QTL ANALYSIS OF BEEF FAT COLOUR AND THE EFFECT OF BCDO2

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SUMMARY

Most agricultural economic traits are controlled by both the environment and genetics. Locating quantitative trait loci (QTL) using molecular markers remains a key approach to identify the genes controlling those traits. In this study, the QTL for fat colour in beef cattle were mapped. The mapping herd was a double-backcross design using two extreme *Bos taurus* breeds (the Jersey dairy breed and Limousin beef breed). Three fat colour traits were measured (beta-carotene concentration in subcutaneous fat and fat colour scores of biopsy and carcass fat), and a total of 16 QTL on 13 chromosomes were detected at the 5% genome-wise significance level.

A potential candidate gene in QTL on BTA 15, *β, β-carotene-9', 10'-dioxygenase* (*BCDO2*) was investigated. A SNP in exon 3 which creates a stop codon was previously identified, and inclusion of this *BCDO2* SNP as a fixed effect in the linkage analysis of the fat colour traits changed the F-values for a number of QTL. As expected, the QTL on BTA 15 was no longer significant. As the presence of the *BCDO2* genotype in the model reduced the residual variance, three additional QTL were detected for biopsy fat colour score and one additional QTL for carcass fat colour score.

INTRODUCTION

Beef with yellow fat is considered undesirable by the consumers in most European and Asian markets. Presumably, this is because beef with yellow fat is perceived as being from old or diseased animals. More than AUS$18 million is lost to Australian beef producers annually due to the rejection of carcasses with high yellow fat colour scores by the Asian markets, such as Japan and Korea (Browne 1992). The yellowness is caused by the deposition of beta-carotene from green feed into the adipose tissue (Kruk 2001). Over the past decade, there has been an increasing emphasis on the development of molecular genetic tools, such as DNA markers, to improve beef production and quality through marker-assisted selection. In this study, a whole genome scan for QTL affecting fat colour related traits in half-sib families generated from Limousin and Jersey breed was conducted.

MATERIALS AND METHODS

Cattle resources. Purebred Jersey (J) and purebred Limousin (L) cattle were crossed to produce three pairs of half brothers F_1 progeny (X). One of the half brothers from each pair was mated to the purebred Jersey or Limousin cows in Australia and the other half brother was mated in New Zealand. A total of 366 backcross progeny (205 XJ and 161 XL) were born in the autumn over 3 years from 1996 to 1998 in Australia. β-carotene concentration was measured in subcutaneous fat sampled at slaughter and in fat biopsy samples taken from around the tail at 12 months of age. Fat colour score of the adipose biopsy samples was estimated on a 5-point scale (l = white to 5 = very yellow) immediately after removing the fat from biopsy site and rinsing with water. Yellowness in the subcutaneous fat was also scored visually after slaughter, assessed on a 10-point (l=white to 10=very yellow) scale by trained assessors using standard colour chips (AUS-MEAT 1990) (Kruk 2001). Fat colour and beta-carotene data were examined for distribution before the analysis. Only the beta-carotene distribution was positively skewed. Therefore, the beta-carotene values were logarithmic transformed to reduce the skewness (Esmailizadeh 2006). DNA was extracted from all

cattle, and approximately 190 microsatellite markers were genotyped per animal, providing a whole-genome scan at roughly even 20 cM intervals.

Model of analysis. Linkage of each trait to markers on the chromosomes was tested using the QTL Express software package (Seaton *et al.* 2002; http://qtl.cap.edu.ac.uk, accessed 3rd November 2006). The analysis uses the multimarker approach for interval mapping in the half-sib families, as described by Knott *et al.* (1996). Within every half-sib family, a QTL was fitted at 1-cM intervals along the chromosome. The least squares regression model was used for the QTL mapping and included the fixed effects of breed of dam (J or L) and cohort (96H, 96S, 97H, 97S, 98H, 98S). 5% genome-wise significance thresholds were determined using permutation methods to account for multiple testing (Churchill and Doerge 1994). In general, an *F* statistic of greater than 7 was set as the threshold (Esmailizadeh 2006). A t-test was calculated for the most likely position of a QTL. The *BCDO2* gene effect (θ_k) was excluded in the first model (model 1) and included in the second model (model 2).

