A directed search in the region of GDF8 for quantitative trait loci affecting carcass traits in Texel sheep[1]

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ABSTRACT: A directed search for QTL affecting carcass traits was carried out in the region of growth differentiation factor 8 (GDF8, also known as myostatin) on ovine chromosome 2 in seven Texel-sired half-sib families totaling 927 progeny. Weights were recorded at birth, weaning, ultrasound scanning, and slaughter. Ultrasonic measures of LM cross-sectional dimensions and s.c. fat above the LM were made, with the same measurements made on the LM after slaughter. Following slaughter, linear measurements of carcass length and width were made on all carcasses, and legs and loins from 540 lambs were dissected. Genotyping was carried out using eight microsatellite markers from FCB128 to RM356 on OAR 2 and analyzed using Haley-Knott regression. There was no evidence for QTL for growth rates or linear carcass traits. There was some evidence for QTL affecting LM dimensions segregating in some sire families, although it was not consistent between ultrasound and carcass measures of the same traits. There was strong and consistent evidence for a QTL affecting muscle and fat traits in the leg that mapped between markers BM81124 and BULGE20 for the four sires that were heterozygous in this region, but not for the three sires that were homozygous. The size of the effect varied across the four sires, ranging from 0.5 to 0.9 of an adjusted SD for weight-adjusted leg muscle traits, and ranging from 0.6 to 1.2 of an adjusted SD for weight-adjusted leg fat traits. The clearest effect shown was for multivariate analysis combining all leg muscle and fat traits analyzed across sires, where the $-\log_{10}$ probability was 14. Animals carrying the favorable haplotype had 3.3% more muscle and 9.9% less fat in the leg relative to animals carrying other haplotypes. There was evidence for a second peak in the region of marker TEXAN2 for one sire group. It seems that a QTL affecting muscle and fat traits exists within the New Zealand Texel population, and it maps to the region of GDF8 on OAR2.

Key Words: Carcass Traits, Growth Differentiation Factor 8, Lamb, Quantitative Trait Locus, Texel

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Introduction

Improving the lean meat yield of lamb carcasses is included in the selection objective for many terminal sire breeds. Identification of QTL influencing this trait and their utilization via marker-assisted selection (**MAS**) has the potential to improve the rate at which genetic progress can be made. Traditional selection methods rely on measurements of predictor traits (ultrasound and computed tomography scanning) because direct measurement is costly and difficult.

Currently the two confirmed QTL affecting carcass traits in sheep are the LM muscling locus (Nicoll et al., 1998) and the Callipyge locus (Cockett et al., 1994). The Texel breed is known for its superior muscling phenotype, and it has been hypothesized that mutations to growth differentiation factor 8 (**GDF8**, also known as myostatin) could be involved in the phenotype, as it is with Belgian Blue and other breeds of cattle (Grobet et al., 1998; Marcq et al., 1998).

Preliminary reports suggest there is indeed a QTL affecting muscling and fat traits in the region of GDF8 on OAR2 based on ultrasound measurements on purebred animals (Broad et al., 2000; Walling et al., 2004) and on carcass measurements from an F_2 and back-

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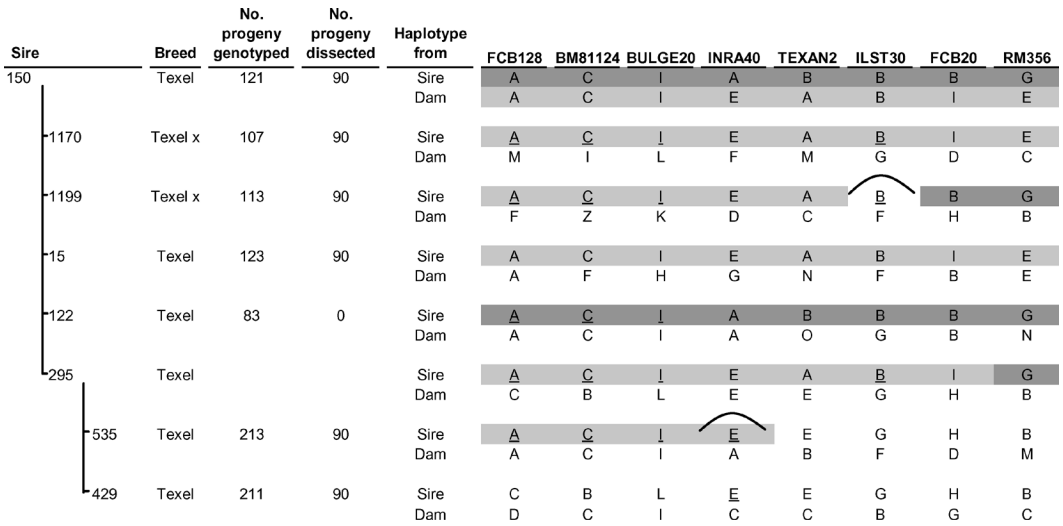

Figure 1. Sire haplotypes (from sire or dam) for markers in the region of interest on OAR 2. Sire 295 was not used in the trial, but is included in this diagram to show the link to sires 535 and 429. Cells highlighted in dark grey and light grey indicate that they were inherited from the sire and dam of 150, respectively. The underscores represent instances where the sire-inherited allele was not determined as the sire was homozygous at that marker. ∩ indicates that crossing over occurred somewhere in the region shown.

cross trial (Marcq et al., 2002; Laville et al., 2004). The study of Broad et al. (2000) showed evidence for a QTL in the region of marker INRA40, whereas Walling et al. (2004) showed evidence for QTL in the region of BM2113, although in both of these studies, the evidence was not strong. Marcq et al. (2002) provided strong evidence for a QTL in the region of markers BM81124 and BULGE20.

The objective of the present study was to use animals identified by Broad et al. (2000) to create half-sib families with all progeny slaughtered, so that detailed dissection of the leg and meat quality analysis of samples from the leg and loin could be carried out, followed by QTL analysis in the region of GDF8 on OAR2. This report presents the findings for the live animal, carcass, and dissection data.

Materials and Methods

Animals

Seven half-sib families were generated using five Texel (15, 122, 429, 535, and 150) and two Texel × Coopworth sires (1170 and 1199). Six of the sires were either sons or grandsons of the foundation sire 150 (Figure 1). Legs and loins of progeny of sire 122 were not dissected because of low numbers (n = 82). Choice of sires used (total available n = 23) was based on heterozygosity at the markers of interest (markers are discussed below). The sires were single-sire-mated to ewes of unrelated breeds (Romney and Coopworth). Based on power calculations, it was estimated that 145 progeny per sire were required to detect a QTL with an effect of approximately one SD, and that 90 progeny were required for the detailed measurements on leg composition and meat quality, which are traits with smaller variance. Actual numbers achieved are presented in Figure 1. All progeny for any one sire were born and raised together on pasture. Further details on the progeny and their management are given in Johnson et al. (2005). The mean carcass weights were 16.7 and 17.6 kg for ewe and ram lambs, respectively.

