

Identification of genetic markers for fat deposition and meat tenderness on bovine chromosome 5: Development of a low-density single nucleotide polymorphism map^{1,2}

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ABSTRACT: As genetic markers, SNP are well suited for the development of genetic tests for production traits in livestock. They are stable through many generations and can provide direct assessment of individual animal's genetic merit if they are in linkage disequilibrium and phase with functional genetic variation. Bovine chromosome 5 has been shown to harbor genetic variation affecting production traits in multiple cattle populations; thus, this chromosome was targeted for SNP-based marker development and subsequent association analysis with carcass and growth phenotypes. Discovery of SNP was performed in a panel of 16 sires representing two sires from each of seven beef breeds and two Holstein sires by PCR amplification and sequencing using primers designed from genomic sequence obtained by low-coverage sequencing of bacterial artificial chromosome (BAC) clones. From 550 SNP, 296 (54%) were tentatively identified as having a minor allele frequency >10%. Forty-five SNP derived from 15 BAC were chosen based on minor allele frequency and were genotyped in 564 steers and their sires. Production and carcass data were collected on the steers as a part of the Germplasm Evaluation (GPE), Cycle VII Project at the U.S. Meat Animal Research Center (Clay Center, NE), which involves of the evaluation of sires from

seven of the most popular U.S. breeds. Haplotypes based on seven SNP derived from a BAC containing the bovine genes HEM1 and PDE1B were associated with traits related to carcass fat. Steers homozygous for the major haplotype had 0.15 ± 0.04 cm less subcutaneous fat, 0.57 ± 0.18 kg less rib fat, 0.18 ± 0.07 lower yield grade, $1.11 \pm 0.35\%$ less predicted fat yield, and $0.79 \pm 0.3\%$ greater predicted retail product yield than heterozygotes. The frequency of the major haplotype was 0.70 in the steers, and it ranged from 0.44 (Limousin) to 0.98 (Simmental and Gelbvieh) in a panel consisting of an average of 20 purebred sires from each of the seven breeds. A second set of haplotypes based on four SNP derived from a BAC containing the genes NOL1 and CHD4 was associated with Warner-Bratzler shear force. Steers homozygous for the major haplotype had 0.27 ± 0.11 kg greater shear force than those heterozygous for the major haplotype and one of two minor haplotypes. The frequency of the major haplotype was 0.59 in the steers and ranged from 0.27 (Hereford) to approximately 0.95 (Angus and Red Angus) in the panel of purebred sires. These results demonstrate the feasibility of targeting QTL regions for SNP-based marker development and that a low level of coverage can identify markers associated with phenotypic traits.

Key Words: Bovine, Carcass Traits, Haplotype, Single Nucleotide Polymorphism

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Introduction

The initial goals of cattle genome research were to identify QTL affecting production traits and to use ge-

netic markers for marker-assisted selection to increase frequency of favorable QTL alleles in target populations. Most QTL identification studies have been performed in large, half-sib families using microsatellite markers to track sire alleles. Unfortunately, results of these studies have found limited application because of the difficulties of transferring the technology to target

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herds, which requires determination of the presence and phase with respect to markers of QTL alleles in the population. Recent studies in humans and mice have demonstrated that haplotype blocks defined by a small number of SNP have the potential to track functional alleles based on genotypes of individuals without the need to reproduce pedigree studies in the target population (Reich et al., 2001; Wiltshire et al., 2003). Currently, at least three SNP-based markers with predictive value for carcass traits in cattle are being evaluated or applied in commercial settings (Buchanan et al., 2002; Barendse et al., 2004; Page et al., 2004).

Until recently, targeted SNP discovery in livestock has been limited by the amount of genomic sequence available. The completion of a bovine bacterial artificial chromosome (BAC) contig map (<http://www.bcgs.c.ca>) and the finding that 30% of the BAC-end sequences could be anchored to the human genomic sequence (Larkin et al., 2003) were the first steps to remove this limitation. In this study, we targeted bovine chromosome 5 (BTA5) for SNP discovery because several studies have indicated the presence of QTL associated with growth and carcass traits (Stone et al., 1999; Casas et al., 2000; Li et al., 2004). Our first objective was to estimate the amount of resequencing required to identify four to five common SNP in a region corresponding to a BAC. Our second objective was to analyze SNP-based marker genotypes for associations with growth and carcass traits.

