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Association analysis of *adiponectin* and *somatostatin* polymorphisms on BTA1 with growth and carcass traits in Angus cattle

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Summary

This study tested positional candidate genes *adiponectin* (*ADIPOQ*) and *somatostatin* (*SST*) for effects on carcass traits in a commercially relevant cattle population. Both genes are located within a region of BTA1 previously reported to harbour quantitative trait loci (QTL) that affect marbling, quality grade, yield grade, ribeye area and weaning weight in *Bos taurus* × *Bos indicus* crosses. Except for the first intron of *ADIPOQ*, both genes, including over 2 kb upstream of the promoters, were sequenced in five registered Angus sires to identify polymorphisms. A variable copy duplication and three single nucleotide polymorphisms (SNPs) in *ADIPOQ* and one SNP in *SST* were genotyped and tested for association with 19 traits in a 14-generation pedigree of 1697 registered Angus artificial insemination sires representing all the major USA lineages of the breed. Linear models that parameterized predicted genetic merits in terms of allele substitution effects were fit by weighted least squares, and goodness-of-fit tests were employed to differentiate causal mutations or polymorphisms in strong linkage disequilibrium (LD) with causal mutations from markers in weak LD with QTL. We confirmed the presence of QTL affecting marbling, ribeye muscle area and fat thickness in the vicinity of *SST* and *ADIPOQ* on BTA1 in Angus; excluded *SST* as underlying the ribeye muscle area QTL; and excluded *ADIPOQ* as underlying the marbling score QTL. However, association analysis provides very limited information about QTL location and has little intrinsic value when performed in the absence of linkage or LD analysis using flanking marker data to localize the QTL effect relative to positional candidate genes.

Keywords adiponectin, Angus, association, beef, carcass traits, linkage disequilibrium, quantitative trait loci, single nucleotide polymorphism, somatostatin.

Introduction

Cai *et al.* (2004) and Kim *et al.* (2003) identified quantitative trait loci (QTL) in the interstitial region of BTA1 affecting marbling, hot carcass weight (CW), quality grade, yield grade, ribeye area and weaning weight (WW) in *Bos indicus* × *Bos taurus* backcross and F_2 progeny. *Somatostatin* (*SST*) was identified as a positional candidate gene for the detected QTL effects. While the gene mapped to the most likely position for the QTL, none of the polymorphisms identified by sequencing *SST* from Angus and Brahman cattle could be shown to be causal for the detected QTL effects (Cai *et al.* 2004).

Using the human–bovine comparative map, we identified *adiponectin* (*ADIPOQ*), which codes for an adipocytokine adiponectin, as an alternative positional candidate gene for the QTL reported by Cai *et al.* (2004). *ADIPOQ* maps 810-kb from *SST* in human and is an excellent functional candidate gene for the marbling QTL because its product, adiponectin, modulates lipid and glucose metabolism in insulin-sensitive tissues. Collagen-like adipocytokine adiponectin is expressed by white adipose tissue and is secreted into the bloodstream, where among other functions it acts as an inhibitor of lipogenesis, a potent insulin sensitizer, an anti-atherogenic agent, an anti-inflammatory agent and a neoglucogenic inhibitor (Chandran *et al.* 2003; Diez & Iglesias 2003; Jacobi *et al.* 2004). It is the only adipose-specific hormone known to be negatively correlated with obesity parameters in human adults (Arita *et al.* 1999; Matsubara *et al.* 2002;

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Kern *et al.* 2003; Shetty *et al.* 2004), children and adolescents (Stefan *et al.* 2002; Weiss *et al.* 2003; Yannakoulia *et al.* 2003) and pigs and mice (Jacobi *et al.* 2004; Lord *et al.* 2005).

Kissebah *et al.* (2000) proposed *ADIPOQ* as the most probable positional candidate gene for an obesity QTL localized to human chromosome 3q27 in Caucasian-Americans. Wu *et al.* (2002) also found strong evidence of linkage for a body mass index QTL at 3q27 in a combined analysis of Caucasian, African and Mexican-American families. These results illustrate adiponectin's crucial role in fat and glucose metabolism and suggest that variation in *ADIPOQ* may impact adiposity traits in beef cattle. As adiponectin has also been implicated in the regulation of bone development (Berner *et al.* 2004; Oshima *et al.* 2005), effects of *ADIPOQ* on yield grade and weight traits are also possible.

