QTL ANALYSES OF GROWTH TRAITS ON CATTLE CHROMOSOME 14

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SUMMARY

Several authors have reported QTL for growth on cattle chromosome (BTA)14, with positions ranging from the centromeric end to just after the midpoint of the chromosome. Previously, we have identified two possible growth QTL on BTA14 from our Jersey-Limousin double back-cross design. In the present study, these QTL have been tested in Angus cattle from a yearling-weight selection trial with selected and control animals. A candidate gene, corticotrophin-releasing hormone (CRH), has been identified which is associated with 600-day live weight in cattle.

Keywords: *Bos taurus,* QTL, growth, CRH.

INTRODUCTION

A collaborative study began in 1995 between AgResearch in New Zealand and the University of Adelaide in Australia to search for DNA markers linked to production, carcass and beef meat quality traits. A group of markers on chromosome (BTA)14 were associated with live weight (Morris *et al.* 2002). We test here whether these micro-satellite markers are segregating in an independent weight-selected line of cattle, and if a candidate gene under the QTL might be associated with live weight.

MATERIALS AND METHODS

Trial design. *Jersey-Limousin.* The trial design involved dams of two of the more extreme *Bos taurus* breeds, Jersey (J) and Limousin (L). Three pairs of first-cross JxL or LxJ half-brothers were generated in this project, and one of each pair was used for mating in New Zealand or Australia with both J and L cows, to produce a total of about 400 heifer or steer back-cross progeny in each country. The marker-search involved identifying sire-derived alleles in the calves whose presence was associated with performance in one or more traits ("phenotypes"). The primary traits of interest were carcass composition and measures of beef meat quality. Other simple traits during the growth phase were also recorded, such as live weights and ultrasound measurements. New Zealand animals grazed mainly on a pasture diet and were weighed monthly until slaughter. For the two calf crops in New Zealand (1996 and 1997 births), the phenotype used in the present study was live weight at approximately 600 days of age (W600), the last live weight recorded before slaughter commenced.

Angus weight-selection lines. A long-term selection trial was initiated in 1971 with Angus cattle (Baker *et al*. 1991) at the then Waikite Lands and Survey block. The adjusted-yearling weight selection line (Selected) and the control line (Control) were continued until 2002. Selection achieved almost 1% gain per year in yearling live weight over the term of this trial. DNA samples were collected in 2000 from cows and heifers born in the herd from 1993 through to 1999.

Corticotrophin-releasing hormone (CRH) has been identified as a candidate gene near BMS108 on BTA14, using animals from a Canadian reference herd (Buchanan *et al.* 2002). A single nucleotide polymorphism (SNP) at nucleotide 240 (amino acid 77) was tested in a population of 429 unrelated bulls selected from Canadian sire summaries, revealing a significant ($P<0.05$) relationship between genotype and weaning- and yearling-weights. This SNP (C240G) and two other SNP (C22G [amino acid 4] and A144G [amino acid 45]) within CRH (Buchanan *et al.* 2005) were tested in our study.

Data analyses. The Angus Selected and Control lines were genotyped for six micro-satellite markers on BTA14, to confirm a growth QTL from the JxL cattle (Morris *et al*. 2002, 2003), testing for line differences in allele frequency using Chi-square. In a more refined analysis, 'Peddrift' was then used (Dodds and McEwan 1997) to account for genetic drift, and founder and sampling effects, and the significance level for the Chi-square statistic was tested using simulation over 1000 iterations. For the candidate gene CRH, SNP (defined above) from within the gene were genotyped in both the Angus cattle (to test for a genotypic association with Selected or Control lines, by Chi-square), and also in the JxL resource (to test for a genotype effect on W600 (1996 calves only), by least squares analysis).

Table 1. Test for similar BTA14-marker frequencies in animals from the Angus Weight-Selection and Control lines, and 'Peddrift' analysis of the lines (probability estimates)

Marker	Position (cM)	Chi-square P-value	Peddrift (1000 iterations) P-value
BMS1678	6.2	3.83×10^{-05}	0.175
RM180	19	4.94×10^{-04}	0.337
ILSTS008	35.2	0.45	0.742
BMS108	50.8	2.63×10^{-23}	0
BM4513	62.5	3.01×10^{-12}	0.013
BL1036	78.7	3.27×10^{-04}	0.432

RESULTS

Association tests in the Angus lines. Micro-satellite marker results on BTA14 in the Angus herd are shown in Table 1, with contingency Chi-square P-values. Map positions were from the MARC map (Kappes *et al.* 1997). Two markers were very highly associated with Selected or Control line. Peddrift results are also given in Table 1, indicating that dissimilar allele frequencies at marker BMS 108 were not a chance event following genetic drift, founder or sampling effects. Six of the seven alleles of BMS108 were completely fixed in one line or the other. Distributions are shown in Table 2. This marker must be closely linked with a gene segregating under selection pressure.

