Beef Tenderness QTL On BTA25 From A Whole Genome Scan With The Bovine SNP50 Beadchip

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Introduction

Beef tenderness is the single most important palatability aspect for consumers (Miller *et al*. (1995)). Numerous studies have shown that consumers are willing to pay for more tender beef (Lusk *et al.* (2001)). Beef tenderness has a significant genetic component (Koch *et al.* (1982); Wheeler *et al*. (1996)).

Capitalizing on the genetic differences that naturally exist for beef tenderness is difficult to achieve by traditional means and will benefit greatly from genomic technologies (Dekkers (2004)). Genomic tests for some genes related to beef tenderness have been developed and are available commercially to producers now as validated through the National Beef Cattle Evaluation Consortium (www.ansci.cornell.edu/nbcec).

Studies of the candidate genes involved in the calpain and calpastain pathway have resulted in the discovery of a number of SNP which have been linked to both calpain enzymes and calpastatin (Page *et al*. (2002); Schenkel *et al*. (2006)). Further work has now identified that calpastatin has many SNP and additional SNP likely remain that could help explain more genetic variation in tenderness (Casas *et al.* (2009)). Despite the extensive work on the calpain-calpastatin system, it is clear that there are many additional genomic regions with a significant association with beef tenderness. This study utilized a whole genome scan with the Illumina Bovine SNP50 Beadchip to reveal QTL affecting beef tenderness within a multiple-breed beef cattle population.

Materials and methods

Animals and phenotypic data. Seven hundred two animals born 1998-2005 were genotyped, using the Illumina Bovine SNP50 Beadchip, including 225 bulls, 34 heifers and 443 steers. Average breed composition consisted of 38% Angus (AN), 9% Charolais (CH), 26% Simmental (SM), 10% Piedmontese (PI), and 17% other breeds. Calves were the result of AI breeding with primarily purebred sires, although some composite sires were also used.

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In general the cow herd is Angus x Simmental and some terminal sires have been used. Animals were born and raised at one of the three University of Guelph cooperating research centres in Ontario, Canada, weaned at approximately 200 days of age, and fed through to slaughter at the Elora Beef Cattle Research Centre. Animals were involved in various post-weaning feeding trials and finished to a subcutaneous fat thickness as determined by ultrasound and slaughtered at the University of Guelph meat science laboratory. Warner-Bratzler peak shear force measurements (kg) were used as an objective method of assessing tenderness on a cooked sample of *longissimus dorsi* 7-day post-mortem (LM7D) (Shackleford *et al.*, 1999). The trait heritability was 0.17, trait mean of 5.12±1.41.

Genotypic data and statistical analysis. A total number of 56,947 single nucleotide polymorphism (SNP) were genotyped across 29 *Bos taurus* autosomal chromosomes (BTA). SNPs that were out of Hardy Weinberg equilibrium (P<0.01) or less than 10% Minor Allele Frequency (MAF) were removed from further analysis. After screening, 38,745 SNPs were utilized. Chromosome 1 had the highest number of SNPs (2,461) whilst chromosome 22 had the fewest (682); nevertheless the average distance between 2 adjacent SNPs was 60kb, and consistent among the chromosomes.

A univariate animal model was used to estimate the allele substitution effect at each locus, $y_{ijk} = \mu + b_j + c_i + t_k + hy + a_{ijk} + \sum_{l=1}^{s} \alpha_l x_l + e_{ijk}$, where μ was the overall mean, b_j the breed effect, c_i the heterosis effect, t_k the trial treatment effect, *hy* the herd:year effect, a_{ijk} the additive genetic effect of individual, x_l the number of copies of the 2nd allele (0, 1, 2) in the genotype for l^{th} marker, α_l the allele substitution effect, e_{ijk} the residual. Age, breeds, heterosis, trial treatments, and allele substitution effect were fixed effects, while herd:year and animal effects were random. ASREML (Gilmour *et al.* (2009)) was used to estimate the effects. Type I error was controlled by the false discovery rate (FDR) (Benjamini and Hochberg (1995)). The FDR threshold was 4.42%, and its calculation was fully described in Mosig *et al.* (2001).

