MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) FOR MUSCULARITY IN BEEF CATTLE

I. Novianti, W.S. Pitchford and C.D.K. Bottema

Cooperative Research Centre for Beef Genetic Technologies
School of Agriculture, Food and Wine, The University of Adelaide,
Roseworthy SA 5371 Australia

SUMMARY

Muscularity is a potential indicator for the selection of more productive cattle. Mapping quantitative trait loci (QTL) for traits related to muscularity is useful to identify the genomic regions where the genes affecting muscularity reside. QTL analysis from a Limousin-Jersey double backcross herd was conducted using QTL Express software with cohort and breed as the fixed effects. Nine QTL suggested to have an association with muscularity were identified on cattle chromosomes BTA 1, 2, 3, 4, 5, 8, 12, 14 and 17. The myostatin gene is located at the centromeric end of chromosome 2 and not surprisingly, the Limousin *myostatin* F94L variant accounted for the QTL on BTA2. However, when the *myostatin* F94L genotype was included as an additional fixed effect, the QTL on BTA17 was also no longer significant. This suggests that there may be gene(s) that have epistatic effects with *myostatin* located on cattle chromosome 17.

INTRODUCTION

Muscularity can be defined as "the thickness of muscle relative to the dimensions of the skeleton" (Boer *et al.,* 1974). To select for muscularity, estimated breeding values for loin eye muscle area (EMA) adjusted to a 300kg carcass can be used (Graser *et al*. 2005). Eye muscle area is also used in calculating retail beef yield (RBY). EMA and RBY are relatively highly heritable (Koots *et al*. 1994) and estimated breeding values for these traits can be applied in selection programs to breed cattle for specific market requirements. However, many carcass traits that are commonly selected, such as hot standard carcass weight, are not sufficient to describe the ability of the animals to produce meat since these traits are also affected by the overall size of the animal. Therefore, other muscularity traits, such as meat percentage (retail beef yield) and meat to bone ratio, would be better descriptors. Unfortunately, such traits are not usually recorded and only a limited number of quantitative trait loci (QTL) for these muscularity traits have been mapped. Finding QTL is necessary in order to identify the regions of the genome that may contain genes affecting the traits of interest.

One gene known to have a significant role in muscle development is *myostatin.* McPherron *et al*. (1997) determined the biological function of *myostatin* by knocking out the gene in mice and demonstrating that the mutant mice were larger than the wild type mice as a result of increased muscle mass. The results proved that *myostatin* has an important role in skeletal muscle development by inhibiting muscle overgrowth.

Studies have also reported that there are many other proteins involved in the *myostatin* regulation pathway of muscle development (McPherron *et al.* 1997; Hill *et al.* 2002; Hill *et al.* 2003; Lee 2004; Dominique and Gerard 2006). Therefore, there are likely to be other genes that interact with *myostatin*. The objectives of this project were to identify QTL for muscularity and related carcass traits and to determine if there are QTL that may be epistatic with the *myostatin* gene.

MATERIALS AND METHODS

Materials. Genotype and phenotype data from the JS Davies cattle gene mapping project were used for this study. Two breeds of cattle were used for this project, Limousin and Jersey. The two breeds (Jersey and Limousin) were chosen in the project to maximise the trait variation in the progeny from their crosses. Limousin is a beef breed of a moderate to large frame, while Jersey is a small frame dairy breed. Limousin cattle have the F94L *myostatin* genotype which affects retail beef yield (Sellick *et al.* 2007).

The first phase on this study was conducted in 1993 by mating 280 purebred Jersey and Limousin cows to produce the first cross progeny, namely Limousin x Jersey F_1, which were born in 1994 and 1995. In the second phase, three Limousin x Jersey F_1 sires were mated to the pure Jersey and Limousin dams in Australia and New Zealand (NZ) to produce double backcross animals, called Limousin cross progeny and Jersey cross progeny herein (Sellick *et al.* 2007). There were 161 Limousin cross progeny and 205 Jersey cross progeny born in Australia.

The phenotypic traits that were used for this study were hot standard carcass weight (HSCW), meat weight, meat percentage, bone weight, bone percentage and meat to bone ratio. All traits except HSCW were estimated using regression equations from previous bone-out trials based on HSCW, fat depth, loin eye muscle area, the weight of 2-3 cuts and 2-3 bones with the protocol differing slightly for each cohort as described in Esmailizadeh *et al. (*2008*)*. This study used the genotype data from 150 microsatellite markers in the 3 F_1 sires and their progeny.