Materials and Methods

Bovine BAC-end sequences with homology to human chromosome 12 (HSA12) and a portion of HSA22 that are syntenic to BTA5 were identified via BLASTN (Altschul et al., 1990) of all BAC-end sequences in GenBank to the human genome. Fingerprint contigs of BAC containing multiple end sequence matches within a small region of the human genome were considered to be mapped to the human genome, and individual end sequence matches within the contig were then taken to predict the approximate position of the bovine BAC on the human genome. The approximate position on BTA5 could then be determined from alignment of the human genome with an integrated radiation hybrid/genetic linkage bovine map (W. M. Snelling, personal communication). Initial efforts to position BAC based on a single end sequence having even high homology with human sequence yielded a correct assignment only approximately 50% of the time; the requirement for multiple BAC-end homologies within a contig proved to be a much more reliable indication of position, even when no single match had high homology. The original intent was to obtain sufficient SNP coverage over all of BTA5 to conduct preliminary analysis for linkage disequilibrium in addition to association analysis. However, the initiation of a project for complete sequencing of the bovine genome presented the prospect for effi-

ciently obtaining more evenly spaced markers for linkage disequilibrium analysis; thus, marker development was confined to the known QTL region, which is approximately the middle one-third of the chromosome.

Bacterial Artificial Chromosome Subcloning

Clones from the CHORI-240 BAC library (BACPAC Resources, Oakland, CA) were streaked onto LB/chloroamphenicol plates, and single colonies were used to inoculate 4 mL of LB/chloroamphenicol broth for overnight growth. Cells were collected by centrifugation, and BAC DNA was purified using a modified alkaline lysis procedure based on the Perfectprep kit (Eppendorf, Hamburg, Germany). The modification was the use of tubes and alcohol precipitation instead of a 96-well format and elution. The DNA was digested with 17 U *Sau3A1* (Promega, Madison, WI) for 30 min at 37°C in 25- μ L reactions before restriction fragments were separated on 1.2% agarose gels. Fragments between 700 and 1,000 bp were excised, purified using glass milk (GENECLEAN, Qbiogene, Carlsbad, CA), and ligated into pBlueScript followed by transformation into competent DH10B cells. For each BAC, 96 transformants were picked and sequenced in both directions using a previously described protocol (Smith et al., 2001). Resulting sequences were screened for *E. coli* vector, and repetitive sequence using BLASTN, and remaining subclones containing unique sequence were the basis for designing amplification primers for SNP discovery using the primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). A total of 23 BAC was selected for subcloning, and the sequences derived from them have been deposited in NCBI GSS database (<http://www.ncbi.nlm.nih.gov/dbGSS/index.htm>; Accession No. CW717116 to CW748471). Primers also were designed at 5,000-bp intervals from a bovine BAC containing the gene SOX10 (AC137534) sequenced as a part of the NISC Comparative Sequencing Initiative (<http://www.nisc.nih.gov>). The consensus animal sequences (sequence tag sites; STS) and SNP have been deposited in database STS (<http://www.ncbi.nlm.nih.gov/dbSTS/index.html>) and database SNP (<http://www.ncbi.nlm.nih.gov/SNP/>), respectively. Clone and primer information pertaining to the SNP that were found to be associated with carcass traits is summarized in Table 1. A single SNP derived from each BAC was genotyped in the MARC reference families to determine their position on the linkage map (Kappes et al., 1997). In some cases, none of the SNP derived from a BAC was segregating in the reference populations; however, in each case, the BAC contained a gene that had been mapped previously. The BAC mapping to the more central portion of BTA5 are presented in Figure 1. These and additional BAC mapping to the ends of BTA5 have been incorporated into in a composite map in which linkage and radiation hybrid maps have been integrated (W. M. Snelling, personal communication).

Table 1. Clone and primer information for markers with single nucleotide polymorphism associated with carcass traits

| QTL | BAC | dbSNP ^a | Primers ^b | |
|------------------|------------------------------|--------------------|------------------------|-----------------------|
| | | | Forward sequence | Reverse sequence |
| Fat ^c | CHORI240-495G12 ^d | 38340689 | AACCTGGTCCCTGAGTCTCC | CGCAGTTGCTAGTCTGCTTG |
| | | 38340690 | AACCTGGTCCCTGAGTCTCC | CGCAGTTGCTAGTCTGCTTG |
| | | 38340691 | AACCTGGTCCCTGAGTCTCC | CGCAGTTGCTAGTCTGCTTG |
| | | 38340692 | AACCTGGTCCCTGAGTCTCC | CGCAGTTGCTAGTCTGCTTG |
| | | 38340805 | TGCAATCTGCTTCCAGAAAAG | GATGCTCTCCCTGCTATGAC |
| | | 38340806 | TGCAATCTGCTTCCAGAAAAG | GATGCTCTCCCTGCTATGAC |
| | | 38340810 | AGACCCGCTCAACAAGGAG | TACGCGGAACCCCTCTTAC |
| Shear force | CHORI240-362B01 ^d | 38339300 | AAACAGGACAAAATGTCAGGTG | GAGAGGCAGCCCACAAGTAG |
| | | 38339182 | CAGGCTGTGAGTCAGGTCAG | AGAGGTGGAAGGACGAATCC |
| | | 38340644 | TTGGGTTTCCCATATACCC | CTCCTGGGAGAGGCTGACTT |
| | | 38340915 | TAGCTGCCTGACCTTTCTGC | GGGGTAGCACTCACCATGTAG |