Somatostatin affects carbohydrate and lipid metabolism through the actions of growth hormone and affects muscle and bone growth via the actions of insulin-like growth factor-1 (Melmed *et al.* 1996). Growth hormone secretion is regulated through alternating cycles of stimulation by growth hormone releasing hormone and inhibition by somatostatin (Holl *et al.* 1988; Hartman *et al.* 1991). Growth hormone increases lipolysis (Gerich *et al.* 1976), stimulates hormone-sensitive lipase and decreases both glucose uptake and lipogenesis (Larsen *et al.* 2003) in adipose tissues throughout the body. Somatostatin reverses these effects by suppressing growth hormone secretion by the somatotrophs. Growth hormone also has a variety of metabolic actions that are independent of the insulin-like growth factor-1 pathway, such as the stimulation of amino acid transport in muscle (Hjalmarson *et al.* 1969) and stimulation of epiphyseal growth and osteoclast differentiation and activity (Larsen *et al.* 2003). Thus, somatostatin effects on adipose tissue are mediated through the growth hormone metabolic pathway cascade.

The objectives of this study were to test whether the QTL previously detected in *B. indicus* × *B. taurus* cattle were segregating within a commercial population of Angus cattle, and, if so, to test whether variation within *ADIPOQ* and *SST* was causal for any of the detected effects.

Materials and methods

Population and traits

Our mapping population comprised a 14-generation pedigree of 1697 registered Angus artificial insemination (AI) sires. The pedigree included 10 male lineages, which were distinct at the time of birth of the oldest bull within each lineage for which DNA was available via frozen semen. However, all these lineages were inter-related through the bulls' maternal pedigrees, and 77.9% of the bulls were also represented in the pedigree as maternal grandsires. The oldest bull for which DNA was available via semen was born in 1955 (Fig. 1a). Pedigree data, expected progeny differences (EPD) and their accuracies (Spring 2005 evaluation) for all bulls and their parents were provided by the American Angus Association (AAA).

Because the accuracies of EPDs can differ substantially among bulls, we elected to analyse only the 19 traits for which accuracies were computed by the AAA. These traits included calving ease direct expressed as a difference in percentage of unassisted births, with a higher value indicating greater calving ease in first-calf heifers; birth weight; WW; yearling weight; yearling height (YH); scrotal circumference (SC); calving ease maternal; maternal milk (MILK), which is the contribution to a calf's WW due to the milk and mothering ability of a sire's daughters; mature weight of a sire's daughters; mature height of a sire's daughters; CW; USDA marbling score (MARB); ribeye muscle area (RE); external fat thickness (FAT) measured between the 12th and 13th ribs; per cent retail product (%RP), which is the per cent of CW that is salable; ultrasound measurement of per cent intramuscular fat in the ribeye muscle; ultrasound ribeye muscle area (URE); ultrasound fat thickness as the weighted average of 60% of the rib fat measured between the 12th and 13th ribs and 40% of the rump fat measurement; and ultrasound per cent RP (U%RP).

Detection of variation and loci genotyped

Five Angus AI sires representing three of the lineages were chosen from the pedigree for sequencing and polymorphism detection. *ADIPOQ* sequencing primers were designed from the bovine *adiponectin* mRNA sequence reported by Hattori *et al.* (2003) (NM_174742). Bacterial artificial chromosome (BAC) clone 225G21 harbouring *ADIPOQ* was identified by overgo hybridization from the CHORI-240 bovine BAC library (http://bacpac.chori.org/bovine240.htm) in order to obtain the sequences of the 5′-untranslated region (UTR), 3′-UTR and regions of intron 1 flanking the neighbouring exons. *SST* sequencing primers were designed based on the genomic sequence reported by Cai *et al.* (2004). Primers used in the polymerase chain reaction (PCR) amplification and sequencing of bovine *ADIPOQ* and *SST* are listed in Table S1.

Three *ADIPOQ* single nucleotide polymorphisms (SNPs) and one *SST* SNP were amplified by allele-specific PCR, and the genotypes were scored visually on 2% agarose gels with a co-amplified *16S rRNA* gene fragment used as a positive control. An *ADIPOQ* variable copy duplication was also scored visually by separating PCR products on 2% agarose gels. The *ADIPOQ* and *SST* SNPs and *ADIPOQ* duplication primers used for genotyping in this study are listed in Table S2. We quality checked all generated SNP and duplication locus genotypes using GENOPROB (Thallman *et al.* 2001a,b) to identify misinheritances and genotype errors and also to predict missing genotypes. We also used GENOPROB to derive the maternal and paternal haplotypes segregating within the registered Angus population.

