823614 had significantly (P < 0.05) larger REA than those inheriting the heterozygous and minor homozygous allele genotypes (Table 7). Breed effects were also a significant (P < 0.0001) contributing factor for REA when evaluating rs42512588 and rs42823614 (Table 6).

Table 6. Level of significance and frequency of animals from each genotype associated with hot carcass weight, yield grade, marbling score and rib eye area.

				Minor	**Het**	**Major Genotype**	**SNP**	**Breed**
Traits	BTA	SNP ID	Allele[5]	Genotype Frequency	Genotype Frequency	Frequency	P-value	P-value
HCW[1]	6	rs42900130	A/G	5	82	211	0.0234	0.1176
HCW	6	rs42961882	T/A	29	115	118	0.0223	0.1624
HCW	6	rs43446022	G/C	19	48	67	0.0015	0.0174
HCW	20	rs41931108	C/G	60	122	86	0.0368	0.0426
YG[2]	20	rs41595713	T/C	28	170	78	0.0226	0.051
MARB[3]	6	rs41653357	A/C	31	98	115	0.0261	0.4872
MARB	20	rs43036576	A/G	24	112	150	0.0369	0.3932
REA[4]	6	rs42823614	A/C	3	50	253	0.0131	<.0001
REA	20	rs42512588	C/T	48	154	104	0.0414	<.0001

Note: [1]HCW=Hot carcass weight; [2]YG=Yield grade; [3]MARB=Marbling score; [4]REA=Rib eye area; [5]Representation of the major allele is located on the left.

Table 7. Single nucleotide polymorphisms associated with carcass traits and least square means estimate comparisons between reported genotypes for hot carcass weight, yield grade, marbling score and rib eye area.

Traits	**BTA**	**SNP ID**	**Allele[5]**	**Major Genotype Mean**	**Het Genotype Mean**	**Minor Genotype Mean**
HCW[1]	6	rs42900130	A/G	357.94±4.80^{a}	343.70±6.04^{b}	374.37±24.89^{ab}
HCW	6	rs42961882	T/A	362.11±4.93^{a}	347.47±4.95^{b}	352.03±8.46^{ab}
HCW	6	rs43446022	G/C	343.27±5.38^{a}	369.25±6.43^{b}	360.39±9.08^{ab}
HCW	20	rs41931108	C/G	357.28±5.39^{ab}	348.76±4.45^{a}	364.84±5.66^{b}
YG[2]	20	rs41595713	T/C	2.330±0.111^{a}	2.182±0.092^{a}	1.806±0.183^{b}
MARB[3]	6	rs41653357	A/C	447.75±13.67^{a}	423.31±13.77^{b}	407.99±19.27^{b}
MARB	20	rs43036576	A/G	416.90±12.60^{a}	443.71±13.21^{b}	428.65±18.77^{ab}
REA[4]	6	rs42823614	A/C	87.52±0.90^{a}	81.98±1.84^{b}	82.46±5.55^{ab}
REA	20	rs42512588	C/T	88.24±1.27^{a}	85.08±1.07^{b}	89.05±1.98^{ab}

Note: $^{a, b}$ Differing superscripts indicate a difference of means at P < 0.05 within rows; [1]HCW=Hot carcass weight; [2]YG=Yield grade; [3]MARB=Marbling score; [4]REA=Rib eye area; [5]Representation of the major allele is located on the left.

DISCUSSION

A total of ten unique SNP located on BTA6 were significantly (P < 0.05) associated with growth, and carcass traits. Six out of the ten unique SNP were significantly associated with growth traits including BW, WW and HH. These results are in agreement with reports that identified significant SNP for BW and WW on BTA6 (Lu et al., 2013). Previous reports also identified SNP located on BTA6 significantly associated with HH which agrees with the study herein (Bolormaa et al., 2014).

Four SNP located on BTA6 were identified as being significantly associated with carcass traits including HCW, MS and REA. These results were in agreement with reports that identified significant SNP for HCW on BTA6 and a second report that identified significant SNP associated with REA located on BTA6 (Lu et al., 2013; Casas et al., 2000). Previous reports also identified significant SNP for MS located on BTA 6 (Lee et al., 2012), which is in agreement with the results presented in the present study. The current study identified no significantly associated SNP for YG located on BTA6. Furthermore, it was previously reported that significant markers

associated with BF were identified on BTA 6 (Li et al., 2004), however, the current study did not identify any significant SNP associated with BF on BTA6.

Of the ten unique SNP identified on BTA6, two were significantly associated with more than one trait in the current study. Marker rs42900130 was significantly ($P < 0.05$) associated with BW and HCW. Furthermore, marker rs42823614 was significantly ($P < 0.05$) associated with REA and was also identified as an SNP significantly associated with incidence of BRD in previous studies (Miller et al., 2016).A total of six unique SNP located on BTA20 were significantly ($P < 0.05$) associated with growth traits, carcass traits and incidence of BRD. Three of the eleven unique SNP were significantly associated with growth traits including BW and WW. These results are in agreement with previous reports that identified significant QTL regions associated with BW and WW on BTA20 (Saatchi et al., 2014). However, the current study failed to validate previous reports that identified SNP on BTA 20 significantly associated with HH (Bolormaa et al., 2014).

Three SNP identified in the current study located on BTA20 were significantly associated with carcass traits including HCW, YG, MS and REA. These results are in agreement with reports that identified significant QTL regions on BTA20 associated with HCW (McClure et al., 2010). The study herein is also in agreement with reports that identified significant SNP for YG, MS and REA located on BTA20 (Saatchi et al., 2014; Garcia et al., 2010). The current study was not in agreement with reports that previously identified SNP on BTA20 that were significant for BF (Garrett et al., 2008).

Of the six unique SNP identified on BTA20, three were significantly associated with more than one trait. Marker rs41595713 was significantly ($P < 0.05$) associated with BW and YG. Marker rs41931108 was significantly ($P < 0.05$) associated with BW, WW and HCW. Furthermore, marker rs42512588 was significantly ($P < 0.05$) associated with REA in the current study, which was also one of the markers identified as significantly associated with incidence of BRD in a previous study (Miller et al., 2016).

Although, several SNP markers located on BTA6 and BTA20 were identified as significantly associated with a variety of economically important traits, two SNP were significantly associated with both REA and BRD incidence on BTA6 and 20 (Miller et al., 2016). These preliminary results verified the initial hypothesis that SNP cold be significant for a variety of traits in a single QTL region and that single SNP could have potential effects on multiple traits. Furthermore, results from the current study would indicate that these two QTL regions located on BTA6 and 20 warrant further investigation to identify SNP significantly associated with multiple economically important traits in beef cattle. Although multiple SNP were identified in the current study, additional experimentation utilizing larger populations of crossbred steers validating markers reported herein and many more markers needs to be conducted prior to implementation into marker assisted selection programs. Additionally, SNP location and function need to be evaluated to determine if the SNP is located on a functional portion of a gene or is being inherited due to genetic linkage because of close genomic proximity to a causative SNP.

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