Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of *Bos taurus*\(^1\)

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**ABSTRACT:** Backfat thickness is one of the major quantitative traits that affect carcass quality in beef cattle. In this study, we have fine mapped a QTL for backfat EBV on bovine chromosome 14, using an identical-by-descent haplotype-sharing analysis, in a commercial line of *Bos taurus*. We also examined the association between gene-specific single nucleotide polymorphism (SNP) markers of the genes diacylglycerol acyltransferase 1 (*DGAT1*) and thyroglobulin (*TG*) and the backfat EBV. The results indicate that the QTL region for backfat identified on chromosome 14 is in agreement with previous studies. However, neither of the two polymorphisms of candidate genes tested, *DGAT1* nor *TG*, showed a significant \((P > 0.10)\) association with the backfat EBV in the cattle populations examined. However, a strong association \((P = 0.0058)\) was detected between a microsatellite marker (CSSM66) lying approximately mid-way between the two candidate genes and the backfat EBV. These results suggest that other SNP of *DGAT1*, *TG*, or other gene(s) in the chromosomal region should be examined to test whether they have a significant effect on lipid metabolism.

Key Words: Backfat, Cattle, Marker Genes, Quantitative Trait Loci


**Introduction**

Recently, two genes with an effect on lipid metabolism have been mapped to the centromeric region of bovine chromosome (BTA) 14. An allele of the thyroglobulin (*TG*) gene was identified as having a significant association with marbling score (Barendse, 1999). A QTL for fat yield and percentage in milk of dairy cattle was mapped to a similar region on BTA 14 (Coppie et al., 1998; Heyen et al., 1999; Riquet et al., 1999), and a mutation in the diacylglycerol acyltransferase 1 (*DGAT1*) gene was proposed as the causative mutation underlying this QTL (Grisart et al., 2002; Winter et al., 2002). The TG alleles identified by Barendse (1999) were defined based on a single nucleotide polymorphism (SNP) at the 5’ untranslated region (~537bp) of the *TG* gene, the “2” allele being “GATC” and the “3” allele being “GATT.” Animals with the genotype “33” had significantly lower marbling scores than those with genotype “22” or “23” (Barendse, 1999). Diacylglycerol acyltransferase 1 encodes acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis (Cases et al., 1998). The alleles identified by Grisart et al. (2002) represent a dinucleotide substitution (AA/GC) in the beginning of exon VIII (6,829 bp), with allele q being “GC” and allele Q being “AA.” The mutation results in a nonconservative AA substitution of a lysine (allele Q) to an alanine (allele q), which may have a direct impact on the activity of the enzyme (Grisart et al., 2002). Animals carrying the “Q” allele had increased milk fat yield and fat percentage, and the polymorphism explained up to 51% of the total variance of fat percentage in milk. A more recent study by Winter et al. (2002) also confirmed that the lysine-encoding allele was associated with higher milk fat content.

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In this study, we have identified and fine-mapped a QTL for backfat EBV in the same region of BTA 14 in a commercial population of *Bos taurus*. We have also examined the association between the two SNP of TG and *DGAT1* described above and the backfat EBV.

**Materials and Methods**

**Animals and Phenotypic Data**

Animals were from the M1, M3, and TX lines of Beefbooster Inc. (Calgary, Canada) and were born in 1998. The M1 line was developed from an Angus base. The M3 line was developed from small cows of various breeds, and the TX line was a terminal market strain. All three lines have been under selection for over 30 yr. The selection criteria for the lines were based on indices described by MacNeil and Newman (1994). A 10-mL blood sample was collected by venipuncture from each male calf and the potential sires, and the DNA from each blood sample was extracted and kept for later parentage identification. Sire identification was carried out by the Saskatchewan Research Council (Saskatchewan, Canada) using DNA microsatellite markers. The backfat EBV of each male calf was calculated based on a BLUP procedure by Beefbooster Inc.

**Genotyping**

One hundred seventy-six male calves and their 12 respective sires (9 to 30 calves from each sire) of the M1 line were genotyped using nine microsatellite markers from BTA14 and a SNP in each of two genes, *TG* and *DGAT1* (Barendse, 1999; Grisart et al., 2002), spanning approximately 74% of the chromosome. The animals from the 12 sires were chosen on the basis of larger family sizes. The nine microsatellite markers were CSSM66, BMS1747, BMS1678, BMS1941, BMC1207, BM1577, BMS108, BMS1899, and RM137. Primers for genotyping the microsatellite markers were designed based on the information published on the USDA Meat Animal Research Center website (http://www.marc.usda.gov/genome/genome.html). The forward primers were labeled with fluorescent dyes and the genotyping of the microsatellite markers was performed using an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA).