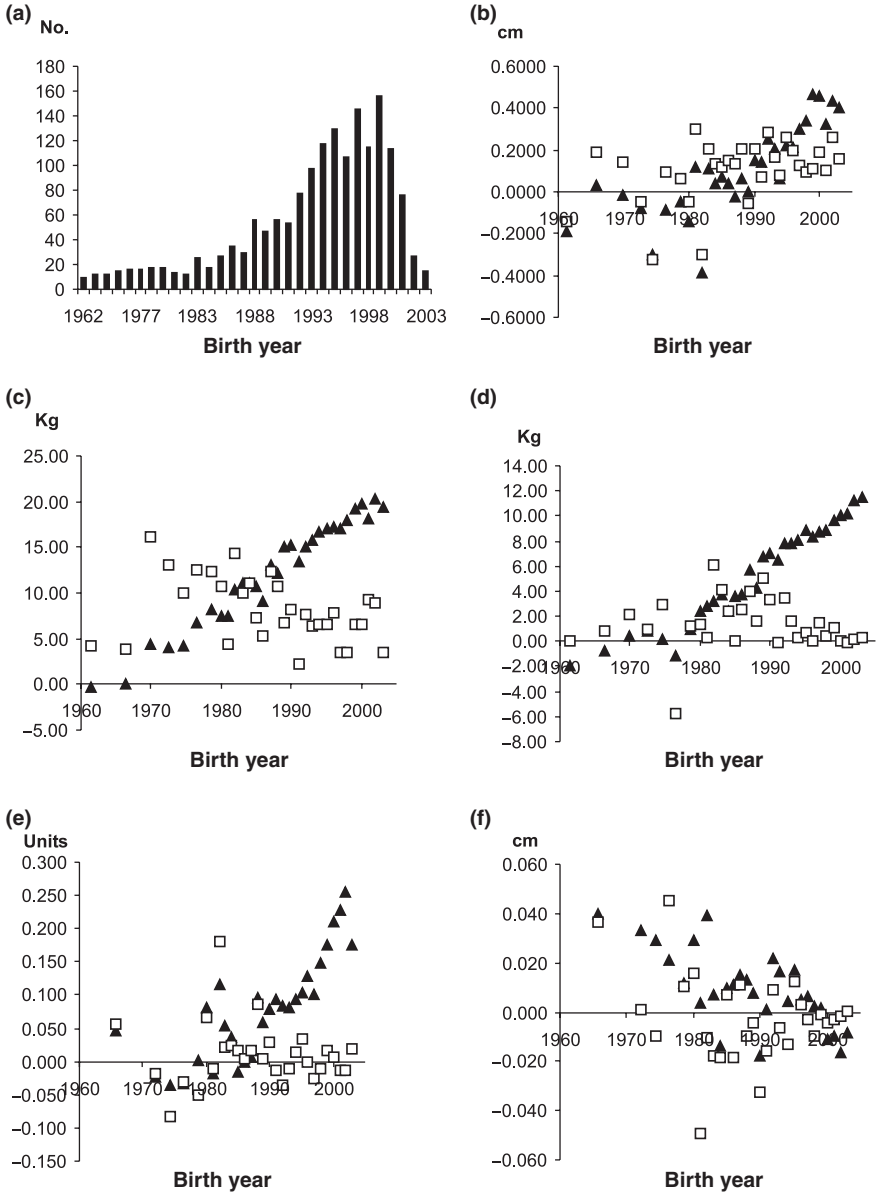

Figure 1 Characteristics of the sample of 1697 registered Angus sires. (a) Distribution of sires by birth year. (b-f) Mean EPDs (▲) and Mendelian sampling effects (□) by year of birth for: (b) scrotal circumference; (c) weaning weight; (d) maternal milk; (e) marbling; and (f) fat thickness.

Statistical analysis

An advantage to performing an association analysis in a population that spans a large number of generations is that any locus that is under directional selection will respond with an increase in the frequency of the favourable allele in time. Loci that are under direct selection are expected to change in allele frequency more rapidly than are closely linked loci even in the presence of strong linkage disequilibrium (LD). Thus, we computed the allele frequencies of all tested loci by sire birth year and used linear regression to estimate the response to selection at each locus. The assumption of linearity requires that the selection intensity applied to each directly selected locus is approximately constant in time.