Mapping QTL. QTL Express software (http://qtl.cap.ed.ac.uk/) was used to map the QTL by regression analysis of phenotypes (HSCW, meat weight, meat percentage, meat to bone ratio) and genotypes obtained from all the backcross progeny. The software is suitable for half-sib outbred populations and F_2 populations (both inbred and outbred crosses) (Seaton *et al.* 2002). A multiple marker approach for interval mapping in the half sib families was used as described by Knott *et al.* (1996) and completed at 1 cM intervals along the chromosome. Based on Knott *et al*. (1996), three steps were applied. Firstly, informative marker alleles from the sires (361, 368 and 398) were identified to determine which allele the progeny inherited (there were 366 progeny in total) so that the sire gametes for the markers could be re-formed. On average, the sires were informative for 189 loci (Esmailizadeh 2006). Secondly, probabilities of the individual progeny inheriting either allele 1 or 2 from the sires were calculated. Then, these probabilities were combined and provided coefficients on which the phenotypic data can be regressed. Cohort (six levels), breed (Limousin cross and Jersey cross), with and without *myostatin* F94L genotype (CC, CA, AA) were included as fixed effects and were nested within the sire. Three covariates were used: HSCW as a covariate for meat weight, bone weight as a covariate for meat weight and bone percentage as a covariate for meat percentage. Significant QTL were defined by selecting the QTL maxima with F-values greater than 4 as the threshold for the 3 sire families (Lander and Kruglyak 1995). F-values greater than 4 represent $P<0.05$ with 3 degree of freedom (for the 3 sire families).

RESULTS AND DISCUSSION

QTL for all the traits (HSCW, meat weight, meat percentage and meat to bone ratio) were detected on BTA 1, 2, 3 ,4, 5, 8, 12, 14 and 17 (Table 1). There were 4 QTL for HSCW, 3 QTL for meat to bone ratio, 4 QTL for meat weight with HSCW as a covariate, 3 QTL for meat weight with bone weight as a covariate, and 3 QTL for meat percentage with bone percentage as a covariate. Of these, 1 QTL was in common for all the traits on BTA 17. All traits except HSCW also had major QTL on BTA 2. The QTL for meat percent and meat-to-bone ratio are of particular interest as they may represent genes that specifically control muscle mass rather than just increased growth.

Table 1. Significant QTL for muscularity related carcass traits with cohort and breed as fixed effects

BTA	Traits	F-value				QTL Location (cM)			
		Nocov	HSCW cov	Bone wt cov	Bone % cov	Nocov	HSCW cov	Bone wt cov	Bone % cov
1	HSCW	4.6				87			
1	MeatWt	4.28				98			
2	MeatWt	6.08	17.27	10.96		6	6	8	
2	Meat%	20.2			17.31	6			5
2	Mttobn	9.24				8			
3	MeatWt		4.06			-	100		
3	Meat%	4.12			4.45	100			100
4	MeatWt		4.28				37		
5	HSCW	6.08				41			
5	MeatWt	4.4				32			
8	MeatWt	5.11		4.19		57		17	
12	Mttobn	4.23				31			
14	HSCW	6.74				36			
14	MeatWt	5.39				35			
17	HSCW	4.09				85			
17	MeatWt		4.87	4.84			37	82	
17	Meat%	6.07			5.11	38			38
17	Mttobn	4.42				82			

Nocov = no covariate, Hscwcov=hot standard carcass weight as covariate, Bone wt cov=bone weight as covariate, Bone % cov=bone percentage as covariate, Hscw=hot standard carcass weight, Meatwt=meat weight, Meat%=meat percentage, Mttobn=meat to bone ratio. Column with shade represent traits that were not analysed using specified covariate. Only significant results are noted

In order to confirm the identified QTL, a second QTL analysis was conducted which included the Limousin *myostatin* F94L genotype as a fixed effect. This QTL analysis could thus identify other chromosomal regions that might contain gene(s) that interact with *myostatin*. The QTL on BTA 1, 3, 5 and 14 were not affected by the inclusion of *myostatin* F94L genotype as a fixed effect. Since the level of significance and the location of the QTL did not change, this suggests that there are genes in these regions which control muscularity but act independently of *myostatin.* There were minor effects for the QTL on BTA 4 and 8 as the F-value slightly decreased (Table 2).