$Y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_l + bx + e_{ijkl}$ (model 1)

$Y_{ijkl} = \mu + \alpha_i + \beta_j + \theta_k + \delta_l + bx + e_{ijkl}$ (model 2)

Where

μ overall mean,

α_i effect of the i^{th} breed (Jersey or Limousin),

β_j effect of the j^{th} cohort (six levels),

θ_k effect of the k^{th} genotype (AA, GA, GG),

δ_l effect of l^{th} sire, b_l is the allele substitution effect of the QTL within family l, x_l is the probability that animal *m* inherited the arbitrarily assigned first haplotype of sire l,

bx b is the allele substitution effect of the putative QTL, x is the probability that animal l inherited the arbitrarily assigned first haplotype of the sire, and

e_{ijkl} is the residual effect.

RESULTS AND DISCUSSION

A nonsense mutation SNP (W240X) was found in the *BCDO2* gene (Tian, *et al* accepted). This SNP was located at base 240 from the start of coding region, and causes an amino acid change at position 80 from a tryptophan (encoded by the G allele) to a stop codon (encoded by the A allele). Association analysis of this nonsense mutation showed significant differences in subcutaneous fat colour and beta-carotene concentration amongst cattle with different *BCDO2* genotypes. Therefore, the *BCDO2* SNP W240X was fitted in the model to detect new QTL for fat colour related traits.

Allele substitution or sire QTL effects, estimated QTL locations and F-statistic value were calculated (Table 1). Without the *BCDO2* SNP W240X in the model, a total of 16 QTL segregating in all the individual sire families on 13 chromosomes were detected at the 5% genome-wise significance level. The largest QTL was for carcass fat colour near 74 cM on chromosome 12.

When the SNP W240X was fitted as a fixed effect in the model, there are some changes the F values and positions of the QTL. A large decrease occurred in the BTA 15 QTL in family 398 for biopsy fat colour. The test statistic diminished at 45cM for BTA15 as expected, since this is the location of the *BCDO2* gene. However, another 4 new QTL were observed, for which the test statistic increased from non-significant to significant on BTA1, BTA7, BTA8 and BTA14.

Vitamin A is essential for mammals. One of the functions of carotenoids is to serve as the precursor of vitamin A. The enzyme *BCDO2*, which specifically acts at the 9' 10'-double bond of β-carotene, results in the formation of β-ionone and two molecules of β-apocarotenal with different chain lengths (Von Lintig and Vogt 2004). Given that the *BCDO2* protein is 530 amino acids in length, this change will result in the truncated polypeptide and presumably the loss of *BCDO2* protein function. So the excentric cleavage pathway of β-carotene will be affected and thus β-

carotene accumulation in the adipose tissue and changes the colour in beef fat. As a potential candidate gene on chromosome 15, this SNP was fitted as a fixed effect in the model. The test statistic for QTL on BTA15 diminished as expected. No other QTL diminished, so there was no evidence of other epistatic QTL in this analysis.