A disadvantage to performing an association study in a temporally variable population is that spurious associations may be generated for any trait and locus that are under selection regardless of whether the locus has a causal effect on variation in the trait. By plotting the mean sire EPDs by sire birth year, we found strong evidence for selection upon all 19 traits for which EPDs are computed by the AAA (e.g. Fig. 1b–f). Thus a locus responding to selection on a trait for which it has a causal effect may produce a spurious correlation with any other trait that is under selection even though it has no direct effect on the trait. To avoid these

artefacts, we analysed Mendelian sampling (MS) terms in all the performed association tests because of the expectation that there should be no time trend in MS even if there is a trend in EPDs due to selection (Bullock *et al.* 2000). The MS term is the deviation of an individual's breeding value from its midparent breeding value (MBV). We estimated the MS effects from EPDs as:

$$\mathrm{MS}_j = 2x\mathrm{EPD}_j - (\mathrm{EPD}_{\mathrm{S}_j} + \mathrm{EPD}_{\mathrm{D}_j}),$$

where MS_j is the Mendelian sampling effect of individual j with sire S_j and dam D_j, and EPD_j, $\mathrm{EPD}_{\mathrm{S}_j}$ and $\mathrm{EPD}_{\mathrm{D}_j}$ are the EPDs of the individual, its sire and its dam respectively. The distribution of sire numbers by birth year, and the mean EPDs and mean MS terms by sire birth year for SC, WW, MILK, MARB and FAT are shown in Fig. 1. While there is strong evidence for selection in all these traits, there is no time trend in the MS term.

Consider a biallelic QTL with alleles Q and q, allele frequencies $F(Q) = p$ and $F(q) = q = 1 - p$ that is in Hardy–Weinberg equilibrium (HWE). If genotypes QQ, Qq and qq are assigned genotypic values of a, d and $-a$, respectively, it is well known that the monogenic EPDs for each genotype are $q\alpha$, $1/2(q-p)\alpha$ and $-p\alpha$ respectively where $\alpha = a + d(q-p)$ is the additive effect of an allele substitution. It is also relatively straightforward to show that the mean MS effect is also $q\alpha$, $1/2(q-p)\alpha$ and $-p\alpha$ for individuals with genotypes QQ, Qq and qq respectively (see Appendix S1).

We analysed MS terms using the additive model

$$\mathrm{MS}_{ij} = \mu + \alpha X_i + e_{ij},$$

where MS_{ij} is the MS effect for the jth individual with the ith genotype at a tested locus, μ is the mean MS effect for heterozygotes and is equivalent to $\mu_s + 1/2(q-p)\alpha$ (where μ_s is the mean MS term which may deviate from zero due to sampling or within-family selection), α is the effect of an allele substitution at the tested locus, X_i takes the values $1/2$, 0 and $-1/2$ for genotypes QQ, Qq and qq respectively and e_{ij} is the residual MS effect due to unexplained polygenic loci influencing the trait.

Henderson (1973) showed that the variance of breeding values predicted under an animal model was G-PEV where G is the covariance matrix among breeding values and PEV is the matrix of prediction error variances. The diagonals of this matrix are approximately $[1 - (1 - \mathrm{Acc})^2]V_\mathrm{A}$, where V_A is the additive genetic variance and Acc is the Beef Improvement Federation accuracy that is reported by the AAA. These variances behave counter-intuitively in comparison to the sampling variances of estimated fixed effects.

In a mixed-model analysis, each individual's phenotypic deviation from its MBV is regressed towards the MBV according to the amount of information on the individual and the heritability of the trait. As Acc increases, the strength of the regression decreases, predicted breeding values converge in probability to true breeding values, and the variance of predicted breeding values converges to V_A from below. However, when Acc is low, individual phenotypic deviations are strongly regressed towards the MBV and the variance of predicted breeding values decreases. Thus, seemingly paradoxically, the variance of predicted breeding values increases as the amount of information on individuals increases, and the variance decreases as information decreases. This is because this variance does not reflect the degree of certainty that we have concerning the prediction of a specific individual's breeding value. Rather, it reflects the variation among a population of predicted breeding values as determined by the information available on those individuals. The point is that this variance is not the appropriate variance with which to weight EPDs or MS terms in a weighted analysis of variance because it would result in inversely emphasizing the predicted merits according to their precision of estimation. Accordingly, following Rodriguez-Zas *et al.* (2002) rather than using the reciprocal of the variance of breeding values as weights, we used the variance proportionality term $[1 - (1 - \mathrm{Acc})^2]$ to weight EPDs and MS terms in all analyses. We tested the significance of the allele substitution effect α using a t-test.

Finally, we performed a goodness-of-fit test for each tested polymorphism × trait combination by fitting the general model $\mathrm{MS}_{ij} = \mu + G_i + e_{ij}$, in which the genotypic effect due to heterozygotes was not constrained to be intermediate to the homozygotes. The general and additive models were compared for goodness-of-fit using an F-test (Searle 1971). In order for a tested mutation to be considered as potentially causal for a QTL effect, genotype effects should differ in the general model; there should be no difference in fit between the general and additive models; and the allele substitution effect α in the additive model should differ from zero.