^aGenBank accession numbers available at <http://www.ncbi.nlm.nih.gov/SNP/>.

^bThe annealing temperature for all primers was 58°C.

^cTraits related to carcass fat are fat thickness, rib fat, predicted fat yield, and yield grade.

^dCHORI240-495G12 contains phosphodiesterase 1B (PDE1B), which maps to 38 cM on bovine chromosome 5 (BTA5). CHORI240-362B01 contains nucleolar protein 1 (NOL1), which maps to 108 cM on BTA5.

Single Nucleotide Polymorphism Discovery

The panel for SNP discovery contained two Holstein sires and 14 F₁ sires resulting from matings between Hereford, Angus, or MARC III dams and five of the seven sire breeds (Angus and Hereford were excluded) in Cycle VII of the GPE (Wheeler et al., 2005). Animal sequences were analyzed using Phred, Phrap, and Consed programs (<http://phrap.org>). The results were evaluated visually using Consed (Gordon et al., 1998). An SNP was tentatively classified as a common SNP when the minor allele was observed three or more times in the panel of 32 chromosomes (>10% frequency).

Genotyping Assays

Genotyping assay design software and the mass spectrometry-based genotyping platform were provided by Sequenom (Sequenom Industrial Genetics, San Diego, CA). Initially, two to five SNP for each of a subset of 15 BAC mapped primarily to the QTL region of BTA5 were selected for genotyping in the Cycle VII resource population (Figure 1). When preliminary analysis suggested an association with a carcass trait, additional SNP corresponding to the same BAC were selected, taking into consideration the tentative haplotype structure that can be deduced from the sequencing of 16 unrelated animals in the discovery panel. Routinely, two 384-well plates consisting of 564 steers from the resource family, their sires, additional purebred sires not used to produce offspring in this study, and crossbred sires being used to produce later generations in GPE Cycle VII were genotyped.

Resource Population

The breeds represented in Cycle VII of the GPE and the phenotypic data analyzed in this study have been reported by Wheeler et al. (2005). Briefly, the seven

breeds in Cycle VII are those with the largest number of registrations in their respective breed registries in the United States: Angus, Hereford, Gelbvieh, Charolais, Limousin, Red Angus, and Simmental. Approximately one-half of the sires used was a sample of the 50 most popular sires within a breed based on registrations, whereas the remaining one-half has been unproven younger sires. Semen from these sires was used to artificially inseminate Angus, Hereford, or composite MARC III dams. Five hundred sixty-four spring-born steers were produced in 1999 and 2000, with a mean of 80 steers per sire breed. Slaughter was at a commercial facility, and rib sections were returned to MARC meats facility for analysis (Wheeler et al., 2005).

Traits analyzed for growth included birth weight, weaning weight, preweaning ADG, postweaning ADG, and live weight at slaughter. Carcass traits analyzed were hot carcass weight; adjusted fat thickness; LM area; marbling score; estimated kidney, pelvic, and heart fat percentage; and USDA yield grade (estimates the amount of saleable meat from the carcass; a yield grade of 1 yields the greatest percentage of retail product, and 5 yields the least). The wholesale rib was obtained from each carcass. The rib was dissected into retail product, fat, and bone to predict retail product yield and was dissected into fat and bone yield percentages using prediction equations described by Shackelford et al. (1995). Total carcass retail product and fat and bone yields were obtained by multiplying the yields from rib dissection by hot carcass weight. Meat tenderness was measured as Warner-Bratzler shear force at 14 d postmortem using a method described by Wheeler et al. (1998).

