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Search for a locus near to myostatin that increases muscling in Texel sheep in New Zealand


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ABSTRACT

In close collaboration with three Texel breeders, we initially genotyped 36 rams with 5 DNA microsatellite markers that are known to map around the myostatin gene in sheep. Of these 36 rams, progeny of 12 polymorphic sires (4 from each flock) were selected for further analysis. This selection was made on the basis of each sire producing more than 40 offspring which were accompanied by parentage records, live weights at weaning and 8 months of age, and ultrasonic measurements of fat depth and rib-eye muscle dimensions. Final progeny numbers from the sires chosen ranged from 45 to 115 per sire. The data was analyzed by regressing the observed measurements on the probability of which paternal grandparental chromosome was inherited at the myostatin locus. The results suggest that the region of the sheep genome near the myostatin locus affects muscling (P<0.05) and fat depth (P<0.05) in the progeny of 4 of 12 sires tested.

Keywords: sheep, muscularity, QTL, myostatin

INTRODUCTION

Previous studies have indicated the presence of major genes for increased muscling in Texel sheep in Belgium and Australia (Marcq et al., 1998; Marshall et al., 1999). It has recently been suggested that, in Belgian Texels, the myostatin gene may be involved (Marcq et al., 1998). In Belgian Blue cattle, and at least nine other European beef breeds, mutations in the myostatin gene are now known to be responsible for dramatically increasing their muscularity (Grobet et al., 1997 & 1998; Kambadur et al., 1997). Stone et al. (1999) separately reported a QTL in cattle affecting meat traits close to, but distinct from, the myostatin locus. Therefore, this study has been undertaken to determine whether myostatin, or a locus near to it, affects the muscling of Texel sheep in New Zealand.

MATERIALS AND METHODS

Blood samples were collected from about 3000 1998-born progeny of Texel rams from three farms in the central Hawkes Bay, near to Rotorua and in Southland. The white blood cells in these samples were harvested after ammonium chloride lysis of erythrocytes (Montgomery and Sise, 1990), and frozen. From breeding and weaning records, 36 rams with more than 40 offspring each were identified. DNA was later extracted from the frozen white cells, by the proteinase K-salt method (Montgomery and Sise, 1990).

Phenotypic data collected included weaning weight (WT), live weight at ultrasonic scanning (LW8), fat depth at position C at scanning (FDM8), L. dorsi muscle width "A" at scanning (EMW8), L. dorsi muscle depth "B" at scanning (EMW8), and estimated L. dorsi area (EMA) calculated as 0.77*EMW8*EMD*.

The paucity of polymorphisms in the ovine myostatin gene (Marcq et al., 1998) has meant that the gene has not been mapped on the sheep genome. Therefore, the following published cattle microsatellite markers mapping close to the gene on cattle chromosome 2 were first tested on sheep genomic DNA: BULGE20, BULGE23, BULGE27, BULGE28, BY5, BY41, and BY42 (Grobet et al., 1998; Sonstegard et al., 1998). Of these, only three (BULGE20 & 23, and BY5) amplified the correct product in sheep. All three markers were confirmed to be located on sheep chromosome 2 by analysis of the cell hybrid panel (Burkin et al., 1998), and were also mapped on this chromosome by linkage analysis on the international mapping flock (Crawford et al., 1995). These markers and two nearby microsatellites, BM81124 and INRA40 flanking myostatin in cattle (Kappes et al., 1997), were screened over the 36 Texel sires. The markers BM81124 (De Gortari et al., 1997) and INRA40 (Dr J. Maddox, Centre for Animal Biotechnology, University of Melbourne, personal communication) had previously been mapped on sheep chromosome 2.