The genotyping of *TG* was carried out as described by Barendse (1999). Briefly, the genomic DNA was amplified using primers TG5U2 (5′ ggg gat gac tac gag tat gag tg 3′) and TG5D1 (5′ tgtg aatc ttt tg agg cttg ta 3′). The PCR products were digested using Mbol (/GATC) (New England Biolabs, Beverly, MA) by incubation at 37°C for 1 h. The fragments were separated on 3% agarose gels (Sigma, St. Louis, MO) by electrophoresis with 1× TBE buffer and stained using ethidium bromide. The genotype of each animal was determined based on the fragment profile, with allele “2” being cut and allele “3” uncut.

The genotyping of the *DGAT1* gene-specific SNP was carried out using an ABI PRISM 7700 sequence detector based on allele discrimination using the 5′ nuclease assay (Applied Biosystems). Briefly, a forward primer (5′ ccg tgtg tttc gta ctttg g 3′) and a reverse primer (5′ ccg cgg tag gtc agg tgg t 3′) were designed to amplify the dinucleotide substitution (AA/GC) region at the beginning of exon VIII based on the sequence of *Bos taurus DGAT1* (GenBank No. AY065621). Two fluorogenic probes were also designed to target the two alleles, with VIC reporter dye for allele q (GC) and FAM reporter dye for allele Q (AA). The sequences of the probes for allele q and allele Q detection were 5′ ccg tgtg gcc gct tt 3′ and 5′ ccg tgtg gcc ttc tta 3′, respectively. A perfect match of a probe sequence to the target sequence will result in the cleavage and release of the reporter dye. Thus, substantial increase in either VIC or FAM dye fluorescence indicates homozygosity for the VIC-specific allele (allele q) or for the FAM-specific allele (allele Q). An increase in both signals indicates heterozygosity. A subset of animals was sequenced across the mutation and the sequence results were used to confirm the genotypes obtained by discrimination assay. In addition to the 176 male calves and their 12 respective sires in the M1 line, 26 additional male calves of six sires from the same M1 line, 174 male calves of 14 sires from the M3 line and 121 male calves of 15 sires from the TX line were also genotyped for *DGAT1*, making the total number of male calves 497 for the single locus association study between the *DGAT1* gene-specific SNP marker and the backfat EBV. Sires were not included in the association analyses but were genotyped in order to verify the allele inheritance of male calves.

**Haplotype Identification and Fine Mapping of QTL for Backfat**

Haplotype identification and fine mapping of QTL for backfat EBV on chromosome 14 was carried out using the identical-by-descent haplotype-sharing analysis as described by Li et al. (2002a,b). Animals from the M3 and TX line, and the 26 additional male calves from the M1 line, were not included in the haplotype analysis since they were only genotyped for *DGAT1*. Genotypes of the microsatellites and the two gene-specific SNP of each of the 176 male calves in the M1 line were checked against the calf’s sire to verify the sire inheritance. Alleles of each locus contributed by the sire as well as by the dam were identified for each calf by examining the genotype of their sires. The haplotypes (allele linkage phases) of each male calf were then established along chromosome 14. The GLM procedure of SAS (SAS Inst., Inc. Cary, NC) was used to test the association between each of the most commonly observed haplotypes and the backfat EBV. The linear model was:

\[ Y_{ij} = \mu + H_i + E_{ij} \]
where \( Y_{ij} \) = the backfat EBV of animal \( j \) for haplotype \( i \), \( \mu \) = overall experimental mean, \( H_i \) = fixed effect corresponding to the haplotype effect under test (1 when the individual has the haplotype or 0 when the individual is without the haplotype), and \( E_{ij} \) = residual error. Because the number of animals carrying two copies of a haplotype was small in the data set, animals carrying two copies of a haplotype were grouped with animals carrying one copy of a haplotype as haplotype class “1.” Animals with uncertain haplotypes were considered to be missing values and were deleted from the analysis. Other identifiable sources of variation, such as herd and age of dam, were not included in the model because their effects were found to be not significant in a preliminary analysis.