Single Nucleotide Polymorphism Analysis

Carcass composition data and data for growth traits were analyzed with the MIXED procedure of SAS (SAS

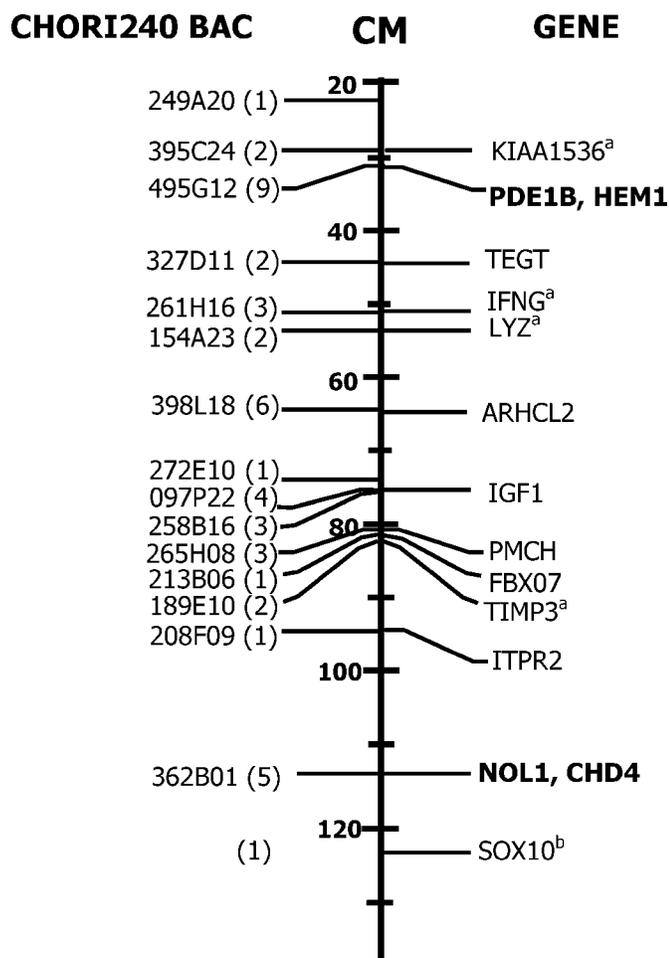


Figure 1. Relative map position of markers on bovine chromosome 5 (BTA5). The bovine bacterial artificial chromosome (BAC) used for each locus is indicated to the left of the ideogram, and the putative gene or genes contained in the BAC is to the right. Numbers in parentheses following BAC clones indicate the number of SNP from the BAC that were successfully genotypes in the Cycle VII animals. The two loci where associations were detected are in bold font. ^aIdentifies marker positioned only by two-point linkage analysis; all others are mapped by multipoint with 3-LOD (logarithm of the odds of linkage) confidence intervals ranging from 2.9 to 9.6. ^bNational Institutes of Health Intramural Sequencing Center comparative sequencing initiative, Accession No. AC137534.

Inst., Inc., Cary, NC). The model included the fixed effects of sire breed, dam breed, the interaction between sire breed and dam breed, year of birth (2000 and 2001), and slaughter group (four slaughter groups per year) within year of birth. Random effects were sire within sire breed and residual. Age at weaning was included in the model as a linear covariate for growth and carcass composition traits. Levels of significance associated with effect of sire breed on carcass and growth traits were tested with the random effect of sire. Each SNP genotype was included in the model one at a time.

Haplotype Analysis

Haplotypes were established for SNP at loci with significant associations using PHASE (Stephens et al., 2001; Stephens and Donnelly, 2003). To assess association of haplotypes with traits, the same statistical model described previously for individual SNP was used. Nesting within sire breed was not significant; thus, results presented are the fixed effects of haplotype in all instances. Each haplotype was included in the model separately for every trait. Animals with rare haplotypes or incomplete genotypic data were eliminated from the analysis. Traits were evaluated with each haplotype in the model. Least squares differences and probability values were estimated for each haplotype within the SNP combination. The overall probability values reported in Tables 2 and 3 are nominal and do not correct for multiple testing. In this particular study, all SNP associated with traits related to carcass fat or shear force could be placed into haplotypes, and very similar results were obtained for SNP and haplotypes. Thus, data for SNP are not presented. At the locus associated with shear force, coalescent analysis of the four haplotypes produced similar results (Seltman et al., 2003).

Results

A strategy of using the comparative map of bovine and human genomes to identify BAC clones carrying bovine orthologs predicted to map in the QTL interval on BTA5 was attempted and proved successful. A total of 23 BAC was subjected to low-coverage sequencing (approximately 0.5-fold coverage, assuming average BAC insert size of 167 kb) to support primer design. The presence of the targeted locus was verified in 21 of the 23 BAC based on comparison of sequences to the human genome. No attempt was made to discover spurious homologies resulting from pseudogenes or gene families.