Haplotype and LD estimation

We used GENOPROB to examine genotypes for Mendelian inheritance and for genotyping errors using map distances among loci set proportional to their physical distance. We extracted all genotypes for which the probability of a correct genotype was at least 95% ($\mathrm{pG}_{\mathrm{mx}} \geq 0.95$) and retained animals with heterozygous genotypes only if the probability that the parental phase could be correctly assigned was at least 90% ($\mathrm{oG}_{\mathrm{mx}} \geq 0.9$). This process resulted in 1550 animals with no missing genotypes and for which both the paternally and maternally inherited haplotypes were precisely estimated. LD was estimated by D' using the set of maternally inherited haplotypes.

Results and discussion

Polymorphism discovery

Eleven SNPs, one variable copy duplication and one microsatellite were identified by sequencing *ADIPOQ* in five

registered Angus sires (DQ156119 and DQ156120). Seven SNPs and one mononucleotide repeat were identified by sequencing *SST* in the same sires (DQ156121). Gene models for *ADIPOQ* and *SST* indicating the locations of the detected polymorphisms are in Fig. S1.

Of the 21 detected variable loci, we selected ADIPOQ:g.1431C>T, ADIPOQ:g.1436_1506dup and ADIPOQ:g.1596G>A (DQ156119); ADIPOQ:g.2606T>C (DQ156120); and SST:g.447A>G (DQ156121) for association analysis in Angus for the following reasons:

1 ADIPOQ:g.1436_1506dup is a variable-copy duplication of a 71-bp segment in the *ADIPOQ* promoter, which harbours a putative *Upstream Stimulatory Factor 1* (*USF1*) binding site. *USF1* is a transcription factor that regulates several genes involved in lipid and glucose homeostasis including *hepatic fatty acid synthase* (Casado *et al.* 1999), *hepatic lipase* (Botma *et al.* 2001), *adipocyte fatty acid synthase* (Wang & Sul 1997), *adipocyte hormone-sensitive lipase* (Smih *et al.* 2002) and *insulin* (Read *et al.* 1993). *USF1* has also been implicated in human familial combined hyperlipidaemia, which is characterized by elevated levels of serum cholesterol and triglycerides (Pajukanta *et al.* 2004). Thus, *USF1* may act as a transcription factor for *ADIPOQ*, and the duplication of its binding site within the promoter may influence transcription.

2 The human-to-bovine genomic *ADIPOQ* alignment revealed a region that contains ADIPOQ:g.1431C>T. It was about, 300-bp upstream of exon 1, possessed 80% homology and potentially harbours regulatory elements.

3 ADIPOQ:g.1596G>A is located in the initiator element of the type II promoter, which is a binding site for transcription factors $TAF_{II}150$ and $TAF_{II}250$. The binding of TAF_{II} transcription factors to the initiator sequence and their interaction with the Sp1 factor bound to the upstream GC boxes enable the anchoring of the transcription pre-initiation TFIID complex to the TATA-less promoter (Weaver 2002). By affecting the binding affinity, of $TAF_{II}150$ and $TAF_{II}250$ to the initiator sequence, the ADIPOQ:g.1596G>A mutation may affect the stability of the transcription initiation complex and consequently, the rate of *ADIPOQ* transcription.

4 ADIPOQ:g.2606T>C is distal to the promoter region and provides a locus that could potentially allow the separation of the location of any detected QTL effects from the *ADIPOQ* promoter loci.

5 One of the SST:g.447A>G alleles creates a binding site for the transcription factor *myocyte-specific enhancer factor 2* (*MEF2A*), while the other allele eliminates this site. The *MEF2A* protein is detected only in skeletal and cardiac muscle nuclei and belongs to a family of transcription factors involved in myocyte differentiation and myocyte-specific gene expression (Yu *et al.* 1992). *MEF2A*-binding elements regulate fast myosin heavy chain transcription *in vivo*, thus effecting muscle fibre composition (Allen *et al.* 2005). Consequently, the binding of a muscle-specific transcription factor could facilitate *SST* transcription in muscle tissues and affect carcass growth and yield traits.