Traits Analyzed

Progeny were evaluated for growth, ultrasound measurements, carcass dimensions, and leg composition. Live weights were recorded at birth, weaning, and at the time of ultrasound measurement. Ultrasound measurements of the LM at the last rib included width (**A**), depth (**B**) and s.c. fat cover over B (**C**). The average BW at scanning was 35 kg. Animals were slaughtered in a commercial meat plant as described by Johnson et al. (2005). Measurements recorded on the whole carcass included cold carcass weight, carcass length, carcass widths at the widest part of the forequarter, thorax and gigot, soft tissue depth 110 mm off the midline in the region of the 12th rib (**GR**; Kirton, 1989) and measurements on LM at rib 13 (A, B, C). Dissection of the leg yielded weights of six individual muscles (semimembranosus, semitendinosus, biceps femoris, adductor, quadriceps femoris, and gluteus medius), total muscle, s.c. fat, intermuscular fat, and pelvic bone, femur bone, and total bone. Partial dissection of the loin (from transverse cuts caudal to rib 13 and at the separation from the leg between the last and second-to-last lumbar vertebrae) yielded weights of the LM and s.c. fat.

Genotyping

The DNA was extracted from blood-soaked Whatman FTA paper as described by Shackell et al. (2001). The GDF8 gene has not been mapped in sheep, but based on conserved synteny, it most likely maps to the region of microsatellite marker BM81124 on OAR2 (Smith et al., 1997; Maddox et al., 2002). Eight microsatellite markers that flank approximately 122 cM of BM81124 on OAR2 were genotyped across all progeny (FCB128, BM81124, BULGE20, INRA40, TEXAN2, ILSTS030, FCB20, RM356), and a further microsatellite was genotyped for sire 15 (TGLA10). With the exception of BULGE20, these microsatellites are mapped on the reference sheep linkage map (Maddox et al., 2002). Marker BULGE20 is a bovine-derived marker previously used in sheep by Broad et al. (2000). The amplification procedure was as described by Crawford et al. (1995). Checks on genotypic data were carried out using CRIMAP (Green et al., 1990). Map distances were the same as those derived by Broad et al. (2000). All distances are reported relative to marker FCB128, which maps to approximately 99.4 cM from the start of OAR2 (Maddox et al., 2002). Sire genotypes are presented in Figure 1.

Statistical Analyses

The information content (**IC**) was calculated based on the variance of conditional probabilities at a given position and was related to the number of informative progeny, the frequencies of the sire alleles in the dam population, and distances between the markers. Analysis of data took place at 2-cM intervals along the 122-cM region of interest. The basis of the analysis was the Haley Knott regression method (Haley and Knott, 1992), further adapted for use with outbred populations (Knott et al., 1996). The analyses were carried out using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Probability (within sire) of the sire passing his paternally inherited allele was fitted in all models. Fixed effects varied for groups of traits, but included sire (all traits), sex (all traits), birth/rearing rank (only pre-slaughter traits), and dissector (dissection traits). Covariates included scan weight (ultrasound traits) and carcass weight (carcass and dissection traits). Interactions between the sire allele probability and the fixed effects were tested but were generally nonsignificant. An exception was the sire × sex interaction that was sometimes significant; it was not included in the model equation when it was not significant. Results for instances where the sex interaction was significant are reported. Multivariate analysis was carried out for groups of traits as specified in the results using the multiple analysis of variance function in the GLM procedure. A single combined analysis across sires was carried out. Evidence for two peaks was tested by fitting sire allele probability for pairs of positions along with their interaction within the models previously described. Tests for linkage disequilibrium within the dam populations accounted for the phenotypic variation that depended on the actual allele received from the dam. This analysis was carried out using single marker analysis by including dam allele as a fixed effect in the model. Haley-Knott regression F-tests are reported as $-\log_{10}$ probability values.

Significance thresholds were estimated using both the formulae of Lander and Kruglyak (1995) and the permutation test described by Churchill and Doerge (1994) using 10,000 replicates. The permutation test requires fewer assumptions (applies to the specific genotyping undertaken, and phenotype distribution) and provides threshold values for each sire/trait. However, when multiple sires/traits were plotted together, individual thresholds could not be shown, so threshold values described by Lander and Kruglyak (1995), which are genome-wide and more conservative, were used. Confidence intervals for the position of the QTL peak were generated using the "bootstrapping" method of Visscher et al. (1996), with 500 replicates. Estimated effects are presented on both the measurement scale, and in residual (adjusted for nongenetic effects) SD (σ_P) units.

A GLM including haplotype (favorable or other) was fitted (along with relevant fixed effects) for the progeny of those sires that were heterozygous for the favorable haplotype (the informative sires), to estimate the across-sire size and significance of haplotype effects.

Results and Discussion

Haplotypes, Numbers, and Information Content

Only three sires were heterozygous for all markers used (1170, 1199, and 429), whereas one sire (122) was heterozygous for only three of the eight markers (Figure 1). The information content of the sires varied considerably; for four sires, the information content remained above 0.8 between markers BM81124 and ILSTS030 (Figure 2). This value is considerably higher than that reported by Broad et al. (2000), which is because the sires and dams are of different breeds in this study and because sires chosen were on average heterozygous at more of the markers than in the study of Broad et al. (2000). This higher information content meant that the likelihood of detecting QTL, if they existed, was increased.

Live and Carcass Weight Traits

No QTL affecting growth rate or live weight traits were detected (results not presented). Work in cattle has shown that mutations to the GDF8 gene, in addition to altering muscle and fat, have an effect on growth, with Casas et al. (1999) showing that Piedmontese bulls carrying at least one copy of the mutation had increased growth rates.