After being used to verify that the desired locus had been targeted, the BAC-derived sequences were used to develop PCR primers to detect SNP in a panel of 16 animals to estimate the amount of resequencing required to identify common SNP for a given locus. Of a total of 450 STS, SNP were identified in 256 (57%). Of a total of 550 SNP, 296 (54%) were tentatively identified as common (minor allele frequency >10%). The mean number of common SNP per STS was 0.75 ± 0.11 , which is equivalent to one SNP every 1,000 bp because the mean size of the STS was approximately 800 bp. Based on variation about the mean number of SNP per STS, to be 95% confident of discovering a single SNP, a fourfold variation (400 to 1,600 bp) in the amount of sequencing is predicted. For comparison, two Holstein bulls were included in the SNP discovery panel. At least one of the two Holstein bulls was heterozygous for 41% of the SNP compared with 55% for two randomly chosen crossbred bulls in the panel, which provides a bench-

Table 2. Contrast of genotypes for haplotypes 1 (CGA) and 2 (AAG) at the phosphodiesterase 1B locus and associated carcass traits

| Trait | Genotype ^a | | | P-value |
|-------------------------|-----------------------|--------------|--------------|--------------------|
| | 1:1 | 1:2 | 2:2 | |
| Fat thickness, cm | 0 | 0.15 ± 0.04 | 0.19 ± 0.11 | 0.001 ^b |
| Rib fat, kg | 0 | 0.57 ± 0.18 | 0.79 ± 0.47 | 0.001 ^b |
| Fat yield, % | 0 | 1.11 ± 0.35 | 1.17 ± 0.90 | 0.005 |
| Yield grade | 0 | 0.18 ± 0.07 | 0.31 ± 0.17 | 0.01 |
| Retail product yield, % | 0 | -0.79 ± 0.30 | -1.32 ± 0.90 | 0.01 |
| Shear force, kg | 0 | 0.18 ± 0.08 | -0.15 ± 0.21 | 0.04 |

^aFrequencies: 1:1 = 303; 1:2 = 169; and 2:2 = 17. Seventy-five steers with incomplete genotypic data or minor haplotypes were eliminated from the analysis.

^bValues are significant after Bonferroni corrections for multiple testing ($0.001 \times \text{two haplotypes} \times 17 \text{ traits} = P < 0.03$).

mark for what might be expected for heterozygosity among other pure breeds.

To verify the prediction of the position on the bovine linkage map, a single SNP for each BAC determined to be informative in the MARC mapping population was genotyped. The complete map of BTA5 and further details will be presented elsewhere in a comprehensive composite map of the bovine genome (Snelling et al., 2005; W. M. Snelling, personal communication). A subset of 15 BAC mapping to the QTL region along with the number of SNP corresponding to each BAC that were successfully genotyped in the Cycle VII animals and the presumptive genes contained within the BAC are shown in Figure 1.

Initially, genotypes were collected on the GPE Cycle VII population for 38 SNP derived from 15 BAC targeting the QTL region of BTA5. The genotype data for each SNP were analyzed for association with a wide range of carcass and growth traits. Significant association with at least one carcass trait was detected with multiple SNP at two loci, phosphodiesterase 1B (**PDE1B**) and nucleolar antigen 1 (**NOL1**). At the PDE1B locus, two haplotypes based on seven SNP (four SNP were redundant in defining haplotypes in this population) derived from a single BAC were associated with traits related to carcass fat. Steers homozygous for the major haplotype CGA (frequency among steers = 0.70) had decreased subcutaneous fat thickness, wholesale rib fat, predicted fat yield, and yield grade, and greater

predicted retail product yield, compared with heterozygotes for the haplotypes (Table 2). Fat thickness and rib fat are significant ($P < 0.03$) after applying Bonferroni corrections for multiple testing (Table 2). Similar results were obtained for total carcass retail product and fat yields, which were estimated by multiplying the yields from wholesale rib dissection by hot carcass weight (data not shown). Because of the low number ($n = 17$) of steers homozygous for the minor haplotype, the only valid contrasts were between heterozygotes and those homozygous for the major haplotype, although the tendency was for a greater difference between those homozygous for the major and minor haplotypes. We conclude that these haplotypes constitute a marker associated with a modest effect on carcass traits related to fat composition. The seven SNP comprising the haplotypes were from three STS bracketing a 41-kb region of HSA12 based on BLAST comparisons with the human sequence. The source BAC, CHORI-240-495G12, mapping to 32 cM contains sequence homologous to PDE1B and hematopoietic protein 1 (**HEM1**), a gene expressed in hematopoietic cells. Two additional SNP from this region (dbSNP Accession No. 38340769 and 38340808) did not fit into any haplotype structure, and there was no association with any traits analyzed.