On the other hand, there were major effects of the *myostatin* genotype detected for the QTL on BTA 2 and 17. The results for BTA 2 verified that the *myostatin* F94L genotype accounted for the QTL on BTA 2. Interestingly, the meat percent QTL on BTA 17 also disappeared with the inclusion of the *myostatin* F94L genotype in the model. The F-values for the other QTL on BTA17 also decreased, although not as dramatically.

A test of co-linearity between genotypes on BTA17 and myostatin was conducted to test whether this disappearance was a random effect. Probabilities of 0.59 for the overall alleles and 0.99 for the sire alleles were found. Thus, the *myostatin* allele and marker alleles were not correlated, implying that the QTL disappearance on BTA 17 is likely to be a consequence of an epistatic effect with *myostatin.*

Table 2. Changes in the QTL level of significance with *myostatin* F94L genotype fitted as a fixed effect with cohort and breed.

BTA	Traits	F-value			
		Nocov	Hscwcov	Bnwtcov	Bn%cov
2	MeatWt	3.07	2.31	2.39	
2	Meat%	2.72			2.44
2	Mttobn	1.44			
4	MeatWt		3.49		
8	MeatWt	3.66		3.92	
17	Hscw	3.66			
17	MeatWt		2.05	3.92	
17	Meat%	2.92			2.65
17	Mttobn	3.63			

Nocov = no covariate, Hscwcov=hot standard carcass weight as covariate, Bnwt=bone weight as covariate, Bn%cov=bone percentage as covariate, Hscw=hot standard carcass weight, Meatwt=meat weight, Meat%=meat percentage, Mttobn=meat to bone ratio. Column with shade represent traits that were not analysed using specified covariate

CONCLUSION

QTL for carcass traits related to muscularity were detected on chromosome 1, 2, 3, 4, 5, 8, 12, 14 and 17. The QTL found on BTA 2 and 17 affected the most traits of interest. The QTL on BTA 2 and 17 were no longer significant when the *myostatin* F94L genotype was included in the model. The QTL affected by the *myostatin* genotype on BTA2 were for meat weight, meat percent and meat-to-bone ratio, while the main QTL on BTA17 affected by *myostatin* was for meat percent. The results for BTA 2 verified that the *myostatin* F94L genotype accounted for the QTL on BTA 2, while the results on BTA 17 suggest that there may be gene(s) that interact or have an epistatic effect with *myostatin* for muscling on this chromosome.

REFERENCES

Boer, H.D., Dumont, B.L., Pomeroy, R.W. and Weniger, J.H. (1974). *Livest. Prod. Sci.* **1**:151.

Dominique, J.E. and Gerard, C. (2006) *Exp. Cell.* Res **312**:2401.

Esmailizadeh, A.K (2006) PhD Thesis. University of Adelaide.

Esmailizadeh, A.K., Bottema, C.D.K., Sellick, G.S., Verbyla, A.P., Morris, C.A., Cullen, N.G. and Pitchford, W.S. (2008). *J. Anim. Sci.* **86**:1038

Graser, H.U., Tier, B., Johnston, D.J. and Barwick, S.A. (2005) *Aust.J.Exp.Agric.* **45**:913

Hill, J. J., Davies, M.V., Pearson, J. H. Wang,J.H., Hewick,R.M., Wolfman, N.M. and Qiu, Y.C. (2002) *J. Biol. Chem*. **277**:40735.

Hill, J. J., Qiu, Y.C, Hewick,R.M. and Wolfman, N.M. (2003) *Mol. Endocrinol* **17**:1144.

Knott, S.A., Elsen, J.M. and Haley, C.S. (1996) *Theor. Appl. Genet.* **93:**71

Koots, K. R., Gibson, J.P., Smith, C. and Wilton, J.W. (1994) *Animal Breeding Abstracts* **62**:309.

Lander, E.S. and Kruglyak L. (1995) *Nat. Genet*.. **11:**241.

Lee, S. J. (2004) *Annu. Rev. Cell Dev. Biol*. **20**:61.

McPherron, A. C., Lawler, A.M. and Lee, S. J. (1997). *Nature* **387**(6628):83.

Seaton G., Haley C.S, Knott S.A., Kearsey M. and Visscher P.M. (2002). *Bioinf* **18:**339.

Sellick, G. S., Pitchford, W. S., Morris, C. A., Cullen, N. G., Crawford, A. M., Raadsma, H. W. and Bottema, C. D. K. (2007). *Anim. Genet.* **38**:440.