There was evidence within two sires for a QTL affecting dressing percent, with positions ranging between

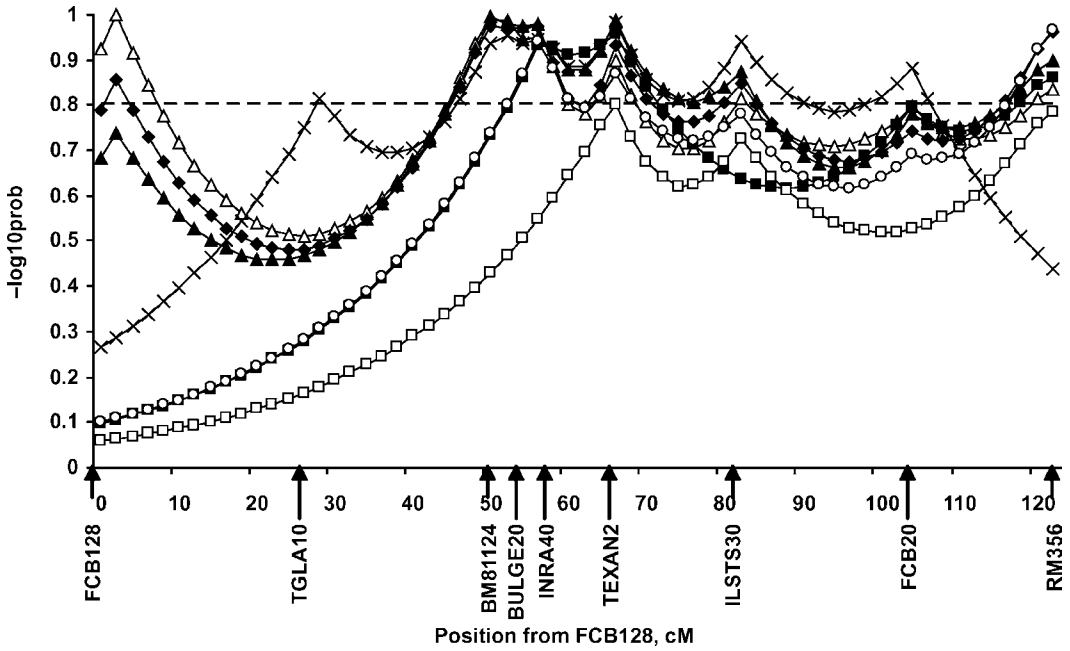

Figure 2. Information content along the region of interest of OAR 2 for half-sib Texel cross lambs within the sire groups 1199 (▲), 1170 (△), 150 (■), 15 (×), 122 (□), 429 (◆), and 535 (○). The dashed line (---) indicates the value above which information content considered to be high (0.8).

72 and 96 cM from marker FCB128, and the size of the effect was approximately 0.3 σ_P. The work of Marcq et al. (2002) found evidence for a QTL increasing dressing percent in the region of markers BM81124-BULGE20. This is in agreement with work from cattle showing that animals with mutations in GDF8 have increased dressing percents (Short et al., 2002).

Linear Traits

There was no consistent across-sire evidence for QTL affecting carcass linear traits (carcass length and width) adjusted for carcass weight (results not presented). In contrast, Marcq et al. (2002) reported evidence for a QTL increasing carcass width in the region of marker BM81124.

Cut Distribution

The presence of significant (using permutation 95% significance threshold) QTL affecting the weight of the leg cut after adjustment for carcass weight was observed for two sires (15 and 1170). The QTL for sire 15 mapped to the region of markers BULGE20 and BM81124, whereas that for sire 1170 mapped to the region of marker TEXAN2, although the confidence intervals did overlap. The size of the QTL effect was approximately 0.5 σ_P. Marcq et al. (2002) also detected a QTL increasing hind quarter percent and also shoulder weight, which mapped to the region of marker BM81124. Cattle exhibiting the muscle hypertrophy phenotype have an increased proportion of the carcass in the leg and loin (Dumont, 1982).

Measurements on the Loin

The measures of muscle and fat taken on the loin in the region of the last rib using ultrasound are the traits most comparable to the previous work of Broad et al. (2000) and Walling et al. (2004). In the current study, the same measurements were also made after slaughter. From results in Table 1, it can been seen that evidence for a QTL affecting loin muscle and fat traits were not consistent between the ultrasound measurements and the carcass measurements, nor were they consistent between sires in terms of either significance or peak position.

The most consistent results for loin traits were for sire 15, which showed evidence for a QTL affecting the linear dimensions of the loin as measured by ultrasound and the absolute weight of the LM within the loin; however, there was no evidence for a similar effect on linear dimensions on the same muscle after slaughter. The peak mapped to the region of markers BULGE20 through TEXAN2, which is not in agreement with either of the previously reported regions for LM QTL by Broad et al. (2000) and Walling et al. (2004). This position is in agreement with the QTL reported by Marcq et al. (2002) and, as will be discussed below, is in agreement with the other QTL detected in this study.

The inconsistency between ultrasound and post-slaughter measurements can be explained in part by the low correlations between the weight-adjusted ultrasound and carcass measurements of the width and depth of the muscle, which were 0.24 and 0.41, respectively. Although not as low, McEwan et al. (1989) re-

Table 1. Details of significant quantitative trait loci for Texel cross lambs using a half-sib QTL analysis for a region of OAR 2 for weight-adjusted loin traits (fresh-tissue basis) measured by ultrasound (US) or on the carcass for four sire groups

Sire	Trait	Mean[a]	σ_P	Esimate ± SE[b]	Estimate in SD units[c]	Permutation[d] 95%	Permutation[d] 99%	Max $-\log_{10}$ prob[e]	Relative position[f]	Confidence interval[g]
1170	US LM depth, mm	20.5	1.9	0.9 ± 0.4	0.5	1.8	2.6	1.8*	122	2 to 122
	US LM area, mm^2	914	141	70 ± 27	0.5	1.8	2.5	2.0*	122	15 to 122
	LM width, mm	53.9	2.8	1.8 ± 0.8	0.6	1.3	1.8	1.5*	38	15 to 122
1199	US fat depth C, mm[h]	1.6	0.5	–0.3 ± 0.1	–0.7	1.9	2.6	2.1*	20	2 to 113
	LM wt in loin, g	193.2	18.4	9.7 ± 3.4	0.5	2.1	2.8	2.3*	52	2 to 122
	GR, mm[h]	4.6	1.8	–1.0 ± 0.3	–0.6	2.2	2.9	2.4*	66	2 to 104
	Loin fat, g	71.8	19.4	–13.4 ± 6.0	–0.7	1.3	1.7	1.6*	54	39 to 104
15	US LM width, mm	58.9	5.1	2.3 ± 0.7	0.5	2.0	2.6	2.8**	66	52 to 104
	US LM depth, mm	23.0	2.3	1.1 ± 0.4	0.5	1.9	2.6	2.8**	54	39 to 104
	US LM area, mm^2	1,058	170	94 ± 26	0.5	2.0	2.6	3.4^{**sug}	62	50 to 104
	LM wt in loin, g	216	20.4	12.4 ± 3.3	0.6	1.9	2.6	3.5^{**sug}	66	39 to 104
	Fat depth C, mm[h]	2.2	0.9	–0.4 ± 0.2	–0.5	1.6	2.2	2.1*	62	34 to 104
429	US fat depth C, mm[h]	1.7	0.7	–0.3 ± 0.1	–0.5	1.9	2.6	2.6*	64	15 to 113
	Loin fat, g	80.9	21.8	–19.1 ± 5.5	–0.9	2.3	3.3	3.2^{*sug}	28	2 to 122