Results and discussion

There were 1,964 significant SNPs at p<0.05, 466 at p<0.01, 46 at p<0.001, and 2 (*CAPN1_1*, and, *HAPMAP48825-BTA-60019* (*SNP60019* hereafter)) at FDR<4.42%. These 2 SNPs were located at 37,525,486Mb on BTA29, and 34,691,758Mb on BTA25. Also considered in this study was *CAPN1_2* at 37,544,057Mb on BTA29 from the current panel, and *UOGCAST1* (Schenkel *et al.* (2006)) genotyped by the commercial provider offering the test (www.igenity.com). *CAPN1_1* and *CAPN1_2* were in the *Calpain* gene and have been described as *CAPN1-316* and *CAPN1-4751*, respectively, previously (Van Eenennaam *et al.* (2007)).

The animal mixed model described earlier was modified to help better understand the effect of subsets of the 4 SNPs. *CAPN1_1* and *CAPN1_2* were analyzed together as haplotypes, while *UOGCAST1* and *SNP60019* were considered independently as they are on different

chromosomes. The results for allele substitution effects and overall allelic frequencies are presented in table 1. Results are similar for *CAPN1_1 – CAPN1_2* haplotypes and *UOGCAST1* SNP as found previously in a large independent validation trial (Van Eenennaam et al. (2007)). Meanwhile the other significant SNP from the WGS was *SNP60019* which belongs to a significant QTL region previously identified for beef tenderness ($p<0.05$) at 31.59-53.37cM (Gutierrez-Gil et al. (2008)), with 2 alleles C and T with frequency of 0.69 and 0.31 population wise, respectively. Replacing 1 copy of C by T at this position independently decreased LM7D by 0.36kg. Results in table 1 are from the joint analyses with all markers fit simultaneously, in which case the effect of the *SNP60019* T allele is -0.25 and is similar in magnitude to *UoGCAST1* and about half the effect of the most favourable *CAPN1* haplotype.

Table 1. Allele substitution (haplotype or SNP) effects on beef tenderness (LM7D, kg)

Marker	Allele	Frequency	Allele Substitution (kg)	SE
CAPN1_1- CAPN1_2	C-C	0.16	-0.42	0.11
	C-T[1]	0.01	-0.77	0.37
	G-C	0.32	-0.08	0.08
	G-T	0.50	0.0	0.0
UOGCAST1	C	0.62	-0.22	0.08
	G	0.38	0.0	0.0
SNP60019	T	0.31	-0.25	0.09
	C	0.69	0.0	0.0

[1]Number of animals with the C-T haplotype too low for an accurate effect estimate

In terms of the proportion of phenotypic variance accounted for by the SNPs, *CAPN1_1* and *SNP60019* individually explained 3.24 and 2.99% of phenotypic variation in LM7D, respectively. The proportion of phenotypic variance explained by *CAPN1_1,2* haplotypes and subsets of *UOGCAST1* and *SNP60019* is presented in table 2.

Table 2. Proportion of LM7D variance explained by haplotypes in *CAPN1_1* and *CAPN_2* along with subsets of SNP in *UOGCAST1* and *SNP60019*

Haplotype / SNP subset	% of LM7D variance
CAPN1_1,CAPN1_2	3.4
CAPN1_1,CAPN1_2, SNP60019	5.0
CAPN1_1,CAPN1_2, UOGCAST1	5.4
CAPN1_1,CAPN1_2, UOGCAST1, SNP60019	6.7

The four SNPs together explained as much as 6.7% of the variation in LM7D, which is 39% of the genetic variance of the trait given the trait heritability of 0.17. The SNP which are currently in use in routine commercial genotyping explained 5.4% of the variation with the addition of *SNP60019* on BTA25, this increased to 6.7%. Further analyses showed that the 4

SNPs did not significantly affect feed intake, average daily gain, body weight, carcass weight, rib-eye area, marbling score, back fat, and rib dissection traits.

Conclusion

A whole genome scan confirmed a QTL for beef tenderness on BTA25. The independent allele substitution effect from these data along with a favourable MAF suggest that this marker is of similar magnitude and impact for beef tenderness as commercially available SNP *CAPN1* and *CAST* that are used in selection and helps explain additional variation in LM7D. Further fine mapping of this region should help identify the causative mutation for implementation in selection programs.

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