Polymorphisms in positional candidate genes on BTA14 and BTA26 affect carcass quality in beef cattle

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ABSTRACT: Several studies have reported the presence of carcass quality QTL on BTA14 and BTA26, with no specific genes being conclusively linked as their cause. The aim of this study was to identify polymorphisms in genes known to affect lipid metabolism in other species and to assess their association with carcass quality traits. Two genes located on BTA14, 2,4 dienoyl CoA reductase 1 (DECR1) and core binding factor, runt domain, α subunit 2, translocated to 1 gene (CBFA2T1), have been previously evaluated in other species and found to contain polymorphisms influencing lipid metabolism. A gene on BTA26, fibroblast growth factor 8 (FGF8), has in recent studies been linked to several QTL affecting obesity in mice, indicating its potential for regulating adiposity in other species. Sequencing analysis identified 9 polymorphisms in DECR1, 4 in CBFA2T1, and 4 in FGF8. Multiple sequence alignment of DECR1 among cattle, humans, and mice showed that 4 of these mutations lie in conserved regions across these species. Using 464 Angus, Charolais, and crossbred animals produced associations with ultrasound marbling score (CBFA2T1, P = 0.019), ultrasound backfat (DECR1, P = 0.012), carcass backfat (FGF8, P = 0.004), and lean meat yield (FGF8, P = 0.005). Quantitative trait loci analysis including a set of previously genotyped markers on BTA14, and 1 DECR1 polymorphism resulted in several significant QTL peaks: ultrasound backfat (UBF) at 91 cM, lean meat yield at 86 cM, carcass gradefat at 15 cM, and yield grade at 87 cM, all at the P < 0.05 level. Using DECR1 as a genetic covariate removed the UBF QTL, indicating that this SNP was contributing to the variation observed in UBF. A similar analysis was performed on BTA26 using 1 of the FGF8 polymorphisms. Results showed significant peaks for lean meat yield at 2 cM and for yield grade at 25 cM, both at P < 0.01, and for carcass backfat at 25 cM (P < 0.05). Removal of FGF8 SNP in further analysis resulted in the disappearance of the carcass backfat QTL. These results suggest that polymorphisms discovered in DECR1, CBFA2T1, and FGF8 may play a role in the lipid metabolism pathway affecting carcass quality traits in beef cattle. However, further studies are needed to confirm that these polymorphisms are responsible for the differences observed in carcass quality in beef cattle.

Key words: Bos taurus, bovine chromosome 14, bovine chromosome 26, core binding factor alpha domain 2,2,4 dienoyl CoA reductase 1, fibroblast growth factor 8

INTRODUCTION

Identification of polymorphisms associated with economically relevant traits in cattle is crucial for understanding the mechanisms underlying their genetic variation. Chromosomes known to harbor carcass quality trait QTL such as BTA14 and BTA26 (Stone et al., 1999; Moore et al., 2003; Casas et al., 2004; Mizoshita et al., 2005) are primary sites for the presence of functionally important genes affecting lipid metabolism.

Among important genes on BTA26 lies the fibroblast growth factor 8 (FGF8). Because of its consistent links to obesity QTL in mice, it was suggested that it might act as a master regulator or interacting element controlling multiple genes that contribute to adiposity in mice (Stylianou et al., 2006), indicating its potential for regulating adiposity in other species.

Two genes on BTA14 have been linked to effects on lipid metabolism in other species: 2,4 dienoyl CoA reductase 1 (DECR1; Amills et al., 2005) and core binding factor, runt domain, α subunit 2; translocated to 1 gene (CBFA2T1; Wolford et al., 1998). In pigs,
DECR1 mapped under a linoleic QTL located on chromosome 4 (Perez-Enciso et al., 2000), and sequencing analysis identified 2 SNP showing associations with linoleic content (Amills et al., 2005). In 2006, CBFA2T1 was part of the human obesity map (Rankinen et al., 2006) for being associated with fat percentage in studies in Pima Indian males (Wolford et al., 1998).

The association between these genes and lipid metabolism in other species make them plausible candidates when searching for associations in cattle and perhaps contributing to carcass quality QTL peaks observed on BTA14 and BTA26. The objectives of this study were to identify polymorphisms in candidate genes previously reported to affect lipid metabolism in other species and to evaluate their associations with carcass production traits in cattle.