[a]Within-sire raw unadjusted phenotypic mean of the trait.
[b]Estimate of size of the substitution effect between the favorable haplotype and alternative haplotypes.
[c]Magnitude of the QTL peak: (estimate/σ_P).
[d]$-\log_{10}$prob thresholds (derived by permutation tests with 10,000 replicates) determined for 95 and 99% confidence levels.
[e]The significance of the QTL peak in terms of $-\log_{10}$ of the nominal probability. Superscripts * and ** mean the result reached the permutation test 95%, and 99% thresholds, respectively. Superscripts ^{sug} and ^{sig} indicate that the result reached the alternative recommended suggestive (sug) and significance (sig) thresholds (2.8 and 4.3, respectively) of Lander and Kruglyak (1995).
[f]Position of the QTL peak relative to marker FCB128 in cM.
[g]95% confidence intervals were derived for the position in cM by bootstrapping with 500 replicates.
[h]C = s.c. fat cover over LM depth; GR = soft tissue depth 110 mm off the mid-line in the region of the 12th rib.

ported correlations of 0.38 and 0.72 for the same two traits. These low correlations mean that matching evidence for both traits would not necessarily be expected.

Evidence for QTL affecting weight-adjusted measurements of fat in the loin region, including C (ultrasound and after slaughter), GR, and loin subcutaneous fat weight (Table 1), was inconsistent. The results were not consistent between sires for significance or peak position (although in some instances confidence intervals were overlapping), and, in the case of C, varied between ultrasound and postslaughter measurements. In the current study, average measures of C based on ultrasound and postslaughter measurements were 1.7 and 2.4 mm, respectively, compared with the 5.6 mm reported by Wolf et al. (2001) for heavier Texel-cross lambs (22 vs. 18 kg mean carcass weights). This difference could provide a possible explanation because, given that the lambs in this study were at an earlier stage of development, their fat depots were relatively small, so any differentiation between genotypes may not have been fully exhibited.

Measurements on the Leg

The leg was dissected into individual muscles, s.c. and intermuscular fat, and bone. The data were analyzed at the across-sire multivariate level for muscle and fat traits combined, at the within-sire multivariate level for muscle traits and fat traits separately, and also within sires for each muscle and fat depot.

Multivariate analysis across all sires, using weights of the main leg muscles and the s.c. and intermuscular fat provided significant evidence ($-\log_{10}$prob of 14) for a QTL 52 cM from marker FCB128 (Figure 3), which places it between markers BM81124 and BULGE20. All muscle traits had positive weightings and fat traits had negative weightings within the multivariate analysis.

Multivariate analysis of leg muscle traits for each sire provided significant evidence for QTL (using the Lander and Kruglyak thresholds) for two sires (1199 and 15), whereas two other sires provided evidence approaching the suggestive threshold (1170 and 429; Figure 4). For all four sires, the position of the main peak was 52 cM from marker FCB128. There also was a second peak approximately 68 to 72 cM from marker FCB128 in the region of marker TEXAN2. Multivariate analysis of leg fat traits for each sire provided significant evidence for a QTL for one sire (1199) and suggestive evidence for one sire (1170), whereas one sire had evidence that approached the suggestive threshold (sire 15; Figure 5). As for the muscle traits, there seems to be two positions to which the fat traits map, at 52 cM and 68 to 72 cM from marker FCB128.

Plots for individual muscle and fat traits for sire 1199 are given in Figures 6 and 7, with further details of the size, significance, and position of peaks provided in Table 2. Sire 1199 was the sire for which there also was the strongest evidence for a second peak, with

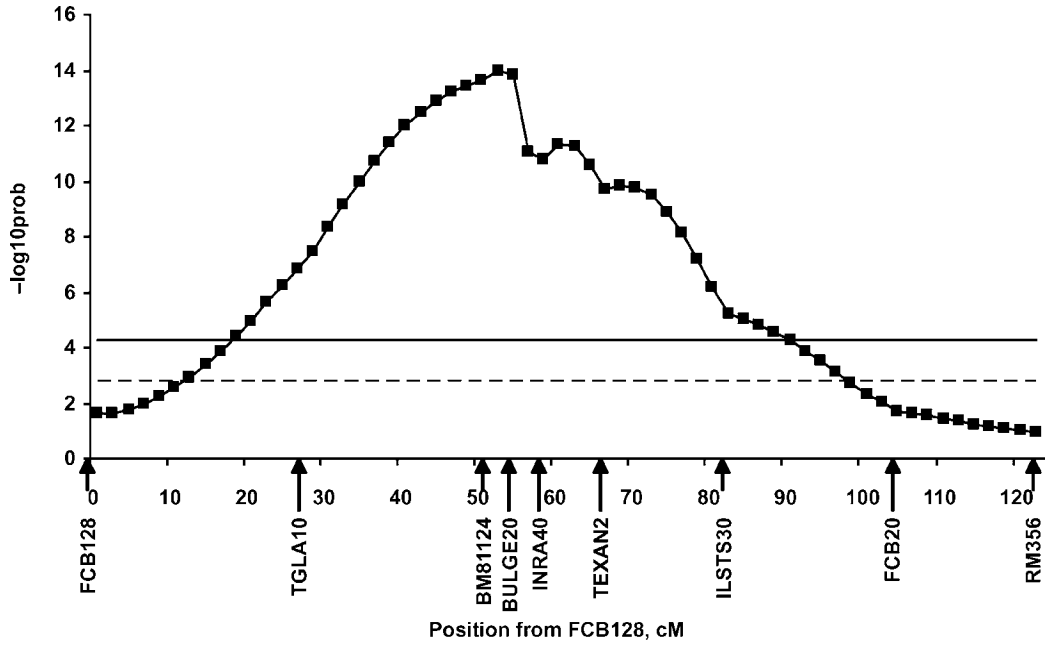

Figure 3. A $-\log_{10}$prob curve for half-sib Texel cross population for a region of OAR 2 from a multivariate analysis across sires for carcass-weight-adjusted weights of muscles and fat depots in the leg. Traits analyzed were weights of semimembranosus, semitendinosus, biceps femoris, quadriceps femoris, adductor, gluteus medius, s.c. fat, and intermuscular fat. Thresholds derived from Lander and Kruglyak (1995) were genome suggestive threshold (– – –) and genome significant threshold (——).

