QTL ANALYSES OF BEEF MUSCLE FIBRE TYPE

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SUMMARY

Beef cattle have been selected in particular for increased growth rates and greater muscle development. The myostatin gene is responsible for the muscle hyperplasia in double muscled animals and it is also responsible in these animals for differences, during foetal development, in the relative proportions of muscle fibre types. A mutation in the myostatin gene in Limousin cattle (F94L) has been found to produce a milder form of double muscling. A whole genome scan identified a highly significant quantitative trait locus (QTL) at the proximal end of chromosome 2 in a Jersey-Limousin double backcross trial for percentage meat and percentage fat in the carcass, and also for proportions of fibre type IIb count and area in the *M. longissimus*. We propose that this same F94L mutation is responsible for the QTL associated with increased proportions of type IIb muscle fibres in the Jersey-Limousin crosses. A significant QTL for fibre type was also found on chromosome 13 ($P<0.05$).

INTRODUCTION

A collaborative study began in 1995 between AgResearch in New Zealand (NZ) and the University of Adelaide in Australia to search for DNA markers linked to production, carcass and beef meat quality traits (Morris *et al.* 2009). The present paper reports on muscle fibre type composition, which is one factor suggested as causing meat quality differences. Fibre-type composition has been reviewed in relation to meat quality by Klont *et al.* (1998). A sub-set of the animals born in NZ were analysed for muscle fibre type and we report here a QTL search performed to identify chromosomal regions with significant linkage to fibre type.

MATERIALS AND METHODS

Trial design. The trial design involved dams of two very different *Bos taurus* breeds, Jersey (J) and Limousin (L). In NZ, three first-cross JxL or LxJ bulls were mated with both J and L cows, to produce a total of about 400 heifer or steer back-cross progeny. The marker-search involved identifying in the calves sire-derived alleles 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. Animals in NZ grazed mainly on pasture. At slaughter, muscle samples were taken to measure meat quality during the aging process. For the first calf crop in NZ (1996 births), the phenotypes used in the present study were measures of fibre type distribution in the *M. longissimus thoracis et lumborum (M. longissimus)* at slaughter.

Sections from a slice of the *M. longissimus* were stained using myosin ATPase histochemistry (Martyn *et al.* 2004) and the counts and areas of each fibre type (I, IIa and IIb) were recorded for 192 animals. From the total samples analysed for each animal, the proportion of the total count and area from all the sections was calculated for each of the three muscle types.

Data analyses. The proportions of total count and area were first analysed by least squares to identify the appropriate model for a subsequent QTL scan. Factors tested were breed, birth type

(the ¾ Limousin were produced by embryo transfer and included many sets of twins), live weight prior to slaughter, sex and hot carcass weight. Sex was confounded with slaughter group as these animals were slaughtered in 18 groups at intervals of a week – animals being allocated to groups before slaughter commenced, with groups being balanced for sire, breed and live weight. Each slaughter group comprised only animals of the same sex with heifers being slaughtered on average earlier than steers. Significant effects were breed and sex, after slaughter group was nested within sex.

Table 1. Numbers of animals, means and residual standard deviations for the proportion of Count and Area for each of the 3 fibre types by breed and sex

			Count (proportion)			Area (proportion)		
			Type I	Type IIa	Type IIb	Type I	Type IIa	Type IIb
Breed	Sex	n	Mean	Mean	Mean	Mean	Mean	Mean
LJJJ	Heifer	54	0.244	0.430	0.326	0.197	0.418	0.385
	Steer	65	0.244	0.385	0.371	0.198	0.359	0.442
LJLL	Heifer	43	0.285	0.365	0.350	0.187	0.336	0.478
	Steer	30	0.281	0.325	0.394	0.182	0.294	0.523
All		192	0.259	0.384	0.357	0.193	0.360	0.447
		rsd*	0.042	0.053	0.057	0.037	0.063	0.073
		cv*	0.162	0.138	0.160	0.191	0.175	0.163