MATERIALS AND METHODS

Animals used in the study were cared for according to the guidelines of the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993).

Animals and Management

Four hundred sixty-four steers from 28 half-sib families from an experimental line of Angus, Charolais, or Alberta Crossbred Bulls and the University of Alberta’s Crossbred dam line described previously by Nkrumah et al. (2004) were used in this study. The dam line was produced from crosses among 3 composite cattle lines, namely beef synthetic 1, beef synthetic 2, and dairy X beef synthetic. Beef synthetic 1 was composed of 33% each of Angus and Charolais, approximately 20% Galloway, and the remainder from other breeds. Beef synthetic 2 was comprised of approximately 60% Hereford and 40% other beef breeds. The dairy X beef line was composed of approximately 60% dairy breeds (Holstein, Brown Swiss, or Simmental) and 40% beef breeds, mainly Angus and Charolais (Goonevardene et al., 2003). The animal test diets were the same for yr 2 and 3, but differed in yr 1 with the substitution of barley and oat grain with dry-rolled corn due to a shortage of feed barley in that particular year; however, both diets had similar ME content. Briefly, the test diet for yr 1 contained 80% dry-rolled corn, 13.5% alfalfa hay pellets, 5% feedlot supplement (32% CP beef supplement), and 1.5% canola oil. Year 2 and 3 diets contained 64% barley grain, 20% oat grain, 9% alfalfa hay pellets, 5% beef feedlot supplement, and 1.5% canola oil. Details of the diet for these animals have been described in Nkrumah et al. (2004).

Traits Analyzed

Ultrasound and carcass merit data were collected on beef steers over a period of 3 yr (November 2002 to June 2005). Carcass traits were evaluated according to the Canadian beef carcass grading system (Agriculture Canada, 1992). Carcass and ultrasound measurements have been described previously by Nkrumah et al. (2004). Briefly, ultrasound measurements of 12th-13th rib fat depth (UBF), LM area (ULMA), and marbling score were obtained with an Aloka 500V real-time ultrasound with a 17-cm, 3.5-MHz linear array transducer (Overseas Monitor Corp. Ltd., Richmond, British Columbia, Canada) at 28-d intervals according to procedures described by Brethour (1992). After these tests, animals were shipped to a commercial plant and carcass grade fat (GRFAT), carcass backfat (CBF), and LM area (LMA) measurements were collected at the 12th/13th rib after a 24-h chill at −4°C. Ultrasound and carcass marbling score are a measure of intramuscular fat, being classified as 1 to <2 units = trace marbling (Canada A quality grade); 2 to <3 units = slight marbling (Canada AA quality grade); 3 to <4 units = small to moderate marbling (Canada AAA quality grade); and ≥4 units = slightly abundant or more marbling (Canada Prime). Lean meat yield (LMY) is an estimate of saleable meat calculated according to Jones et al. (1984). Yield grade (YGRADE) classes are based on the proportion of lean meat and are classified as YGRADE1 > 59%, YGRADE2 = 54 to 59%, and YGRADE3 < 54%.

DNA Isolation and Genotyping

A 10-mL blood sample from 464 steers and their sires was collected by jugular venipuncture and kept at 4°C or −80°C until DNA extraction. Genomic DNA was subsequently extracted using a standard saturated salt phenol/chloroform procedure (Miller et al., 1988). Sequences from DECR1 (gene ID: LOC509952), CBFA2T1 (gene ID: LOC538628), and FGF8 (gene ID: LOC326284) were blasted to the bovine genome assembly using the NCBI BLAST (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9913) tool to design primers for both intronic and exonic regions of those genes. Primer design for CBFA2T1, DECR1, and FGF8 sequences was carried out using primer3 (http://frodo.wi.mit.edu/) with the following settings (min opt max): primer size: 22 24 26; primer tm: 58 60 62; primer GC%: 40 50 60, respectively. Genomic DNA was amplified using standard PCR conditions. The PCR products were subjected to a clean-up stage consisting of an equal mixture of Exonuclease I and Shrimp Alkaline Phosphatase (2/1 concentration) enzymes (Invitrogen, Carlsbad, CA) for 15 min at 37°C and 15 min at 85°C. Clean PCR products were sequenced using BigDye-terminator chemistry (Applied Biosystems, Norwalk, CT) and a 3730 DNA sequencer (Applied Biosystems). Genotyping of microsatellites was performed by automated fragment analysis using an ABI PRISM 3730 DNA sequencer (Applied Biosystems). Genotyping of SNP was carried out using the Illumina GoldenGate assay on the BeadStation 500G Genotyping System (Illumina Inc., San Diego, CA).
Table 1. Summary of DECR1, CBFA2T1, and FGF8 genes including SNP database (dbSNP), nucleotide position, and SNP alleles