results for individual traits peaks either at approximately 52 cM from marker FCB128 or at 68 to 72 cM from marker FCB128. From the graphical evidence, there always was a second peak at the latter location. The size of the QTL effect was approximately 0.8 σ_P for the muscle traits and 1.2 σ_P for the fat traits for

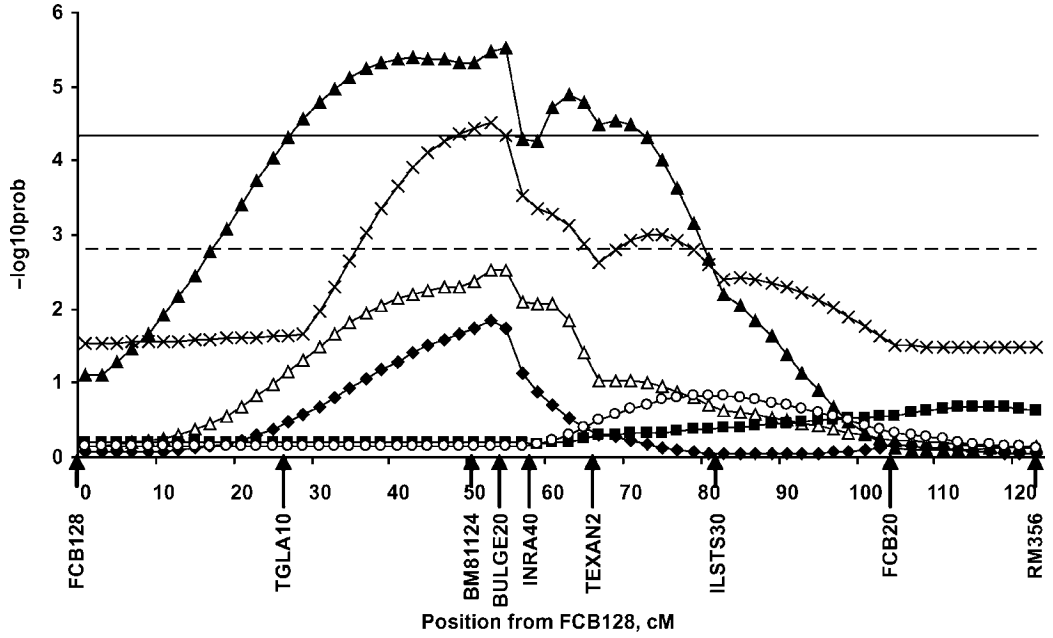

Figure 4. The $-\log_{10}$prob curves for half-sib Texel cross population for a region of OAR 2 from multivariate analyses for carcass-weight-adjusted weights of muscle in the leg. Muscles were semimembranosus, semitendinosus, biceps femoris, quadriceps, adductor, and gluteus medius. Sires were 1199 (▲), 1170 (△), 150 (■), 15 (×), 429 (◆), and 535 (○). Thresholds derived from Lander and Kruglyak (1995) were genome suggestive threshold (– – –) and genome significant threshold (——).

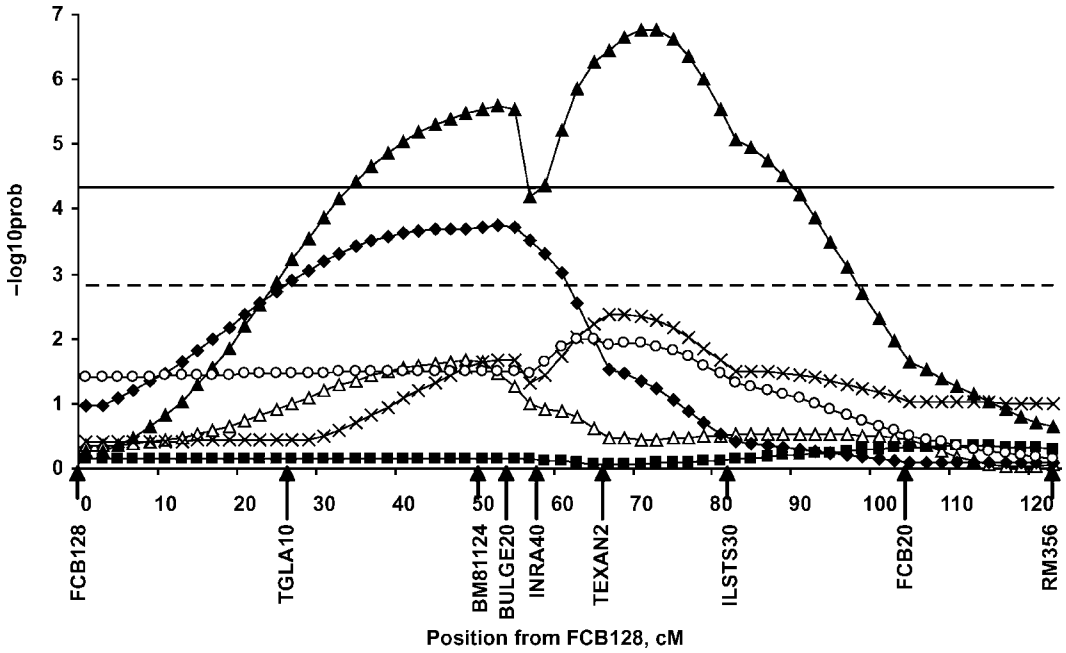

Figure 5. The $-\log_{10}$prob curves for half-sib Texel cross population for a region of OAR 2 from multivariate analyses of carcass-weight-adjusted weights of leg s.c. and intermuscular fat. Sires were 1199 (▲), 1170 (△), 150 (■), 15 (×), 429 (◆), and 535 (○). Thresholds derived from Lander and Kruglyak (1995) were genome suggestive threshold (– – –) and genome significant threshold (——).

this sire. For other sires (results not presented), the size of the effect varied, but it was approximately 0.5 σ_P to 0.9 σ_P for leg muscle traits (or 4 to 7% of the phenotypic mean) and 0.6 σ_P to 1.2 σ_P for leg fat traits (or 12 to 20% of the phenotypic mean; Table 3). The main positions of the significant peaks for individual