Type-III sum of squares was used in all \( F \)-tests. The haplotype effect in SD was estimated by dividing the difference of backfat EBV least squares means between haplotype classes “1” and “0” by the SD of the trait. The comparison- and chromosome-wise thresholds of the \( P \)-value were generated empirically from the permutation method outlined by Churchill and Doerge (1994) and described by Li et al. (2002b). A type-I error of 0.05 and 0.10 was used to calculate comparison- and chromosome-wise \( P \)-value thresholds, respectively.

**Single-Locus Association Analyses Between Gene-Specific SNP of DGAT1, TG and Microsatellite CSSM66, BMS1747, and Backfat**

Single-locus association analysis between the genotype of genes DGAT1 and TG and the backfat EBV were performed using the GLM:

\[
Y_{ij} = \mu + G_i + E_{ij}
\]

where \( Y_{ij} \) = the backfat EBV of animal \( j \) for genotype \( i \), \( \mu \) = overall experimental mean, \( G = \) the fixed effect of animal genotype for TG (22, 23, or 33) or for DGAT1 genotypes (qq, Qq, QQ or qq, Qq+QQ [two genotypes combined]), and \( E_{ij} \) = residual error. For DGAT1, a fixed line effect (M1, M3, and TX) was included in the model for the across-line association analysis. Other identifiable sources of variation, such as herd and age of dam, were not included in the model because their effects were found to be not significant in a preliminary analysis. In addition to the two candidate genes, single-locus association analyses were also carried out for genotypes of a single microsatellite marker that was present in the haplotype showing a significant effect on the backfat EBV. The genotypes of a single microsatellite marker were defined as, for example, 198/198, 198/A, A/A of CSSM66. The allele “198” represented the allele of CSSM66 to be tested. The allele “A” designates all other alleles of the microsatellite marker. The analyses were performed using SAS for each of the alleles with higher frequencies (>8%), and Type-III sum of squares was used in each \( F \)-test.

**Results**

On average, nine alleles were detected for each microsatellite locus of BTA 14 in the M1 line, with a range of 2 to 13 alleles per locus. A haplotype is defined by alleles at adjacent loci along BTA 14. For loci DGAT1 and CSSM66, the haplotype q-198 represents a segment of chromosome having allele q at DGAT1 (DGAT1-q) and allele 198 at CSSM66 (CSSM66-198).

Associations between an individual haplotype and the backfat EBV were only analyzed for the common haplotypes with a frequency of above 8.0%. Among them, two haplotypes, DGAT1-q, CSSM66-198 and CSSM66-198, BMS1747-98, were found to have significant associations with backfat at the comparison-wise \( P \)-value threshold on bovine chromosome 14, and both reached the chromosome-wise \( P \)-value threshold (Figure 1). The two haplotypes were located at the chromosomal region of 5 to 25 cM of BTA 14 and have frequencies of 28.0% and 16.6%, respectively. Haplotype DGAT1-q, CSSM66-198 had a significant negative effect of 0.50 SD on backfat at a \( P \)-value of 0.0051 (Table 1). Haplotype CSSM66-198, BMS1747-98 also had a significant negative effect at a \( P \)-value of 0.0034, decreasing the backfat EBV by 0.89 SD (Table 1).

The association analysis between the DGAT1 gene-specific SNP and backfat EBV showed no significant genotype effects (Table 2). This was confirmed across three independent breeding lines (M1, M3, and TX) from Beefbooster Inc. Pooling the samples across lines also showed that no significant association existed. The association analysis between the TG gene-specific SNP and backfat EBV was also not significant for the genotype effect in the M1 line (Table 2). There was a trend for allele “3” to decrease the backfat EBV; the average backfat EBV for genotype “22,” “23,” and “33” was 0.02321, −0.0459, and −0.0701, respectively (Table 2, Figure 2).

A significant association was found at a \( P \)-value of 0.0058 between allele 198 of the microsatellite marker CSSM66 and the backfat EBV when tested against all other alleles combined in the M1 line (Table 2, Figure 2). The average backfat EBV of the genotype “198/198” and “198/A” were significantly lower than those of other genotypes of CSSM66. No significant association, however, was detected for the marker BM1747.