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>Gene name</th>
<th>BTA</th>
<th>GenBank accession No. and base position (Btau_3.1)</th>
<th>Type of mutation</th>
<th>Minor allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ss95214665:C&gt;T</td>
<td>CBFA2T1</td>
<td>14</td>
<td>NW_001502787.1 – 83885 Intron 0.07 (C)</td>
<td>Intron</td>
<td>0.07 (C)</td>
</tr>
<tr>
<td>ss95214666:G&gt;T</td>
<td>CBFA2T1</td>
<td>14</td>
<td>NW_001502787.1 – 84976 Intron 0.15 (G)</td>
<td>Intron</td>
<td>0.15 (G)</td>
</tr>
<tr>
<td>ss95215669:G&gt;T</td>
<td>CBFA2T1</td>
<td>14</td>
<td>NW_001493259.1 – 665097 Intron 0.14 (G)</td>
<td>Intron</td>
<td>0.14 (G)</td>
</tr>
<tr>
<td>ss95214676:C&gt; G</td>
<td>DECR1</td>
<td>14</td>
<td>NW_001493259.1 – 733746 Intron 0.28 (C)</td>
<td>Intron</td>
<td>0.28 (C)</td>
</tr>
<tr>
<td>ss95214679:C&gt;G</td>
<td>DECR1</td>
<td>14</td>
<td>NW_001493259.1 – 733467 Intron 0.45 (C)</td>
<td>Isoleucine to valine 0.49 (T)</td>
<td>0.49 (T)</td>
</tr>
<tr>
<td>ss95214678:G&gt;T</td>
<td>DECR1</td>
<td>14</td>
<td>NW_001493259.1 – 733748 Intron 0.43 (T)</td>
<td>Alanine to alanine 0.49 (T)</td>
<td>0.49 (T)</td>
</tr>
<tr>
<td>ss95214579:A&gt;G</td>
<td>DECR1</td>
<td>14</td>
<td>NW_001493259.1 – 733639 Intron 0.49 (A)</td>
<td>Valine to methione 0.37 (T)</td>
<td>0.37 (T)</td>
</tr>
<tr>
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<td>DECR1</td>
<td>14</td>
<td>NW_001493259.1 – 733659 Intron 0.49 (C)</td>
<td>Intron</td>
<td>0.49 (C)</td>
</tr>
<tr>
<td>ss95214673:A&gt;T</td>
<td>DECR1</td>
<td>14</td>
<td>NW_001493259.1 – 733639 Intron 0.49 (A)</td>
<td>Intron</td>
<td>0.49 (A)</td>
</tr>
<tr>
<td>ss95214674:G&gt;T</td>
<td>FGF8</td>
<td>26</td>
<td>NW_001494359.1 – 166776 Glycine to glycine 0.14 (T)</td>
<td>Glutamic acid to lysine 0.37 (G)</td>
<td>0.37 (G)</td>
</tr>
<tr>
<td>ss95214675:A&gt;G</td>
<td>FGF8</td>
<td>26</td>
<td>NW_001494359.1 – 166804 Glycine to glycine 0.14 (T)</td>
<td>Glutamic acid to lysine 0.37 (G)</td>
<td>0.37 (G)</td>
</tr>
<tr>
<td>ss95214676:C&gt;G</td>
<td>FGF8</td>
<td>26</td>
<td>NW_001494359.1 – 167062 Intron 0.23 (C)</td>
<td>Intron</td>
<td>0.23 (C)</td>
</tr>
<tr>
<td>ss95214677:C&gt;G</td>
<td>FGF8</td>
<td>26</td>
<td>NW_001494359.1 – 170040 Intron 0.01 (C)</td>
<td>Glycine to arginine 0.01 (C)</td>
<td>0.01 (C)</td>
</tr>
</tbody>
</table>

1 CBSFA2T1 = core binding factor, runt domain, α subunit 2; translocated to, 1 gene; DECR1 = 2,4 dienoyl CoA reductase 1; FGF8 = fibroblast growth factor 8.