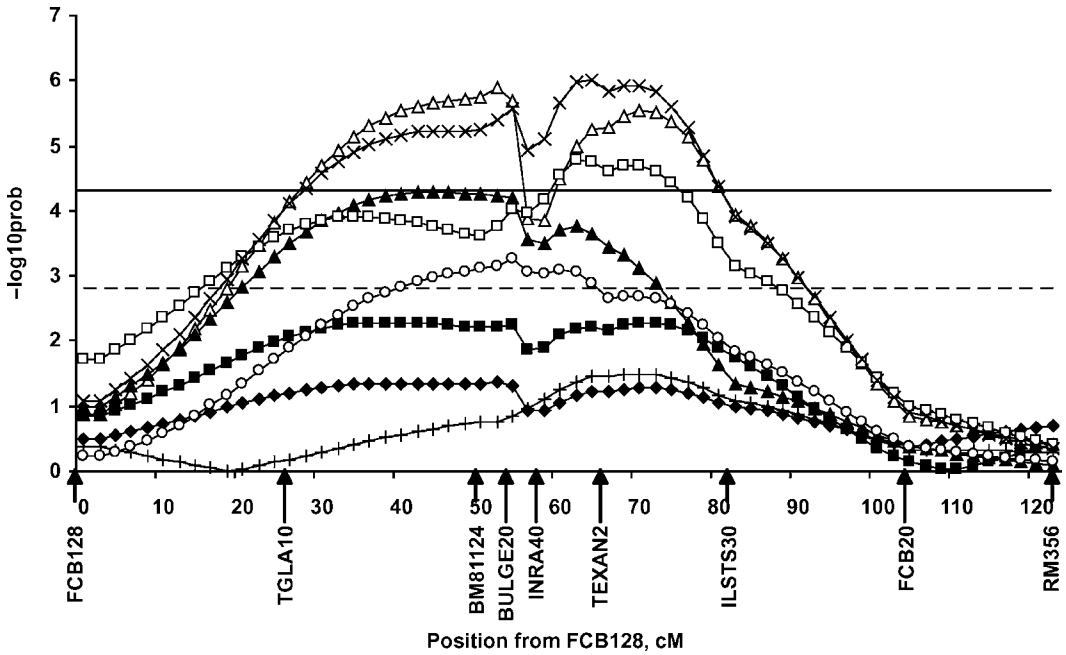

Figure 6. The $-\log_{10}$prob curves from a QTL analysis on OAR 2 of carcass-weight-adjusted weights of leg muscles from progeny of sire 1199. Traits were semimembranosus weight (△), semitendinosus weight (▲), biceps femoris weight (■), quadriceps weight (+), adductor weight (◆), gluteus medius weight (○), muscle trim weight (□), and total muscle in the leg weight (×). Thresholds derived from Lander and Kruglyak (1995) were genome suggestive threshold (– – –) and genome significant threshold (——).

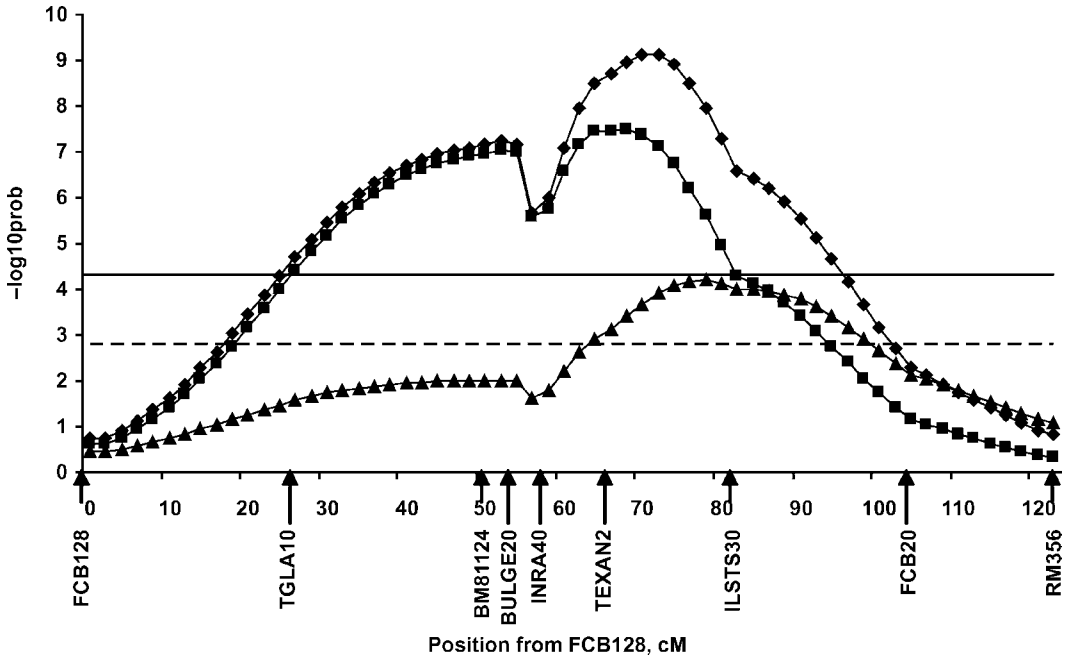

Figure 7. The $-\log_{10}$prob curves from a QTL analysis on OAR 2 of carcass-weight-adjusted weights of leg fat traits from progeny of sire 1199. Traits were s.c. fat in the leg weight (■), intermuscular fat in the leg weight (▲), and total fat in the leg weight (◆). Thresholds derived from Lander and Kruglyak (1995) were genome suggestive threshold (– – –) and genome significant threshold (——).

muscle- and fat-related traits for sires other than 1199 were approximately 52 cM from marker FCB128.

The individual and multivariate analyses combined provide strong evidence for at least one QTL affecting leg muscle and fat traits in the region of GDF8. The likely position of the peaks was between markers BM81124 and BULGE20, although there also was evidence for a second peak in the region of marker TEXAN2 for one sire. This is in general agreement with Marcq et al. (2002), where dissection of the shoulder into its component parts showed strong evidence for a QTL in the region of markers BM81124 and BULGE20, although their results explained more of the variation in muscle than fat, which is contrary to

Table 2. Peaks from a quantitative trait loci analysis of significant carcass-weight-adjusted leg muscle and fat fresh weight traits for 90 progeny of sire 1199

Trait	Mean[a]	σ_P	Estimate ± SE[b]	SD unit[c]	Permutation[d] 95%	Permutation[d] 99%	Max $-\log_{10}$ prob[e]	Relative position[f]	Confidence interval[g]
Muscle traits									
Semimembranosus, g	232.8	18.8	16.0 ± 3.2	0.9	2.2	3.0	5.9**sig	52	28 to 82
Semitendinosus, g	80.9	8.1	7.8 ± 1.9	1.0	1.8	2.4	4.3**sig	44	28 to 66
Biceps femoris, g	224.1	18.0	12.4 ± 4.4	0.7	1.6	2.2	2.3**	38	15 to 122
Gluteus medius, g	155.8	15.1	10.9 ± 3.1	0.7	1.8	2.4	3.3**sug	54	39 to 82
Muscle trim, g	620.0	46.0	38.4 ± 8.7	0.8	2.0	2.8	4.7**sig	62	15 to 74
Total leg muscle, g	1,762.7	115.1	94.4 ± 8.8	0.8	1.9	2.6	6.0**sig	64	28 to 74
Fat traits									
Subcutaneous fat, g	201.1	35.3	–43.2 ± 7.6	1.2	2.3	3.1	7.4**sig	68	39 to 74
Intermuscular fat, g	141.0	23.4	–24.0 ± 5.9	1.0	2.0	2.6	4.2**sug	78	39 to 93
Total leg fat, g	342.1	49.2	–66.2 ± 10.3	1.3	2.4	3.3	9.1**sig	70	50 to 74