* rsd = residual standard deviation; cv = coefficient of variation

Initial Haley-Knott linkage (Knott *et al.* 1996) runs in SAS identified a large QTL at the proximal end of BTA2. Earlier published work (Sellick *et al.* 2007) had identified a mutation (F94L) in the myostatin gene derived from Limousin, which had a very large effect on the percentages of meat and of fat in a carcass. Records from 183 animals that had fibre type data and also carcass composition data from a butcher's dissection were run through a Haley-Knott routine with a model fitting breed, slaughter group within sex, and sire, to identify QTL. Marker positions were taken from the map of Ihara *et al* (2004). Permutation tests were conducted to determine thresholds for the significance of QTL. With the use of haplotypes (Sellick *et al.* 2007), the maternally inherited haplotype was also identified and we fitted both sire- and dam-derived haplotypes in an analysis of variance to determine the additive and dominance estimates for the myostatin mutation on fibre type distributions. (Some Limousin dams carried the wild-type allele, possibly reflecting the genetic background of some Limousin animals in NZ which were bred up by back-crossing). Similarly, the maternal haplotypes from mainly un-related J and L dams were used to test for linkage disequilibrium. To further test that this mutation was responsible for the differences described, the F94L genotype was fitted as a fixed effect in a re-run of the Haley-Knott scans, with the expectation that the highly significant QTL for fibre type would disappear.

RESULTS

The Haley-Knott graph for chromosome (BTA) 2 is shown in Figure 1 which also included the QTL for percentage of meat in the carcass. There were highly significant QTL identified for both Count and Area of type IIb fibres and lesser significant QTL for both type IIa and type I fibres. Given the fact that the three proportions sum to unity, this is not surprising but the residual correlations between pairs varied widely. For proportions of Total Count and of Total Area, type IIa and IIb have correlations of -0.71 and -0.85 respectively; type I correlations with types IIa and IIb are -0.33 and -0.43 for Count and -0.04 and -0.48 for Area.

A QTL, significant at the 5% level, was found on BTA13 (type I) for one family, and QTL at the suggestive level were found on BTA5 (types I and IIa), BTA7 (type IIb), BTA12 (types IIa and IIb), BTA13 (types I and IIb), BTA14 (type I), BTA20 (type I) and BTA22 (type I).