### Sequencing Analysis

After sequencing analysis was performed on test animals, sequence products were blasted to the bovine sequence assembly Btau_3.1 using the NCBI BLAST tool to verify that the correct gene sequences were being analyzed. Multiple sequence alignment for humans, mice, and bovine sequences was performed using the online tool ClustalW2 (available at http://www.ebi.ac.uk/Tools/clustalw2/index.html).

### Quantitative Trait Loci Analysis

A total of 75 SNP markers and 3 microsatellites were used for QTL analysis on BTA26, in addition to ss95214675:A>G FGF8 SNP. Another QTL analysis was performed on BTA14 using 112 SNP markers and 11 microsatellites, in addition to ss95214671:C>T DECR1 SNP and ss95215669:G>T CBFA2T1 SNP (Supplemental Table 1; http://jas.fass.org/content/vol87/issue8/). Markers on both chromosomes were selected because they showed the greatest number of heterozygous sires and minor allele frequency of 0.14 (CBFA2T1) and 0.37 (DECR1 and FGF8; Table 1). Marker locations across both chromosomes were obtained by Snelling et al. (2007). Gene SNP locations were estimated by analyzing the location of their flanking markers. Both DECR1 and CBFA2T1 blasted between the same markers, and, therefore, were given similar centimorgan estimates of approximately 92.7 cM on BTA14. The FGF8 gene was estimated to be around 27.0 cM on BTA26.

The total number of animals genotyped in our study was 464 belonging to 28 families. However, 396 animals belonging to 20 half-sib families (range 10 to 56 progeny) were used for QTL analysis. The remaining animals belonged to families with less than 2 animals and therefore were not utilized for the purpose of QTL analysis. The QTL analysis performed in this study used the multiple marker interval mapping approach described by Knott et al. (1996). The conditional probabilities that a calf inherited the first allele of a putative QTL from its sire were obtained from QTL Express (Seaton et al., 2002), which uses the information from the closest informative flanking markers at 1-cM intervals. This analysis is similar to that used by de Koning et al. (1999) in which the conditional probabilities of inheriting the sire allele were nested within half-sib families. This is because not only the linkage phase between a marker and a QTL can differ between families, but also because not all sires are heterozygous for the QTL. In addition, sire effects were also included as random effects (Nagamine and Haley, 2001; Nkrumah et al., 2007; Van Eenennaam et al., 2007). The conditional probabilities from QTL express were input into SAS (SAS Inst. Inc., Cary, NC), and QTL analysis was performed using the mixed model described by

\[
Y = X\beta + Gs + Q\alpha + e
\]

where \(Y\) is a vector of observations on the progeny of each sire, \(X\) is the known incidence matrix relating observations to their fixed effect levels, \(\beta\) is the vector of fixed effects (breed, test batch, and age), \(G\) is the known incidence matrix relating observations to random sire effects, \(s\) is the vector of random additive polygenic effects of sires, \(Q\) is a vector of the conditional probabilities, at each interval, that a calf inherited the first allele of a putative QTL from a sire, \(\alpha\) is the regression coefficient corresponding to the fixed allele substitution effect for a putative QTL within half-sib families. Significance thresholds at 5 and 1% were determined using
25,000 permutation tests in SAS by randomly shuffling the phenotypic records of the 396 animals and maintaining the QTL probabilities unchanged, according to the procedure described by Nkrumah et al. (2007). The permutation procedure was carried out for when the SNP were included or excluded from the QTL analysis. Exclusion of SNP from the analysis was carried out by removing the SNP genotypes of all animals and obtaining new conditional probabilities for each calf according to procedures described above. The reported permutation threshold was an average between the thresholds for each trait when analyzed separately. The difference between them was at most 0.02, which was not enough to modify the significance of the QTL after the average was calculated. The same was performed for the inclusion and exclusion of the candidate gene SNP.