Major allele frequencies for SST:g.447A>G, ADIPOQ:g.1436_1506dup, and ADIPOQ:g.2606T>C by sire birth year within the US Angus population are shown in Fig. 2. The trends in major allele frequencies for ADIPOQ:g.1431C>T and ADIPOQ:g.1596G>A, which flank ADIPOQ:g.1436_1506dup, were almost identical to ADIPOQ:g.1436_1506dup (Table 1). The expected major allele frequencies predicted by the weighted regression of allele frequency on birth year with weights being the number of observations within each year are also shown in Fig. 2. All five loci are clearly under selection for the minor allele at each locus; however, the selection has not been sufficient to perturb any of the loci from HWE ($P > 0.55$). Because this genomic region has previously been shown to harbour a marbling QTL and there has been significant historic selection for marbling (Fig. 1e), we expected *a priori* to find evidence for selection within this genomic region. The rate of allele frequency change for ADIPOQ:g.1436_1506dup was greater than for all other loci except ADIPOQ:g.1431C>T (Table 1). Despite the fact that this locus is flanked by ADIPOQ:g.1431C>T and ADIPOQ:g.1596G>A, which are separated by only 166 bp and the LD among all three pairs of loci was at least 0.97 (Table 1), these data suggest that ADIPOQ:g.1436_1506dup is under direct selection in Angus.

Association of polymorphisms with carcass traits

When we fit the general model to EPDs in the weighted analysis of variance of the 95 trait × marker combinations, we found 68 (71.6%) associations ($P < 0.05$). By eliminating those associations for which the additive model was rejected ($P < 0.1$), 39 (41.1%) of the trait × marker combinations remained significant (data not shown). Conversely, when we analysed the MS terms only 14 (14.7%) trait × marker combinations were significant ($P < 0.05$), and this was reduced to eight (8.4%) combinations after elimination of the associations for which the additive model was rejected (Table 2). Clearly, performing association analyses in populations in which the trait values and marker loci are both under selection resulted in numerous spurious associations even when the analysis required additivity of the marker effects on EPDs to declare an association. Conversely, analysis of the MS terms for which there was no time trend (Fig. 1b–f) appeared to protect against spurious associations particularly when additivity of marker effects was required to declare an association.

There is considerable variation in the amount of information available on the different traits for which EPDs are calculated in commercial cattle populations. We defined N_g to be the sum of the weights $1 - (1 - \text{Acc})^2$ for all animals with an EPD for a given trait (Table 2). In a sample of N

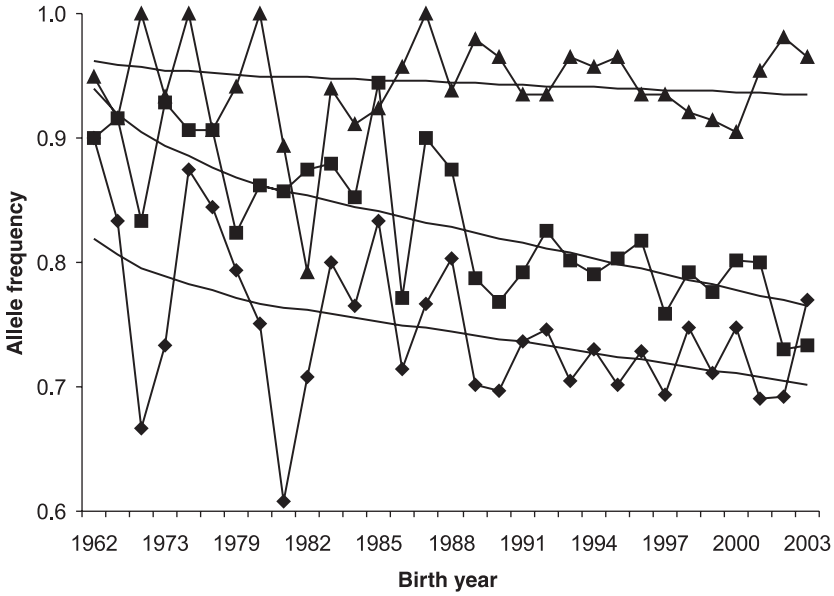

Figure 2 Actual and expected major allele frequency by year of birth for SST:g.447A>G (▲), ADIPOQ:g.1436_1506dup (■) and ADIPOQ:g.2606T>C (◆). Frequencies for ADIPOQ:g.1431C>T and ADIPOQ:g.1596G>A were similar to those for ADIPOQ:g.1436_1506dup (data not shown).

Table 1 Population statistics for *adiponectin* (*ADIPOQ*) and *somatostatin* (*SST*) polymorphisms in Angus.