The major haplotype at a second locus (NOL1) was associated with increased shear force ($P < 0.04$) and ADG ($P < 0.04$). The contrast between steers homozygous for the major haplotype CGC (frequency among steers = 0.59) and those heterozygous for the major haplotype and one (TAT) of two minor haplotypes was -0.27 ± 0.11 kg (Table 3). There was a tendency for an association with weight at slaughter ($P < 0.08$). Associations between phenotypes and genotypes were not significant when a second minor haplotype (TGT) was included in the analysis. Our interpretation is that the TGT haplotype is associated with both QTL alleles for shear force. The development of more robust markers will be pursued in regions flanking this marker, which is based on STS from a BAC, CHORI-240-363B01, that maps to 108 cM and contains sequence similar to NOL1 and helicase DNA binding protein 4 (**CHD4**). The four SNP comprising the haplotype alleles are from separate

Table 3. Contrast of genotypes for haplotype 1 (CGC) and 2 (TAT) at the nucleolar antigen 1 locus and associated traits

| Trait | Genotype ^a | | | P-value |
|----------------------|-----------------------|--------------|--------------|---------|
| | 1:1 | 1:2 | 2:2 | |
| Shear force, kg | 0 | -0.27 ± 0.11 | -0.31 ± 0.21 | 0.04 |
| ADG, kg | 0 | -0.05 ± 0.02 | -0.02 ± 0.04 | 0.04 |
| Slaughter weight, kg | 0 | -13.1 ± 5.8 | -5.5 ± 11.0 | 0.08 |

^aFrequencies: 1:1 = 195; 1:2 = 111; and 2:2 = 19. Two hundred thirty-nine steers with other minor haplotypes or with incomplete genotypic data were eliminated from the analysis.

Table 4. Breed frequencies for haplotypes^a

| Breed | No. | Haplotypes fat ^{bd} | | | Haplotypes WB shear force ^{ce} | | | |
|-----------|-----|------------------------------|------|------|---|------|------|------|
| | | CGA | AGG | AAG | CGC | CGT | TGT | TAT |
| Angus | 21 | 0.75 | 0 | 0.25 | 0.98 | 0 | 0 | 0.02 |
| Charolais | 23 | 0.75 | 0 | 0.25 | 0.36 | 0 | 0.01 | 0.54 |
| Gelbvieh | 23 | 0.98 | 0 | 0.02 | 0.43 | 0.01 | 0.13 | 0.43 |
| Hereford | 21 | 0.69 | 0 | 0.31 | 0.27 | 0.13 | 0.33 | 0.27 |
| Limousin | 21 | 0.44 | 0 | 0.46 | 0.69 | 0.02 | 0.14 | 0.20 |
| Red Angus | 21 | 0.46 | 0.31 | 0.26 | 0.94 | 0 | 0 | 0.06 |
| Simmental | 21 | 0.97 | 0 | 0.03 | 0.61 | 0.02 | 0.13 | 0.24 |

^aSemen from purebred sires was obtained from the Germplasm Evaluation Cycle VII project. Two sires were not used to produce the F₁ steers genotyped for association analysis.

^bThe haplotypes observed in this population were defined by seven SNP, CGcAaca, AAtGctg, and AGcGaca. The SNP that were redundant in defining haplotypes in this population are in lower case.

^cThe haplotypes observed in this population were defined by four SNP, CGCg, CGTg, TGTg, and TATa. The single SNP that was redundant in defining haplotypes in this population is in lower case.

^dSteers homozygous for the CGA haplotype allele had less fat thickness than CGA/AAG heterozygotes, $P < 0.001$. Traits related to carcass fat are fat thickness, rib fat, predicted fat yield, and yield grade.

^eSteers homozygous for the CGC haplotype allele had higher shear force values than CGC/TAT heterozygotes, $P < 0.04$. WB = Warner-Bratzler.

STS, which bracket a 27-kb region for HSA12. A fifth SNP (dbSNP access no 38340644) was not associated with any traits analyzed and was from an STS predicted to be 14 kb away from the 27-kb bracket based on human sequence.