Table 1. QTL segregating in single sire families

BTA	*Trait*	*family*	*with/without SNP W240X*	*QTL position*	*F value*	*sire QTL effects*
1	Fcam[1]	sire 361	model without SNP W240X	5cM	7.68	-0.49 (±0.18)
1	Fcam	sire 361	model with SNP W240X	5cM	7.14	-0.48 (±0.18)
1	Fcbiop[2]	sire 398	model without SNP W240X	23cM	2.47	-0.25 (±0.16)
1	Fcbiop	sire 398	model with SNP W240X	0cM	6.72	-0.34 (±0.13)
2	Bcbiop[3]	sire 361	model without SNP W240X	64cM	7.61	0.23 (±0.09)
2	Bcbiop	sire 361	model with SNP W240X	64cM	7.11	0.23 (±0.09)
6	Fcam	sire 361	model without SNP W240X	54cM	7.02	0.47 (±0.18)
6	Fcam	sire 361	model with SNP W240X	54cM	6.69	0.46 (±0.18)
6	Fcbiop	sire 361	model without SNP W240X	46cM	9.03	-0.26 (±0.09)
6	Fcbiop	sire 361	model with SNP W240X	46cM	8.96	-0.26 (±0.09)
6	Fcbiop	sire 368	model without SNP W240X	68cM	7.96	-0.25 (±0.09)
6	Fcbiop	sire 368	model with SNP W240X	67cM	7.62	-0.25 (±0.09)
7	Bcbiop	sire 368	model without SNP W240X	46cM	5.58	0.16 (±0.07)
7	Bcbiop	sire 368	model with SNP W240X	43cM	7.21	0.18 (±0.07)
8	Bcbiop	sire 398	model without SNP W240X	0cM	3.65	-0.26 (±0.13)
8	Bcbiop	sire 398	model with SNP W240X	0cM	7.33	-0.33 (±0.12)
8	Fcam	sire 361	model without SNP W240X	53cM	11.8	0.74 (±0.22)
8	Fcam	sire 361	model with SNP W240X	52cM	12.29	0.77 (±0.22)
11	Fcbiop	sire 368	model without SNP W240X	98cM	15.29	0.3 (±0.08)
11	Fcbiop	sire 368	model with SNP W240X	104cM	16.89	0.35 (±0.09)
12	Bcbiop	sire 361	model without SNP W240X	98cM	8.46	-0.35 (±0.12)
12	Bcbiop	sire 361	model with SNP W240X	98cM	8.38	-0.35 (±0.12)
12	Fcam	sire 368	model without SNP W240X	74cM	17.87	-0.73 (±0.17)
12	Fcam	sire 368	model with SNP W240X	74cM	18.23	-0.73 (±0.17)
14	Fcam	sire 398	model without SNP W240X	0cM	2.34	0.39 (±0.26)
14	Fcam	sire 398	model with SNP W240X	21cM	10.39	0.7 (±0.22)
14	Fcbiop	sire 361	model without SNP W240X	0cM	15.45	-0.34 (±0.09)
14	Fcbiop	sire 361	model with SNP W240X	0cM	15.75	-0.34 (±0.09)
15	Fcbiop	sire 398	model without SNP W240X	45cM	7.23	0.54 (±0.2)
15	Fcbiop	sire 398	model with SNP W240X	89cM	4.25	0.27 (±0.13)
16	Bcbiop	sire 361	model without SNP W240X	51cM	7.64	-0.23 (±0.08)
16	Bcbiop	sire 361	model with SNP W240X	51cM	7.66	-0.23 (±0.08)
16	Bcbiop	sire 368	model without SNP W240X	85cM	6.94	-0.18 (±0.07)
16	Bcbiop	sire 368	model with SNP W240X	85cM	6.91	-0.18 (±0.07)
17	Bcbiop	sire 368	model without SNP W240X	70cM	7.53	-0.19 (±0.07)
17	Bcbiop	sire 368	model with SNP W240X	70cM	6.83	-0.18 (±0.07)
20	Fcam	sire 368	model without SNP W240X	17cM	7.82	0.48 (±0.17)
20	Fcam	sire 368	model with SNP W240X	17cM	9.04	0.51 (±0.17)
24	Fcbiop	sire 361	model without SNP W240X	46cM	6.9	-0.31 (±0.12)
24	Fcbiop	sire 361	model with SNP W240X	46cM	6.84	-0.31 (±0.12)

[1]Fcam: fat colour of carcass on a 10 point scale. [2]Fcbiop: fat colour of biopsy samples on a 5 point scale. [3]Bcbiop: β-carotene concentration of fat biopsy samples (μg/g fat)

CONCLUSION

In summary, inclusion of the *BCDO2* SNP as a fixed effect in the linkage analysis of the fat colour traits changed the F-values for a number of QTL and four new QTL for fat colour traits were identified. The new QTL were presumably observed because the *BCDO2* SNP accounted for some of the residual variation in these fat colour traits. Based on these QTL, a number of additional candidate genes have been selected and are currently being investigated for their potential association with beef fat colour.

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