Of the 36 rams with more than 40 progeny born in 1998, a total of 12 rams (four per property) were identified on the basis that their genotypes with five microsatellite markers showed them to be heterozygous with at least one of these markers. Where possible, rams with large numbers of progeny were also selected. The DNA from all the progeny of these 12 rams were then genotyped with the markers that were heterozygous in their sire. The farms were encoded with numbers 1-3 to disguise their identity. Data were initially analysed using the sire allele inherited for each marker regressed against the live weight and ultrasonic measurements. Subsequent analyses used the following approach. Firstly, BLUP breeding values were calculated for all the dams (dam EBV) of the progeny genotyped using data from all available animals born prior to 1998 via genetic parameters and procedures described by Jopson et al. (1995). Secondly, the probability of inheriting a particular sire chromosome at a particular position (phase) was calculated from the available genotype data using the method of Knott et al. (1996) covering a 10 cM region around the myostatin locus. The analysis used a least-squares regression for each trait examined and two models were used. The first was an age-adjusted model which included fixed effects of sex, dam age, birth and rearing rank and sire, with birthdate, dam EBVs for the trait and phase probability nested within sire fitted as covariates. The second model was a liveweight-adjusted model that included sex and sire as fixed effects with live weight at ultrasonic scanning, dam EBVs and phase probability fitted as random effects.
nested within sire as covariates.

It was subsequently discovered that some dams in Farms 2 and 3 had a large number of missing dam EBVs, either because they had been screened into the flock, or, alternatively, they were not measured themselves and had no progeny that had been measured. Consequently, to retain adequate progeny numbers for analysis, the dam EBV effect was dropped from both these farms’ analyses.

**RESULTS**

**Sires and markers**

Only two of the 36 sires examined (in all three Texel flocks) were heterozygous for all 5 microsatellites, only one sire was informative for four markers, and three were informative for three markers. This illustrates the low level of polymorphism around the myostatin locus observed in this study. Table 1 summarises the markers used and their positions relative to the most proximal marker (nearest to the centromere) and to myostatin.

<table>
<thead>
<tr>
<th>Marker name</th>
<th>Estimated position (cM)</th>
<th>Assumed position relative to MSTN (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM81124</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>BY5</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>Bulge23</td>
<td>3</td>
<td>+2</td>
</tr>
<tr>
<td>Bulge20</td>
<td>5</td>
<td>+4</td>
</tr>
<tr>
<td>INRA40</td>
<td>10</td>
<td>+9</td>
</tr>
</tbody>
</table>

To illustrate the information content of the marker data, results from Farm 2 that are representative of the others, are shown in Figure 1.

**Muscularity effects**

The effect of the sire phase inherited at the myostatin position was then examined by regression. The following results (shown in Table 2) were obtained.

Data for two sires – numbers 1 and 4 – in Farm 1 were consistent with a QTL segregating in the region of the myostatin locus that affects both fat and muscle depth. Sire 1 increased fat depth (FDM8) by 1.1 mm and eye-muscle depth (EMD8) by 2.9 mm, with both effects being significant at the 5% level (Table 2). The effects in sire 4 were significant at the 1 % level for FDM8 (+1.45 mm) but were not significant for the muscling measurements. When the potential live weight effects were separated from effects on carcass composition traits by use of a model including live weight (not presented), additional information was revealed. For sire 1, the effect on FDM8 was not significant, suggesting the effect observed was mediated via live weight, but the effect on EMD8 (+2.42 mm) remained significant at the 5 % level. For sire 4, the increased FDM8 (+1.45 mm) was still significant, but at the 5 % level. However, for sire 2 there was a significant decrease in FDM8 (-0.61 mm, P <0.05) but a significant increase in both eye muscle width (EMW8, +3.2 mm, P <0.01) and area (EMA, +1.09, P<0.05). Overall, there was a probability for the sires of P=0.0092 for a QTL affecting FDM8 and of P=0.015 for an effect on EMD8.