**Discussion**

The successful application of marker-assisted selection in commercial animal populations will depend on a number of factors. Among these are the ability to identify the genes or closely linked markers to the genes underlying the QTL, the ability to test if allelic variations at these loci are segregating in the population, and an understanding of how these genes interact with the environment or with other genes affecting economic traits. All this must be done in an efficient and cost-
Figure 1. Haplotypes with lowest $P$-values between two adjacent loci along bovine chromosome 14 for backfat EBV in the M1 commercial line of *Bos taurus* from Beefbooster Inc. (Calgary, Alberta, Canada). Haplotypes were defined by two alleles of a pair of loci. For example, haplotype q-198 of diacylglycerol acyltransferase 1 (*DGAT1*) and CSSM66 represented a segment of chromosome having allele q of *DGAT1* and allele 198 of CSSM66. The genetic map distance was indicated in centimorgans. The dashed line represents the comparison-wise $P$-value threshold level, whereas the solid line represents the chromosome-wise $P$-value threshold level.

effective manner in order for the technology to be adopted by the livestock industries.

Identity by descent (IBD) QTL mapping using haplotype sharing has been successfully demonstrated in humans (de Vries et al., 1996; Fallin et al., 2001) and cattle (Riquet et al., 1999; Li et al., 2002a,b). The method takes advantage of linkage disequilibrium in populations with limited outbreeding, in which common chromosome segments are shared by individuals in populations that originated from a few common founders. Thus, chromosome segments that house the QTL can be identified through direct haplotype comparison. This strategy of fine mapping overcomes the limitation of interval-based QTL mapping, which requires large numbers of progeny of a single sire and which may be difficult or costly to implement in domestic animal species.

The feasibility of using haplotype-mapping methods as well as single-locus association analyses depends on the extent of the linkage disequilibrium. Farnir et al. (2000) reported that linkage disequilibrium in a Holstein-Friesian dairy cattle population extended over several tens of centimorgans. In this study, we also observed a level of linkage disequilibrium similar to that in dairy cattle, and some haplotypes between two adjacent markers had much higher frequencies than others in the M1 line (data not shown). Such a phenomenon may be attributed to the introduction of a limited

<table>
<thead>
<tr>
<th>Haplotype$^b$</th>
<th>Haplotype effect, mm$^c$</th>
<th>$F$-statistic</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>DGAT1</em>-q, CSSM66-198</td>
<td>$-0.50$ SD (0.1581)</td>
<td>8.80</td>
<td>0.0051$^{**}$</td>
</tr>
<tr>
<td>CSSM66-198, BMS1747-98</td>
<td>$-0.89$ SD (0.2815)</td>
<td>9.01</td>
<td>0.0034$^{**}$</td>
</tr>
</tbody>
</table>

$^a$Beefbooster, Inc., Calgary, Alberta, Canada.

$^b$The haplotypes were named by two alleles of a pair of loci. For example, haplotype diacylglycerol acyltransferase 1 (*DGAT1*)-q and CSSM66-198 represented a segment of chromosome having allele q of *DGAT1* and allele 198 of CSSM66.

$^c$A negative sign represented the negative effect. The actual haplotype effects on the backfat EBV (mm) were shown in parentheses.

$^{**}$$P$-values that were significant above the chromosome-wise threshold.
Table 2. Average backfat estimated breeding values of diacylglycerol acyltransferase 1 (DGAT1), CSSM66, BMS1747, and thyroglobulin (TG), and tests of genotype effects in commercial lines of Bos taurus.

<table>
<thead>
<tr>
<th>Gene/line</th>
<th>Average backfat EBV of genotype</th>
<th>F-statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGAT1</td>
<td>QQ</td>
<td>0.0637</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>Qq</td>
<td>-0.0824</td>
<td></td>
</tr>
<tr>
<td></td>
<td>qq</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1 line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3 line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX line</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Across lines</td>
<td></td>
<td>0.0680</td>
<td>-0.0262</td>
</tr>
<tr>
<td>CSSM66</td>
<td>198/198</td>
<td>0.0689</td>
<td>-0.0224</td>
</tr>
<tr>
<td></td>
<td>198/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1 line</td>
<td>-0.2801</td>
<td>-0.0791</td>
<td>0.0244</td>
</tr>
<tr>
<td>BM1747</td>
<td>98/98</td>
<td>98/B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>98/B</td>
<td>B/B</td>
<td></td>
</tr>
<tr>
<td>M1 line</td>
<td>-0.1937</td>
<td>0.0105</td>
<td>-0.0110</td>
</tr>
<tr>
<td>TG</td>
<td>22</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>M1 line</td>
<td>0.02321</td>
<td>-0.0459</td>
<td>-0.0701</td>
</tr>
</tbody>
</table>