[a]Within-sire raw unadjusted phenotypic mean of the trait.
[b]Estimate of size of the substitution effect between the paternally inherited sire haplotype and alternative haplotypes.
[c]Magnitude of the QTL peak: (estimate/adjusted SD).
[d]$-\log_{10}$prob thresholds (derived by permutation tests with 10,000 replicates) determined for 95% and 99% confidence levels.
[e]The significance of the QTL peak in terms of $-\log_{10}$ of the nominal probability. Superscripts * and ** mean the result reached the permutation test 95 and 99% thresholds, respectively. Superscripts sug and sig mean that the result reached the alternative recommended suggestive and significance thresholds (2.8 and 4.3, respectively) of Lander and Kruglyak (1995).
[f]Position of the QTL peak relative to marker FCB128 in centimorgans.
[g]95% confidence intervals were derived for the position in centimorgans by bootstrapping with 500 replicates.

Table 3. A summary of significant (using permutation 95% significance threshold) quantitative trait loci detected by trait group, shown separately for informative sires and uninformative sires

Trait group (No. of traits)[a]	Sire	No. of traits with significant peaks	Position of the peak(s)[b]	Average size and significance of the effect at the peak
Informative sires				
Live weight (6)	429	1	48	0.4*
Ultrasound muscle traits (3)	1170	2	122	0.5*
	15	3	54, 62 to 66[d]	0.5**
Ultrasound fat traits (1)	1199	1	20	–0.7*
	429	1	64	–0.7*
Dressing percent (1)	15	1	72	0.3**
	429	1	96	0.3*
Carcass linear measures (4)	15	1	34	0.5*
	429	1	64	0.5*
Leg muscle traits (9)	1199	6	52 to 54, 62 to 64[d]	0.9**
	1170	6	52 to 56	0.6* to **
	15	8	52 to 56, 72 to 74[d]	0.6* to **
	429	4	54	0.6* to **
Other muscle related traits (3)	1199	3	54, 62 to 68[d]	1.3**
	1170	2	54, 62[d]	0.8**
	15	3	52, 62[d]	0.8* to **
	429	3	52, 54	1.0* to **
Loin muscle traits (4)	1199	1	52	0.5*
	1170	1	56	0.6*
	15	1	66	0.6*
Leg fat traits (4)	1199	4	54, 70 to 78[d]	–1.2**
	1170	2	50	1.0**
	15	2	58, 70[d]	–0.6* to **
	429	3	54	–0.8*
Loin fat traits (3)	1199	2	54, 66[d]	–0.7*
	1170	1	38	–0.5*
	15	1	62	–0.5*
	429	1	28	–0.9*
Uninformative sires				
Dressing percent (1)	122	1	76	1.0*
Leg muscle traits (9)	150	1	96	0.7*
Loin muscle traits (4)	122	1	96	0.8*

[a]Trait groups were live weight traits (birth, weaning, ultrasound scanning, slaughter, ADG birth to weaning, and ADG weaning to scanning), ultrasound muscle traits (LM width, depth, and area), ultrasound fat traits (fat depth over the LM), dressing percent, carcass linear traits (carcass length, carcass width at the shoulders, carcass width at the thorax, carcass width at the gigots), leg muscle traits (weights of leg, semimembranosus, semitendinosus, biceps femoris, adductor, quadriceps femoris, gluteus medius, and muscle trim), other muscle related traits (leg muscle percent, leg muscularity, and leg muscle-to-bone ratio), loin muscle traits (muscle width, and LM depth, area, and weight), leg fat traits (weights of subcutaneous fat, intermuscular fat, and total fat), and loin fat traits (fat depth over LM, GR (soft tissue depth 110 mm from mid-line in the region of the 12th rib), and loin subcutaneous fat).
[b]Centimorgans from marker FCB128.
[c]Magnitude of the QTL peak: estimate/σ_P.
[d]Evidence for two peaks.

the results reported above. The effect of the QTL varied between muscles in the current study, which is consistent with the work of Dumont (1982), who reported considerable variation in the degree of hypertrophy expressed by muscles in the pelvic limb of muscle-hypertrophied cattle.

Alternative Analyses

Two-QTL analyses showed evidence for two QTL within the region studied for sire 1199 for leg muscle and fat percent ($P < 0.05$, compared with the single QTL model for peaks at 50 to 52 cM and 70 to 74 cM from marker FCB128). The interaction fitted was not significant ($P > 0.10$), which suggests that the two QTL are independent (i.e., not epistatic). Two-QTL tests for sire 15 (the other sire for which there was some graphical evidence for two peaks) did not achieve significance.

The search for sex × sire genotype-probability interactions was first carried out by Knott et al. (1998) looking for QTL for growth and fat traits between an outbred wild boar and Large White pig cross. If a significant interaction is detected, it implies that the QTL effects differ between sexes. In the current study, evidence for a sex × sire genotype-probability interaction