The haplotype frequencies observed in the pure bred sires assembled for the GPE Cycle VII are summarized in Table 4. The frequency of the major haplotype (CGA) associated with lower carcass fat ranged from 0.41 in Limousin to 0.98 in Simmental and Gelbvieh. The frequency of the minor haplotype (AAG) ranged from 0.31 in Hereford to <0.05 in Limousin and Simmental. A third haplotype (AGG) was unique to Red Angus sires. The nine sires with this haplotype were all descendants of one widely used sire born in 1965. Inheritance of a minor haplotype (AAA) observed in four Limousin sires could be verified in only two offspring; 14 remaining offspring were missing genotypic data or were ambiguous. The frequency of the major haplotype (CGC) associated with increased shear force ranged from 0.27 in Herefords to about 0.95 in Angus and Red Angus. The frequency of the minor haplotype (TAT) associated with decreased shear force ranged from 0.54 in Charolais to 0.02 in Angus. A second minor allele (TGT) presumed to be associated with both QTL alleles is at a low frequency in all breeds except Hereford. A fourth minor allele (CGT) was observed at low frequency in multiple breeds. Although the frequencies of these minor haplotypes were not sufficient to determine associations in this study, they could be important in other populations, especially those heavily influenced by a single breed or sire.

Discussion

The number of whole genome QTL scans in domestic animals has been limited because substantial resources are required to obtain a small sample of the genetic

variation using microsatellite markers in large half-sib families. The abundance and evolutionary stability of the latest generation of markers, SNP, coupled with genomic sequence as a template for marker development and advances in high throughput genotyping brings new opportunities for the use of markers in domestic animals as selection and production tools. To our knowledge, the data presented herein represent the first effort to target a broad chromosomal region with random SNP in an attempt to discover genotype-phenotype associations and develop haplotype-based markers with the potential for application across a wide range of populations in beef cattle. Although the number of animals sequenced for SNP discovery in this study was limited, it is important to note that we found little evidence for wide disparities in SNP allele frequencies among the seven *Bos taurus* breeds sampled. In a separate study, 100 randomly chosen SNP were genotyped in a panel of 95 animals representing 17 beef breeds (Heaton et al., 2001), and no SNP was observed to be correlated to any breed (T. P. L. Smith, personal communication). This suggests that with the exception of historically isolated populations, a wide range of breeds within *Bos taurus* will not need to be sampled to define most of the common SNP in a region. This is not to suggest that the frequencies of haplotypes based on common SNP will not vary widely among breeds (see Table 4).

In this study, we targeted a region of BTA5 encompassing previously identified QTL for marker development and subsequent analysis for associations with carcass and growth traits. Forty-five common SNP identified from 15 BAC loci were used to perform association analysis with carcass and growth traits in 564 steers representing seven breeds, which identified 11 SNP with statistical support for association with carcass traits. Seven of these SNP at the PDE1B locus were associated with traits related to carcass fat. Three of

the SNP defined three segregating haplotypes (CGA, AGG, and AAG), whereas the other four were redundant in defining the haplotypes observed in this population. We consider two haplotypes (CGA and AAG) to be a possibly useful marker system for carcass fat. The third (AGG) was too rare to test adequately in this population. Steers homozygous for the major haplotype (CGA) had less subcutaneous fat thickness ($P = 0.001$) and rib fat ($P = 0.001$) when contrasted to heterozygous animals (Table 2). In addition, the yield grade was decreased, and the predicted retail product yield was increased. The results from analyzing individual SNP are not presented because they were very similar to the results obtained by analyzing haplotypes. Because the desirable haplotype is at a relatively high frequency (mean = 0.73; Table 4) in the breeds represented, rapid progress in decreasing the frequency of the undesirable haplotype could be expected. It is notable that the haplotype seems to be nearly fixed in two continental breeds (Simmental and Gelbvieh) known to produce leaner carcasses than British breeds (Wheeler et al., 2005). The allelic effect of 0.15 cm of fat thickness compares with a 0.45-cm difference between the means for British and continental breeds (Cundiff et al., 2004, Wheeler et al., 2005). The haplotype AGG, unique to Red Angus sires with a frequency of 0.3, serves to illustrate the need to determine the haplotypes present in a target population before extensive genotyping. In this example, genotyping only the first or last SNP (see Table 4) would not distinguish this haplotype, which could severely affect interpretation of data from populations with a strong Red Angus influence, depending on the status of linkage disequilibrium between the haplotype and QTL alleles. Similarly, Casas et al. (2000) described a QTL for fat thickness on BTA5 in a half-sib family involving a Piedmontese \times Angus sire, which we have found to be homozygous for a haplotype not observed in the Cycle VII population (CAA).