CRH candidate gene tests in the Angus lines and Jersey-Limousin back-crosses.
Allele frequencies for SNP A144G and C240G were significantly associated with Angus lines (Table 3). In contrast, the SNP C22G and A144G were associated with W600 in the JxL resource (Table 4).

Table 2. Allele frequencies for BMS108 in the Angus Weight-Selected and Control lines

Line	Allele size (base-pairs)						
	104	106	108	110	112	114	116
Selected	1	4	0	17	0	14	36
Control	0	0	38	0	23	15	0

Table 3. Genotype counts for three CRH SNP, and their distributions in Angus Weight-Selected or Control lines, with Chi-square tests (P-values) for differing distributions by line

SNP	Genotype	Line		P-value
		Selected	Control	
C22G	CC	9	9	0.87
	GC	22	22	
	GG	7	5	
	Freq 'C'	0.526	0.556	
A144G	AA	1	0	0.00067
	AG	20	5	
	GG	17	33	
	Freq 'A'	0.290	0.066	
C240G	CC	1	6	0.00133
	CG	11	20	
	GG	26	10	
	Freq 'C'	0.171	0.444	

Table 4. Effects of 3 SNP in CRH for sire-derived, dam-derived or calf genotype on live weight at 600 days of age (W600) in the Jersey-Limousin resource; P-values in brackets (n=141 calves)

SNP	Genotype	n	W600 (kg)	Sire-derived	Dam-derived	Sire- & dam-derived
C22G	CC	43	393	C-G	C-G	CC-GG
	CG	66	382	12.6±4.3 (.003)	11.3±6.2 (.07)	19.5±9.7 (.05)
	GG	25	376			
A144G	GG	77	391	G-A	G-A	GG-GA
	AG	56	375	12.6±4.3 (.003)	16.4±7.7 (.04)	16.2±6.6 (.016)
	AA	8	379			
C240G	CC	9	393	C-G	C-G	CG-GG
	CG	42	390	17.0±8.5 (.048)	5.7±6.7 (.39)	11.1±8.8 (.21)
	GG	88	381			

DISCUSSION

The almost complete fixation of BMS108 alleles in the Angus Selected or Control line indicates that selection for live weight has changed the frequency of alleles at this site, and this marker must be

very closely linked to a gene associated with differences in yearling weight under selection. CRH is a candidate for growth, according to Canadian studies. The question of which SNP to use as a marker for growth is still unclear. C240G was identified in the original Canadian work. In our Angus lines, both A144G and C240G showed significant line differences in genotype frequencies, while C22G was almost equally distributed. However, in the JxL animals genotyped so far, C240G showed much less effect on W600 than did either C22G or A144G. The A144G SNP in the Angus population showed that the 'A' allele was at greater frequency in the Selected line, but there was only one animal with an 'AA' genotype. Presumably our superior weight-selected animals are 'AG' and this raises the question why there were so few 'AA' animals in our population. Mating and calving data suggest that it is not due to a homozygous lethal condition. Peddrift analyses of both A144G and C240G indicate that allele frequency differences between the two Angus lines could have occurred through genetic drift. We also derived sire and dam haplotypes for the three SNP in the JxL population. The effects of haplotypes observed in the sires were similar to those observed in the dams (though non-significant, probably because of low numbers), when fitted together (data not shown). The 'CGC' haplotype was consistently heaver than 'GAG'. Further genotyping of more recent calf crops from the Selected line in this Angus herd should clarify the situation, along with completion of genotyping all animals in the JxL trial.

The data here do not point conclusively to any one SNP from CRH being linked to live weight, and it is possible that another gene in the same region may be responsible for the observed effects. In fact the A144G and C240G SNP both appear to be associated with growth in opposite directions in the two resources. CRH has many roles, particularly associated with stress and appetite, both of which could have major effects on live weight.

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