**Association Analysis**

Associations of the genotypes for each polymorphism and carcass merit were analyzed by regressing phenotypes on genotypes using the MIXED procedure of SAS. Four hundred and sixty-four animals were available for this analysis. The statistical analyses model included fixed effects of SNP genotype, test batch, breed, and age of animal at the beginning of the test, and random effects of sire of animal. Allele substitution effect was calculated by regressing phenotypes on the number of copies of one allele for each SNP using the mixed model procedure in SAS.

**False Discovery Rate**

A false discovery rate procedure was applied to our analysis to minimize false positives according to procedures described previously (Benjamini and Hochberg, 1995; Weller et al., 1998). Briefly, the procedure takes into consideration the number of tests performed, the ranking of the markers within the analysis, and their significance (P-value) rank from least to greatest. Because FDR assumes independence between traits and because these traits are correlated, FDR was calculated within each trait according to the formula

$$FDR = \frac{n \times P(k)}{k},$$

where \( k \) is the ranking of each marker, \( P \) is the P-value associated with the marker, and \( n \) is the number of markers analyzed.

**RESULTS AND DISCUSSION**

**Polymorphisms Detected**

In total, 4 polymorphisms were detected in intronic regions of CBFA2T1. Single nucleotide polymorphisms detected in the CBFA2T1 gene were initially blasted against the bovine sequence assembly mRNA reference sequence, with none of the SNP blasting to this mRNA sequence. Initial blasting analysis of the first 2 SNP showed sequence complementarity to an unknown contig (NW_001502787.1). However, when the same sequence segment that did not contain the SNP was blasted to the mRNA reference sequence, this sequence blasted to exonic regions, implying that our sequence was indeed part of the CBFA2T1 gene. It is known that there are still segments in BTA14 that are showing sequence complementarity to multiple regions or to unknown regions in the latest sequence assembly (Marques et al., 2007). To confirm that, in fact, our sequences were misassigned, CBFA2T1 mRNA (NW_001099385) sequence was blasted to the genomic sequence. Parts of the mRNA sequence were blasted to the same unassigned contig where the first 2 SNP blasted, suggesting that the SNP resided in the CBFA2T1 gene.

Sequencing analysis of DECR1 identified 9 SNP. Sequences were blasted to the bovine sequence assembly to determine if they were coding or noncoding mutations. Four of the SNP were blasted on exonic regions of DECR1. Two of these SNP were shown to change the AA constitution of DECR1: isoleucine to valine and valine to methionine substitutions, whereas the other 2 were silent mutations (Table 1). Multiple sequence alignment between human, mouse, and bovine DECR1 showed that all 4 AA changing polymorphisms are found in conserved sequence regions across those species (Figure 1).

Sequencing results of FGF8 detected 4 SNP. One of the SNP was intronic and the other 3 exonic. Two of the exonic SNP produced AA substitutions (glycine to arginine and glutamic acid to lysine). Only 1 exonic SNP produced a silent mutation (glycine to glycine). Table 1 summarizes the list of SNP, including the allele frequency and location in the genome. Multiple sequence alignment between human, mouse, and bovine FGF8 showed that none of the SNP reported are part of the conserved sequence across those species (data not shown).

**Association Analysis**

Single locus association analysis for CBFA2T1 showed that 2 out of the 4 SNP in that gene were associated with at least 1 carcass quality trait. The ss95214667:C > G was found to be significant \( P = 0.012 \) with ULMA and ss95215669:G > T with UBF \( P = 0.019 \) and ULMA \( P = 0.006 \). Table 2 lists the trait estimates, overall P-value, and the allele substitution effects for significant SNP.

The same regression analysis was performed for DECR1. Results showed associations for 6 of the 9 SNP with UBF \( P = 0.010 \) to \( P = 0.026 \); Table 2. Association tests were also performed on other carcass traits, with no significant levels being achieved (data not shown). Other significant associations were observed between 2 of the SNP in FGF8 and other carcass traits. For example, ss95214675:A > G showed significant as-
associations with LMY ($P = 0.005$), CBF ($P = 0.004$), GRFAT ($P = 0.011$), and LMA ($P = 0.005$), whereas ss95214676:C > G presented significant associations with UBF ($P = 0.048$), GRFAT ($P = 0.033$), LMY ($P = 0.042$), and LMA ($P = 0.005$).