<table>
<thead>
<tr>
<th>Farm 1</th>
<th>N animals</th>
<th>WWT (Kg)</th>
<th>LW8 (Kg)</th>
<th>FDM8 (mm)</th>
<th>EMW8 (mm)</th>
<th>EMD8 (mm)</th>
<th>EMA (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sire 1</td>
<td>264</td>
<td>0.16NS</td>
<td>0.91NS</td>
<td>1.10*</td>
<td>2.90*</td>
<td>0.54NS</td>
<td>1.73NS</td>
</tr>
<tr>
<td>Sire 2</td>
<td>2.29+</td>
<td>-0.38NS</td>
<td>4.07**</td>
<td>1.32+</td>
<td>1.48*</td>
<td>0.84NS</td>
<td>0.54NS</td>
</tr>
<tr>
<td>Sire 3</td>
<td>2.75NS</td>
<td>3.15NS</td>
<td>1.45**</td>
<td>-0.07NS</td>
<td>1.42NS</td>
<td>0.63NS</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Farm 2</th>
<th>N animals</th>
<th>WWT (Kg)</th>
<th>LW8 (Kg)</th>
<th>FDM8 (mm)</th>
<th>EMW8 (mm)</th>
<th>EMD8 (mm)</th>
<th>EMA (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sire 1</td>
<td>266</td>
<td>-0.91NS</td>
<td>0.27NS</td>
<td>-1.30NS</td>
<td>-0.36NS</td>
<td>-0.37NS</td>
<td></td>
</tr>
<tr>
<td>Sire 2</td>
<td>2.29+</td>
<td>0.00NS</td>
<td>-1.30NS</td>
<td>0.40NS</td>
<td>0.47NS</td>
<td>0.84NS</td>
<td>0.54NS</td>
</tr>
<tr>
<td>Sire 3</td>
<td>2.79+</td>
<td>0.49NS</td>
<td>-0.22NS</td>
<td>1.15NS</td>
<td>0.65NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results for Farm 2 were much less conclusive (Table 2). None of the sires tested had significant effects on the body composition traits. However, sire 1 in Farm 1 was also used in Farm 2 in which he appears as sire 4. It is interesting to note that his progeny had among the largest effects on the muscling traits measured (see Table 2).

**FIGURE 1.** Information content across the region of interest for each of the sires (range 0 equals no information, 1 equals complete information from the markers).
For Farm 3, the results provide some support for a QTL segregating in the region of the MSTN locus and affecting muscle depth in sire 3. For this sire, the QTL is estimated to increase EMW8 by 2.34 mm, EMD8 by 1.43 mm and EMA by 1.15 cm² (see Table 2). All were significant at the 5% level. To separate potential live-weight effects from effects on carcass composition, the traits in Table 2 were analysed by a model including live weight (not presented). The results are consistent with those observed previously (Table 2) with a putative QTL affecting fat and muscle depth in sire 3, but the effect did not reach significance over all sires.

**DISCUSSION**

In rams from two of the three Texel flocks tested, evidence was obtained that a QTL is likely to be present in the region of the myostatin gene on sheep chromosome 2 that affects both fat depth and the L. dorsi dimensions. Animal numbers were insufficient to categorically state that a QTL clearly exists, but if it does then its effects would be economically significant.

In the case of Farms 2 and 3, insufficient numbers of animals per sire are available to provide unambiguous results for most sires given the estimated magnitude of the effect. We suggest that the combination of low body weights of the progeny at scanning (31 kg) and dams with missing EBVs in the data from Farm 2 has conspired to make the analysis inconclusive. The estimates for the sire potentially segregating for the QTL are of a similar magnitude to the Carwell locus (Nicol et al., 1998). That is, the effect ranged from 9-13% of the L. dorsi dimensions for the three sires with significant effects on muscling. However, unlike Carwell, which had no effect on fat depth, the QTL around myostatin may also affect deposition of fat (Table 2). It must also be remembered that the QTL in this region is thought to affect conformation and meat yield (Marcq et al., 1998), so the traits measured in the present experiment may not be very sensitive to these aspects of body composition.

Work is under-way to independently validate that the putative QTL identified is centred on the region of sheep chromosome 2 around myostatin. In cattle, Stone et al. (1999) identified a QTL that increased muscling that appears to be located 35 cM distal to myostatin. It is, therefore, important to determine the precise location of the presently detected QTL in sheep. This involves further genotyping with markers flanking the gene both proximally and distally by up to 60 cM. We are also attempting to discover new microsatellite marker(s) located within 100 kb of the gene for additional genotyping around the gene.

**ACKNOWLEDGMENTS**

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