The PED1B gene encodes a phosphodiesterase that catalyzes the conversion of cAMP and cGMP to their respective monophosphates. The map position on BTA5 is consistent with previously determined conserved synteny with HSA12, where human ortholog lies. The connection between PED1B activity and the observed phenotypic variation is not obvious; however, PDE1B lies within 0.5 megabases of a number of genes, including a homeobox gene cluster and a gene encoding a G protein-coupled receptor, for which it is easier to theorize a connection to fat deposition. The map position of PDE1B (30 cM) suggests that this QTL is distinct from a QTL for fat thickness at 65 to 70 cM described by Li et al. (2004).

At a second locus (NOL1), four SNP were associated with shear force ($P < 0.04$) and ADG ($P < 0.04$), with less support for weight at slaughter ($P < 0.08$). Animals homozygous for the major haplotype CGC had a 0.27 kg greater shear force than those heterozygous for the major haplotype and one (TAT) of two minor haplotypes (Table 3). When a second minor haplotype (TGT) was

included in the analysis, there was not an association of genotype with phenotype. Thus, the currently defined haplotypes have limited predictive value without further marker development. Assuming that the TGT haplotype is in phase with both of the QTL alleles, the goal of further marker development will be the discovery of an SNP that splits the presumptive TGTQ and TGTq haplotypes, where Q,q are the QTL alleles, or to develop additional marker loci in flanking regions with the intent of finding other haplotypes that are in linkage disequilibrium with the QTL alleles. As discussed previously, the variation in minor haplotype allele frequencies among the breeds can be important. By eliminating the TGT allele from the analysis of shear force, we are eliminating a disproportionate number of Herefords because the allele frequency is 0.33 compared with the next highest frequency of 0.13 in two other breeds (Table 4).

The pronounced expression of NOL1 in tumor cells provides no obvious connection to variation in carcass or growth traits; however, within 0.3 megabases in this block of conserved bovine/human synteny, there is a member of the G protein-coupled receptor gene family (GRP93) and a member of the guanine nucleotide binding protein gene family (GNB3). Sequence variation in the latter has been associated with body mass index in humans (Siffert et al., 1999).

In an earlier QTL scan involving a *Bos taurus* \times *Bos indicus* sire, the most significant statistical support for QTL on BTA5 was effects on percentage of bone in the wholesale rib and dressing percentage, with the peak being roughly in the middle of the region targeted here (Stone et al., 1999). The failure to detect any associations with these traits in this study may be due to the lack of segregating QTL alleles or inadequate marker coverage. Although either alternative is possible, 46 SNP in 15 clusters unevenly distributed over one-third of a chromosome is not sufficient to rule out segregating QTL, especially considering that genotypic data were obtained for one or two SNP at several of the loci (Figure 1). We consider the findings presented here as a preliminary description of haplotype-based markers in linkage disequilibrium with QTL. Because of the distribution of haplotypes across breeds, we have limited power to test the possibility that linkage disequilibrium between marker and QTL alleles has been lost to recombination in some fraction of the sires. It is hoped that as additional populations are targeted, the question of phase across breeds and populations can be addressed adequately. Furthermore, any association based on linkage disequilibrium must be viewed as only a statistical relationship, as are EPD, within a population, which implies a lack of strict concordance between marker genotype and genetic merit of an individual in the population.

Thus far, a small number of SNP-based markers have been shown to be associated with quantitative carcass or growth traits in cattle. A polymorphism in the 5' leader sequence of the thyroglobulin gene associated

with marbling is being commercially applied (Barendse et al., 2004), as is a CT transition in the leptin gene that is associated with carcass fat (Buchanan et al., 2002). Two SNP in the coding region of CAPN1 (μ calpain) have been found to be associated with tenderness and are being evaluated in commercial settings (Page et al., 2002, 2004). The emerging bovine genome sequence is making development of targeted SNP markers much more efficient, improving the prospects of discovering additional associations between genotype and phenotype. The results thus far, including the work described herein, suggest that development and application of DNA markers as tools in selection programs generally will require markers based on haplotypes comprised of multiple SNP, that have been characterized across multiple diverse populations.

Implications

We targeted a region of BTA5 for SNP-based marker development where previous results suggested the presence of QTL affecting carcass and growth traits. These markers were genotyped in 564 steers and their sires, representing seven of the most popular *Bos taurus* breeds in the United States. Combinations of SNP segregating together, haplotypes, were associated with traits related to carcass fat and tenderness at separate locations on the chromosome. These data demonstrate that with low-level marker coverage of a broad chromosomal region, associations between genotype and phenotype can be detected. In that these findings were based on a population consisting of seven breeds, it is plausible to assume that these associations between genotype and carcass traits can be verified in additional populations. Presently, these markers are being further evaluated in additional populations at the U.S. Meat Animal Research Center.

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