This study evaluated 17 SNP across 9 traits and detected 16 significant associations for the fixed effects model and 9 significant associations for the allele substitution model, both at $P < 0.05$. Because the carcass quality traits used in this study were correlated, the most appropriate procedure was to perform FDR tests within each trait, as presented in Table 2. It is important to note that the candidate genes presented here were selected based on their known functions and evidence of links to lipid metabolism pathways.

Minor allele frequencies of the SNP identified ranged from 0.28 to 0.49 for $DECR1$, 0.07 to 0.15 for $CBFA2T1$, and 0.01 to 0.37 for $FGF8$.

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Table 2. Estimates of allele substitution effect for meat quality in beef cattle for SNP on core-binding factor, runt domain, α subunit 2; translocated to 1 ($CBFA2T1$), mitochondrial 2,4 dienoyl CoA reductase 1 ($DECR1$), and fibroblast growth factor 8 ($FGF8$).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>BTA</th>
<th>Trait1</th>
<th>Fixed effect</th>
<th>Allele substitution effect2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall $P$-value3</td>
<td>$FDR$4</td>
</tr>
<tr>
<td>ss95215669:G &gt; T</td>
<td>$CBFA2T1$</td>
<td>14</td>
<td>UMAR</td>
<td>0.019</td>
<td>0.318</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULMA</td>
<td>0.006</td>
<td>0.099</td>
</tr>
<tr>
<td>ss95214667:C &gt; G</td>
<td>$CBFA2T1$</td>
<td>14</td>
<td>ULMA</td>
<td>0.012</td>
<td>0.099</td>
</tr>
<tr>
<td>ss95214758:G &gt; T</td>
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<td>14</td>
<td>UBF</td>
<td>0.016</td>
<td>0.075</td>
</tr>
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<td>UBF</td>
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<td>0.075</td>
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<td>14</td>
<td>UBF</td>
<td>0.026</td>
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<td>ss95214762:C &gt; T</td>
<td>$DECR1$</td>
<td>14</td>
<td>UBF</td>
<td>0.013</td>
<td>0.075</td>
</tr>
<tr>
<td>ss95214763:A &gt; T</td>
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<td>14</td>
<td>UBF</td>
<td>0.010</td>
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<tr>
<td>ss95214765:A &gt; G</td>
<td>$FGF8$</td>
<td>26</td>
<td>LMY</td>
<td>0.005</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CBF</td>
<td>0.004</td>
<td>0.070</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GRFAT</td>
<td>0.011</td>
<td>0.281</td>
</tr>
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<td>$FGF8$</td>
<td>26</td>
<td>LMY</td>
<td>0.048</td>
<td>0.075</td>
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<td>0.033</td>
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<td>LMY</td>
<td>0.042</td>
<td>0.353</td>
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<tr>
<td></td>
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<td></td>
<td>LMA</td>
<td>0.005</td>
<td>0.084</td>
</tr>
</tbody>
</table>

1UMAR = ultrasound marbling (score), ULMA = ultrasound LM area (cm²), UBF = ultrasound backfat (mm), LMY = carcass lean meat yield (%), CBF = carcass backfat (mm), GRFAT = carcass gradefat (mm), LMA = carcass LM area (cm²).

2Allele substitution effect was calculated by regressing phenotypes on the number of copies of 1 allele for each SNP.

3$P$-value from overall $F$-test.

4False discovery rate (FDR) calculated as $FDR = \frac{n \times P(k)}{k}$, where $k$ is the ranking of each marker when the $P$-values are ranked from least to greatest, $P$ is the $P$-value associated with the marker, and $n$ is the number of markers analyzed (Benjamini and Hochberg, 1995; Weller et al., 1998).

5Estimate of the effect expressed in units of the trait.

6NA = $P$-values were not significant at the $P < 0.05$ level; therefore, $FDR$ was not calculated.