	Polymorphism[1]				
	SST:g.447A>G	ADIPOQ:g.1431C>T	ADIPOQ:g.1436_1506dup	ADIPOQ:g.1596G>A	ADIPOQ:g.2606T>C
MAF[2]	0.9414	0.8114	0.8065	0.8076	0.7299
$\Delta F/yr$[3]	−0.0006483[a]	−0.0040593[b]	−0.0042040[b]	−0.0037564[c]	−0.0028217[d]
SE[3]	0.0000911	0.0001050	0.0001025	0.0000950	0.0001221
Genotypes[4]					
MM	1476	1088	1089	1079	870
Mm	181	505	514	504	651
mm	7	59	66	65	117
Total	1664	1652	1669	1648	1638
D´					
ADIPOQ:g.1431C>T	0.9263				
ADIPOQ:g.1436_1506dup	0.9268	0.9733			
ADIPOQ:g.1596G>A	0.9268	0.9822	0.9823		
ADIPOQ:g.2606T>C	0.4802	0.9596	0.9749	0.9699	

[1]Major allele > minor allele is defined in locus designation. For ADIPOQ:g.1436_1506dup, the two-copy 71-bp tandem repeat is the minor allele.
[2]Major allele frequency.
[3]Regression of major allele frequency on birth year of sire ± SE. Values with different superscripts differ by $P < 0.05$.
[4]M, major allele; m, minor allele.

individuals, $0 \leq N_g \leq N$ provides a measure of the extent of genetic information available on the individuals within the sample. While our sample size ranged from 1208 to 1546 bulls with EPDs, the equivalent number of bulls with perfectly known genetic merit ranged from 419.45 to 961.3 (Table 2). This difference demonstrates the difficulty that producers face in obtaining carcass data in commercial cattle populations and indicates that large samples from commercial populations (such as in this study) are required for QTL detection for carcass traits.

Significant associations were detected for SST:g.447A>G with marbling score and YH (Table 2). While two of the four *ADIPOQ* loci yielded significant marbling associations in the general model, the additive model was rejected for all four loci, suggesting that *ADIPOQ* has no direct effect on marbling but that there may be a marbling QTL on BTA1 in

Table 2 Associations between adiponectin (*ADIPOQ*) and somatostatin (*SST*) polymorphisms and Mendelian sampling (MS) terms for growth and carcass traits of Angus cattle.

Locus	Trait[1]	N[2]	N_g[3]	$\alpha \pm$ SE[4]	P_G[5]	P_{GOF}[6]	P_α[7]	Genotypic values[8]		
SST:g.447A>G				*A–G*				*AA*	*AG*	*GG*
	YH	1542	956.3	−0.202 ± 0.065	0.0032	0.1891	0.0018	0.055	0.141	0.476
	MARB	1233	476.1	−0.137 ± 0.053	0.0090	0.1042	0.0092	0.007	0.091	−0.072
	FAT	1233	427.3	−0.011 ± 0.007	0.2543	0.8295	0.1008	−0.003	0.002	0.012
	URE	1541	914.8	−0.016 ± 0.051	0.2200	0.0873	0.7475	0.090	0.112	−0.138
ADIPOQ:g.1431C>T				*C–T*				*CC*	*CT*	*TT*
	YH	1529	950.8	0.030 ± 0.039	0.7437	0.9690	0.4420	0.070	0.056	0.039
	MARB	1224	473.2	0.015 ± 0.032	0.0282	0.0085	0.6403	0.013	0.033	−0.100
	FAT	1224	424.7	0.015 ± 0.004	0.0011	0.2459	0.0004	0.001	−0.008	−0.008
	URE	1531	911.1	0.107 ± 0.030	0.0017	0.8079	0.0004	0.112	0.062	−0.002
ADIPOQ:g.1436_1506dup				*1–2*[9]				*11*	*12*	*22*
	YH	1545	961.3	0.022 ± 0.038	0.7764	0.6998	0.5498	0.072	0.055	0.065
	MARB	1236	477.4	0.012 ± 0.031	0.0604	0.0193	0.7093	0.014	0.033	−0.078
	FAT	1236	428.4	0.013 ± 0.004	0.0027	0.1482	0.0018	0.000	−0.008	−0.006
	URE	1546	919.9	0.103 ± 0.029	0.0017	0.6957	0.0004	0.113	0.065	−0.002
ADIPOQ:g.1596G>A				*G–A*				*GG*	*GA*	*AA*
	YH	1527	948.4	0.025 ± 0.039	0.6676	0.5366	0.5139	0.070	0.049	0.071
	MARB	1223	472.4	0.005 ± 0.032	0.0736	0.0227	0.8679	0.012	0.034	−0.072
	FAT	1223	424.1	0.016 ± 0.004	0.0003	0.3058	0.0001	0.001	−0.009	−0.010
	URE	1526	907.7	0.107 ± 0.030	0.0013	0.7890	0.0003	0.114	0.063	−0.002
ADIPOQ:g.2606T>C				*T–C*				*TT*	*TC*	*CC*
	YH	1518	941.0	−0.015 ± 0.035	0.3365	0.1582	0.6662	0.055	0.083	0.032
	MARB	1208	467.4	0.010 ± 0.029	0.0006	0.0001	0.7326	0.004	0.044	−0.092
	FAT	1208	419.5	0.009 ± 0.004	0.0765	0.7966	0.0242	0.000	−0.005	−0.008
	URE	1517	896.0	0.063 ± 0.027	0.0086	0.0470	0.0182	0.104	0.096	0.003