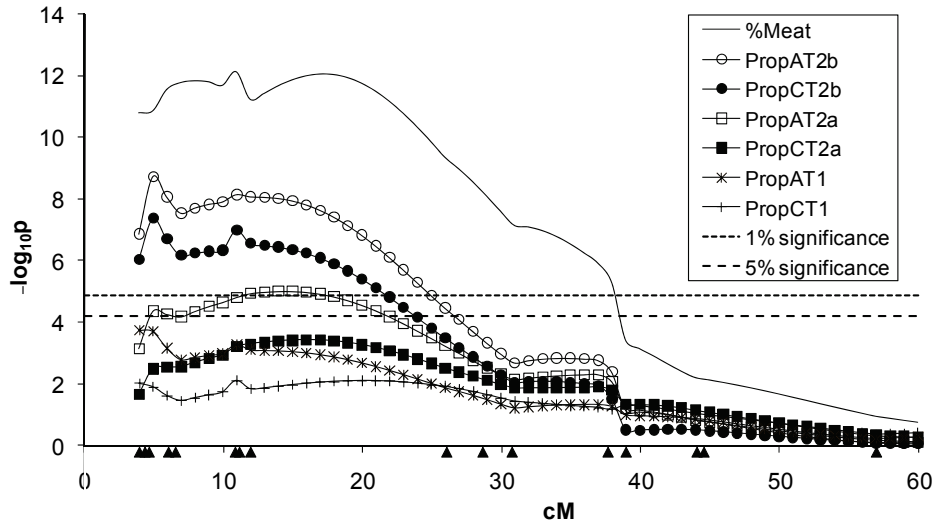

Figure 1. Haley-Knott plot for the first 60 cM of BTA2 with $-\log_{10}(p)$ values from across-family results. Trait abbreviations are Prop (Proportion), C or A (Count or Area) and T1 or T2a or T2b (Fibre Types I, IIa or IIb). Informative markers are shown (triangles) in relation to their distance (cM) along the chromosome, from left to right BY5, BULGE23, BTAFJ1, MSTN, BULGE20, ILSTS26, INRA40, TGLA431, TEXAN2, OARHH30, TGLA377, URB042, ILSTS30, OAFCB20, NEB, RM356.

The results from an ANOVA fitting additive effects (where the Limousin mutation was defined as positive) and dominance effects for the F94L genotype showed an additive effect but no dominance for 5 of the 6 fibre type traits. There was no effect on Count of type I fibres but a negative effect on Area of type I ($P < 0.01$). The Limousin allele had a large positive effect ($P < 0.0001$) on both measures for type IIb fibres and conversely a large negative effect on type IIa reflecting the high negative correlation between these phenotypes.

For the ANOVA fitting both the sire- and dam-derived haplotypes, effects for both parental alleles were again highly significant for all the type II measures. For type I, there was only a dam effect for Count ($P < 0.001$), whilst there were sire ($P < 0.01$) and dam effects ($P < 0.05$) for Area.

The re-runs of the Haley-Knott scans fitting, in addition, the myostatin F94L genotype reduced the test statistics to below the 5% significance threshold across families for 5 of the 6 fibre type traits on BTA2. The exception was type I Count which was significant at the 5% threshold and this was due to one sire family. This same family also still had a suggestive QTL for type I Area and IIb Count and meat percentage. In the complete data-set, this sire has more QTL for muscling and fatness than the other 5 sires – possible explanations are suggested in Morris *et al.* (2009).

DISCUSSION

Differentiation of muscle fibre type occurs during the foetal stage in mammals but it is possible that their relative proportions may be modified after birth by exercise. The J-backcross animals in this trial were hand-reared whilst the L-backcross animals were reared on their dams; both backcrosses grazed together from about 6 months of age in sex mobs until slaughter.

Highly double-muscled animals in breeds such as Belgian Blue and Piedmontese have been shown to have more type IIb fibres and less type I fibres, and this partly explains the overall increase in the whiteness of meat from such animals (Holmes and Ashmore 1972). The *M. longissimus* is a darker muscle than the *M. semitendinosus* and this is probably due to a higher proportion of the white type IIb fibres in the *M. semitendinosus*.

Our QTL trial with J and L breeds did not show QTL around myostatin for striploin (*M. longissimus*) percentage (Sellick *et al.* 2007) or striploin tenderness (Esmailizadeh *et al*. 2008) in Australia or NZ. However, in Australia, where tenderness was also measured on the *M. semitendinosus*, there were QTL in the myostatin region for both tenderness (Esmailizadeh 2006) and silverside weight as a percentage of carcass weight. This difference may be explained by differences in the proportion of type II fibres between the two muscles. Meat percentage in the whole carcass has a correlation of 0.35 with the proportion of type IIb fibres in the *M. longissimus* (and negative with the other two types); this correlation may be higher, for example, if calculated with the proportion of type IIb fibres in the *M. semitendinosus*.

The lack of any dominance in the effect of the F94L mutation on fibre types in the *M. longissimus* as opposed to a relatively large negative dominance effect on meat percentage and silverside percentage (Sellick *et al.* 2007) suggests that the three NZ sires were similar for other gene effects on fibre type.

Myosin genes also have a role in muscle development and some myosin genes appear under the other QTL reported here.

In conclusion, it appears that the F94L mutation found in Limousin cattle does have an effect on fibre type distributions in the *M. longissimus*, though its effect may be less than that of the inactivating mutations found in the Belgian Blue and Piedmontese breeds.

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