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**Figure 1.** Multiple sequence alignment for 2,4 dienoyl CoA reductase 1 ($DECR1$) among cattle (NP_001068891.1), humans (NP_001350.1), and mice (NP_080448.1) using ClustalW2 online tool (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The dark boxes indicate polymorphism locations as described in Table 1. Symbols: * indicates that the residues in the column are identical across all sequences, : indicates that conserved substitutions were identified, and . indicates that semi-conserved substitutions were identified according to the ClustalW2 online tool.
Figure 2. Across family $F$-statistic profiles for a) lean meat yield (LMY), yield grade (YGRADE), ultrasound backfat (UBF), and grade fat (GRFAT) using 123 markers in addition to ss95214671:C > T 2,4 dienoyl CoA reductase 1 ($DECR1$) SNP on BTA 14. Across family $F$-statistic profiles when including and excluding $DECR1$ from the analysis for (b) yield grade (YGRADE) and (c) ultrasound backfat (UBF). The horizontal line represents the chromosome-wise threshold from 25,000 permutations. Relative $DECR1$ SNP position is indicated by an arrow on the horizontal axis.
Figure 3. Across family $F$-statistic profiles for (a) carcass backfat (CBF), yield grade (YGRADE), and lean meat yield (LMY) using 80 markers in addition to ss95214675:A > G fibroblast growth factor 8 ($FGF8$) SNP on BTA 26. Across family $F$-statistic profiles when including and excluding $FGF8$ from the analysis for (b) YGRADE and (c) CBF. The horizontal line(s) represents the chromosome-wise threshold from 25,000 permutations. Relative $FGF8$ SNP position is indicated by an arrow on the horizontal axis.
duced frequency of minor alleles for some of these SNP, it is expected that some of the associations reported are biased. For instance, ss95215669:G > T has a minor allele frequency of 0.14, meaning that among 464 animals analyzed, 9 are homozygous GG, which could explain the nonsignificant association for this SNP for the allele substitution effect (Table 2).

Single point mutations conferring AA substitutions were further evaluated to indicate what types of interactions with other proteins or receptors are being affected. In the case of DECR1 and FGF8, the encountered AA substitutions are of further interest, because the AA involved have different properties. Substitutions involving AA with similar properties such as isoleucine and valine will most likely not cause major changes to the protein. Valine and isoleucine are hydrophobic and possess an additional nonhydrogen substituent attached to their Cβ carbon. The other substitution, valine to methionine, may have a larger impact. Even though valine and methione are apolar, methionine is less lipophilic because of its thiogroup, which might reduce stability overall. Functional studies can elucidate how these changes in AA sequence will affect the functions of DECR1 and FGF8 in cattle and, in turn, why and how they affect variation in the traits studied.

QTL Analysis

Quantitative trait loci analysis was performed to further validate the effects of the SNP on the traits under study. The objective of this analysis was to determine if the polymorphisms discovered were near a QTL peak, as well as to evaluate any changes in the QTL peak. A QTL scan was performed using a set of 123 markers previously genotyped in our beef population, in addition to 123 DECR1 SNP (see Materials and Methods). This QTL analysis on BTA14 yielded significant results for UBF at 91 cM, LMY at 86 cM, GRFAT at 15 cM, and YGRADE at 87 cM, all at P < 0.05 (Figure 2a). These results are consistent with previously reported QTL analysis on this chromosome (MacNeil and Grosz, 2002; Moore et al., 2003; Casas et al., 2004). In addition, YGRADE and UBF showed the greatest F-value only 2 to 4 cM from DECR1 (Figure 2a and 2b). For YGRADE, the most significant location was 87 cM, whereas for UBF it was at 91 cM (DECR1 location: 92.7 cM). This is consistent with association results between DECR1 and UBF in single locus analysis (Table 2).

When DECR1 was subsequently removed from the QTL analysis, no UBF QTL was present at this location (Figure 2c), providing additional support for the effect of DECR1 SNP on this trait. The same was done for YGRADE, LMY, and GRFAT. In these cases, the QTL peaks shifted to 80 cM (Figure 2b) for YGRADE and to 68 cM (figure not shown) for LMY compared with the previous peaks, with no change for GRFAT. Evaluation of the SNP effect on the UBF QTL included using SNP ss95214671:C > T as a covariate in the analysis. This step resulted in a decrease in threshold for this QTL (F-statistic = 2.03 vs. 1.83), indicating that this term explained some of the QTL variation. When CBFA2T1 SNP were included in the analysis, similar QTL profiles were observed (data not shown). This could also be due to the effects of DECR1 SNP because both genes are relatively close to each other.