[1]YH, yearling height in inches; MARB, USDA marbling score; FAT, fat thickness in inches; URE, ultrasound ribeye muscle area in square inches. Trait and polymorphism combinations for which the additive model was not rejected ($P > 0.10$) are indicated in bold face.

[2]Number of genotyped animals that had Mendelian sampling (MS) terms for the trait.

[3]Number of additive genotypic value equivalents for the trait defined as $N_g = \sum_{i=1}^{N} 1 - (1 - \text{Acc}_i)^2$.

[4]Allele substitution effect and standard error.

[5]P-value for $F_{2,N-2}$ test of the hypothesis of no genotypic class differences under the general model.

[6]Test of goodness-of-fit of the additive to the general genotype class models.

[7]Test of significance of the allele substitution effect under the additive model.

[8]Genotype class effects estimated under the general model. Numbers of genotypes in each class are in Table 1.

[9]Alleles are defined by either one (*1*) or two (*2*) copies of the 71-bp sequence.

the vicinity of *SST*. On the other hand, none of the *ADIPOQ* loci detected an association with YH in the general model, which suggests that this association may be a type I error considering the strong LD between SST:g.447A>G and the three most closely linked of the *ADIPOQ* loci (Table 1). Significant associations were also detected between the cluster of three *ADIPOQ* loci separated by 166-bp with fat thickness and URE. The lack of an association with ribeye muscle area determined in the carcasses of progeny of these bulls is of some concern, but may simply be due to different samples of progeny with carcass phenotypes as opposed to ultrasound evaluations. N_g was at least 895.9 for the association tests involving URE but was no more than 441.1 for the tests involving carcass ribeye muscle area. The strongest statistical effect and the largest allele substitution effects on fat thickness and URE were detected for ADIPOQ:g.1596G>A (Table 1), which is located in the initiator element of the type II promoter and may affect the rate of transcription of *ADIPOQ*. However, the regression of allele frequency on year of sire birth (Table 1) indicates that of these three loci, ADIPOQ:g.1436_1506dup is under the strongest selection, and this suggests that ADIPOQ:g.1596G>A probably does not have a causal effect upon these traits. Nevertheless, this might also be due a complex pattern of selection in this region of the genome considering that several distinct QTL influencing different traits appear to be nearby and that all these traits are under selection in Angus.

We confirmed the presence of QTL affecting marbling, ribeye muscle area and fat thickness in the vicinity of *SST* and *ADIPOQ* on BTA1 in Angus cattle. By utilizing MS terms as the dependent variables in our association tests, we reduced the false-positive rate of detection of associations and were able to test for the additivity of polymorphism

effects. This approach allowed us to exclude *SST* as underlying the QTL effect detected by Cai *et al.* (2004) on ribeye muscle area and also to exclude *ADIPOQ* as underlying the detected effect on marbling score. Association analysis *per se* provides very limited information concerning the location of any QTL and is most useful when performed within the context of a positional candidate gene study. Once a QTL has been located to a chromosomal region by linkage or LD analysis, mutations in positional candidate genes can be placed on the genetic map, aligned with the detected QTL position and their effects on phenotype tested. Only then are functional studies warranted to elucidate the mode of action of a putative causal mutation. Association studies performed in the absence of linkage or LD analyses, which use flanking marker data to localize QTL relative to positional candidate genes, have little intrinsic value.

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Supplementary Material

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Figure S1 Gene models for *ADIPOQ* and *SST* indicating the locations of polymorphisms detected in Angus.

Table S1 *ADIPOQ* and *SST* PCR and sequencing primers.

Table S2 *ADIPOQ* and *SST* genotyping primers.

Appendix S1 The mean MS effect is $q\alpha$, $1/2(q-p)\alpha$ and $-p\alpha$ for individuals with genotypes *QQ*, *Qq* and *qq* respectively.

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