aBeefbooster, Inc., Calgary, Alberta, Canada.
bAllele “Q” and “q” of DGAT1 represent lysine and alanine, respectively. Allele “2” and “3” of TG represent GATC and GATT, respectively. Allele “A” of CSSM66 and allele “B” of BMS1747 represented other alleles of the two microsatellite markers. N/A, the mean was not available.
c,dTests for differences among means of backfat EBV of different genotypes. Tests of between means of genotype (QQ + Qq) and genotype q for DGAT1 were presented in the parentheses.

**The difference of means is significant at P = 0.01.

Figure 2. Average backfat EBV for genotype QQ, Qq, and qq of diacylglycerol acyltransferase 1 (DGAT1) for the M1, M3, TX, and cross lines (above), genotype 198/198, 198/A, and A/A of CSSM66, genotype 98/98, 98/B, and BB of BMS1747, and genotype 22, 23, and 33 of thyroglobulin (TG) (below). Allele “198” of CSSM66 and allele “98” of BMS1747 are defined in Figure 1. Allele “A” of CSSM66 and allele “B” of BMS1747 represented other alleles of the two microsatellite markers. The standard errors of the means are indicated by bars. The different letters above the bars indicate that the means differ at the 0.05 level.

In our previous studies, we successfully fine-mapped QTL for birth weight, preweaning ADG, and ADG on feed in both the M1 and M3 commercial lines of Beefbooster Inc. using the identical-by-descent haplotype-sharing analysis, and narrowed down some of the QTL regions to less than 10 cM (Li et al., 2002b). The identical-by-descent haplotype-sharing analysis detected the same, but better-defined, QTL regions in comparison with the interval-mapping method (Li et al., 2002a). In addition to the actual phenotypic data, we have also used the birth weight EBV data for QTL fine mapping and found that the QTL regions for birth weight identified using EBV data were in very good agreement with those detected using the primary phenotypic data (Li et al., 2002a,b). In dairy cattle, Winter et al. (2002) also used the breeding values of milk fat content for a QTL mapping and the association study, and confirmed that the dinucleotide substitution (AA/GC) of DGAT1 was significantly associated with milk fat content, as identified by Grisart et al. (2002) using the primary milk fat measurements. This supports the use of EBV data when the primary measurement of a trait is not available.

The QTL for backfat EBV reported in this study supports the QTL for fat depth reported on BTA14, in the region of 10 to 20 cM, by Casas et al. (2000). Two strong candidate genes involved in lipid metabolism are pres-
A significant single-marker association was detected with the backfat EBV at the CSSM66 locus. This marker falls approximately midway between \( DGAT1 \) and \( TG \) on the cattle linkage map (Grisart et al., 2002). Given the strong evidence of a QTL for backfat in this region based on both haplotype analysis and the direct marker trait association of CSSM66, it seems likely that both a gene(s) affecting lipid metabolism is located close to CSSM66. Indeed, the peak of the QTL region for backfat in beef cattle detected in the study by Casas et al. (2000) and in this study, is located approximately 15 cM from the centromere, distal to that reported for the QTL for milk fat in dairy cattle or the \( DGAT1 \) gene (Grisart et al., 2002; Winter et al., 2002). Examination of the comparative maps between cattle and humans (http://bos.cvm.tamu.edu/bovgbase.html) indicates no strong candidate genes yet identified in the region. The human draft sequence (http://genome.ucsc.edu/index.html) shows that the syntenic region of human chromosome 8, midway between \( DGAT1 \) and \( TG \), houses few identified genes. Identifying the gene(s) underlying this QTL will therefore require further gene mapping data, either in cattle or through identification of more genes and their functions in human or mouse.

**Implications**

A quantitative trait locus for backfat in beef cattle has been confirmed and fine-mapped on bovine chromosome 14 in a commercial line of Bos taurus. Gene-specific single nucleotide polymorphisms of two candidate...
genes, diacylglycerol acyltransferase 1 and thyroglobulin, as well as of two microsatellite markers, CSSM66 and BMS1747, underlying the quantitative trait locus region were examined for their associations with backfat. The results should provide a valuable reference for further positional candidate gene research and marker-assisted selection.

**Literature Cited**