A QTL scan was also performed on BTA26 using 78 markers in addition to 1 of the FGF8 SNP (see Materials and Methods). The results showed the presence of peaks for LMY at 2 cM and for YGRADE at 25 cM, both at P < 0.01, and for CBF at 25 cM (P < 0.05; Figure 3a). When FGF8 was subsequently removed from the analysis, LMY was the only one that did not change. A YGRADE QTL was still present, although at a decreased F-value (2.04 compared with 2.51; Figure 3b). No QTL was observed for CBF at this position when the FGF8 SNP was removed (Figure 3c). Using SNP ss95214675:A > G genotype as a fixed effect, along with the most significant QTL position in CBF, resulted in a nonsignificant QTL (P = 0.0524), indicating that the SNP had a significant contribution in this QTL. When SNP were used as covariates for YGRADE and LMY, the QTL significance did not change.

The mitochondrial enzyme encoded by DECR1 participates in the β-oxidation pathway catalyzing the reduction of trans-2-cis-4-dienoyl-CoA to 3-enoyl-CoA (Kunau and Dommes, 1978), and it is therefore an interesting candidate influencing the genetic variation observed in carcass quality. The FGF8 androgen-induced property was first discovered in earlier experiments by Tanaka et al. (1992). That study reported that a mouse mammary carcinoma cell line was stimulated to secrete several FGF when induced by androgens. These FGF, in turn, demonstrated growth-like properties on this carcinoma cell line. Isolation and characterization of the activity determined that FGF8 contributed to some of the growth effects. In humans, it is present in increased concentrations in breast cancer cells (Zammit et al., 2002), and it is therefore an interesting candidate influencing the genetic variation observed in carcass quality. The FGF8 androgen-induced property was first discovered in earlier experiments by Tanaka et al. (1992). That study reported that a mouse mammary carcinoma cell line was stimulated to secrete several FGF when induced by androgens. These FGF, in turn, demonstrated growth-like properties on this carcinoma cell line. Isolation and characterization of the activity determined that FGF8 contributed to some of the growth effects. In humans, it is present in increased concentrations in breast cancer cells (Zammit et al., 2002), and it is therefore an interesting candidate influencing the genetic variation observed in carcass quality. The FGF8 androgen-induced property was first discovered in earlier experiments by Tanaka et al. (1992). That study reported that a mouse mammary carcinoma cell line was stimulated to secrete several FGF when induced by androgens. These FGF, in turn, demonstrated growth-like properties on this carcinoma cell line. Isolation and characterization of the activity determined that FGF8 contributed to some of the growth effects. In humans, it is present in increased concentrations in breast cancer cells (Zammit et al., 2002), and it is therefore an interesting candidate influencing the genetic variation observed in carcass quality.
Nonetheless, there are several pieces of information that point to one of the polymorphisms in DECR1 as a major contributor to the variation observed in backfat thickness. 1) This mutation affects the AA composition (valine to methionine) at a highly conserved region among humans, mice, and cattle. 2) The QTL becomes significant when DECR1 is added to the analysis. 3) The QTL is partially removed when correcting the analysis for the effect of DECR1 SNP ss95214671:C > T. 4).

The DECR1 participates in the β-oxidation pathway reducing trans-2-cis-4-dienoyl to 3-enoyl-CoA (Kuman and Dommes, 1978). It is true that additional analysis including a larger set of SNP needs to be carried out to verify the pattern of linkage disequilibrium under the QTL. Even though the evidence suggests that polymorphisms in DECR1 may play a role in affecting carcass quality traits in beef, these markers could be in linkage disequilibrium with the actual causative mutation. The evidence for FGF8 being a causative mutation is not as strong as for DECR1 because the function of FGF8 is not clearly linked to a lipid metabolism pathway in cattle and its polymorphisms did not affect a conserved region among the 3 species.

Our study has provided additional information to the research community on specific SNP from 3 functional positional candidate genes associated with carcass quality traits in beef cattle. Further analysis, including evaluation on the pattern of linkage disequilibrium near the polymorphisms coupled to functional analysis, will be crucial for determining if these markers are in fact causative mutations.

**LITERATURE CITED**