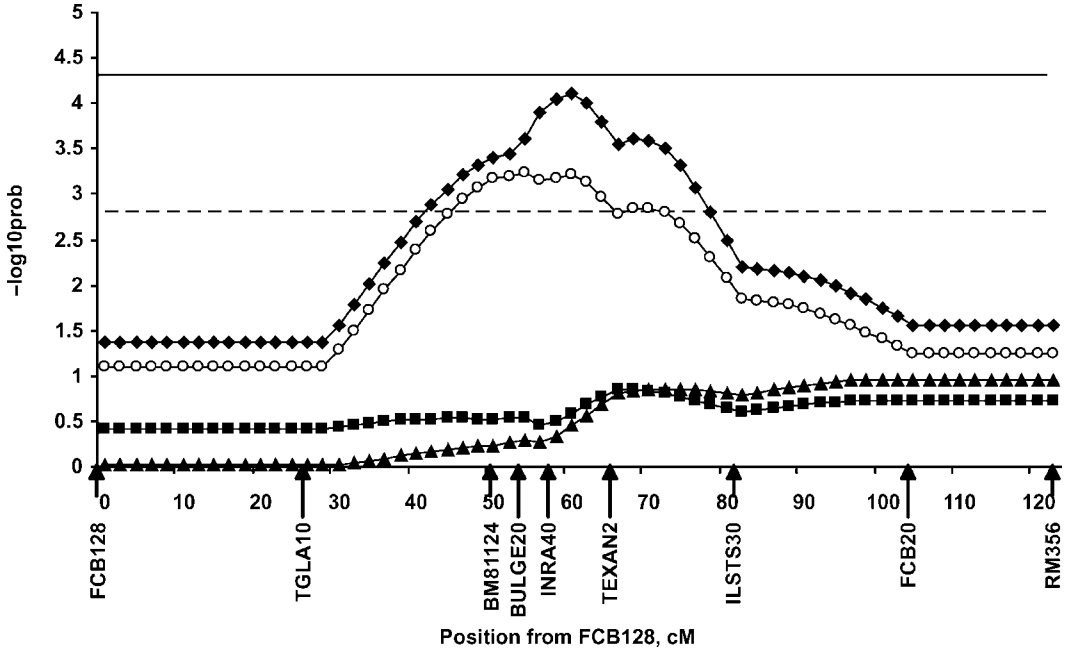

Figure 8. The $-\log_{10}$prob curves from a sex × QTL interaction analysis on OAR 2 based on carcass-weight-adjusted laboratory measurements of LM dimensions for progeny of sire 15. Trait were ram LM width A (■), ewe LM width A (○), ram LM area (▲), and ewe LM area (◆). Thresholds derived from Lander and Kruglyak (1995) were genome suggestive threshold (– – –) and genome significant threshold (——).

was not conclusive across traits or sires. An example of such an interaction in Figure 8 shows that a QTL affecting ultrasound LM dimensions was present for the ewe lambs but not the ram lambs for sire 15, although this was not supported by the postslaughter measurements, possibly because of smaller numbers of lambs per sex (n = 45).

Linkage disequilibrium analysis revealed no evidence for a phenotype association with any of the dam alleles (results not presented). The ability to detect linkage disequilibrium within the dam population was limited by very low frequencies for some of the dam alleles, and the marker spacings were large compared with the likely level of linkage disequilibrium expected in the dam breeds (McRae et al., 2002).

Summary of QTL Analyses

A summary of significant (using permutation 95% significance threshold) results from all QTL analyses, many of which are not reported here in detail, are given in Table 3 within 10 trait groups. Table 3 gives the number of significant QTL detected per sire, the positions of those peaks and the size of the effect (in σ_P units) averaged over a trait group.

We found strong evidence that for four sires (1199, 1170, 15, and 429), the same haplotype (combination of alleles) at markers BM81124 and BULGE20 was associated with the favorable QTL in terms of increased muscle and decreased fat in the leg. From Figure 1, it can be seen that this was the sire-inherited haplotype for three of the sires and the dam-inherited haplotype for the fourth. The three sires for which there was no evidence for a QTL were homozygous for this haplotype (Figure 1). This haplotype is not consistent for the two markers tested on either side of markers FCB128 and INRA40. These observations combined provide further support for the suggestion that the QTL is located in the region of markers BM81124 and BULGE20. It should be noted that this haplotype is extremely rare in the three unrelated Coopworth and Romney dam breed flocks used, with only nine progeny from 927 (approximately 1%) lambs inheriting this haplotype from the dam. The associated confidence intervals as determined by bootstrapping, however, do not provide strong support for this theory as they were often large. Bootstrapping relies heavily on the information content. For these sires, the information content outside of the region of these two markers was lower (Figure 2), particularly between markers FCB128 and BM81124, meaning that the ability to obtain accurate confidence intervals is decreased. Ideally, more highly informative markers in the region of markers BM81124 and BULGE20 should be genotyped to decrease the confidence intervals.

The second peak for sire 1199 mapping to the region of marker TEXAN2 was not apparent for other sires with the same allele at this marker (Figure 1), so it would seem that this region is not identical by descent in these sires. Further markers need to be genotyped in the region to clarify the position of this QTL.

Marcq et al. (2002) found positive results in their F_2 trial, but not in the backcross trial, which suggests that interactions with other Texel (vs. Romanov) genes

Table 4. Least squares mean (±SEM) for the effects of inheriting the favorable haplotype vs. other haplotypes for progeny of those sires that were heterozygous in the region of interest

Trait	Favorable haplotype[a]	Other haplotype[b]	Significance[c]	% Change[d]
Pre-dissection traits				
No.	169	259		
Slaughter weight, kg	41.37 ± 0.29	40.98 ± 0.26	—	0.95
Dressing percent	40.68 ± 0.24	40.13 ± 0.21	*	1.37
Carcass weight, kg	16.82 ± 0.15	16.42 ± 0.13	*	2.44
Dissection traits (fresh weights)				
Number	117	181		
Trimmed leg weight, g[e]	2,632.8 ± 9.6	2,611.4 ± 8.7	*	0.82
Leg muscle weight, g[e]	1,879.6 ± 9.4	1,819.3 ± 8.4	***	3.30
Leg fat weight, g[e]	320.1 ± 4.6	355.5 ± 4.2	***	–9.94
Leg bone weight, g[e]	414.4 ± 3.4	416.2 ± 3.1	—	–0.43
Leg muscle:bone ratio	4.58 ± 0.04	4.38 ± 0.03	***	4.57
Leg muscularity	0.460 ± 0.002	0.447 ± 0.002	***	2.22

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
[a]Alleles C and I at markers BM81124 and BULGE20.
[b]Alleles other than C and I at markers BM81124 and BULGE20.
[c]Nonsignificance is indicated by a "—"
[d]Percentage of change for favorable haplotype relative to other haplotype.
[e]Data adjusted for carcass weight.

are important. Given that the current study used non-Texel dams, caution must be used at extrapolating current results to other breed crosses.

Testing the Haplotype

Results of an alternative analysis comparing lambs from the informative sires with the favorable haplotype relative to those from the same sires with other haplotypes are presented in Table 4. These results support the findings of the QTL analysis, in that the inheritance of the favorable haplotype resulted in legs which had more muscle (+3.3%) and less fat (–9.9%) but with little change in bone. In addition, this analysis pointed to a small positive effect on dressing percent, which was not observed in the QTL analysis. This effect in combination with a small, nonsignificant increase in slaughter weight resulted in lambs with the favorable haplotype having significantly higher carcass weights.

Candidate Genes

At the outset of this study, the candidate gene used was GDF8. This work, along with that of Marcq et al. (2002), strongly suggests that a QTL affecting carcass composition maps to the region of GDF8, which is thought to map between markers BM81124 and BULGE20. However, Marcq et al. (1998) reported no difference in the coding sequence of the GDF8 gene between Texel and Romanov controls. This finding suggests that either another gene is involved or that the mutation occurs within one of the surrounding GDF8 regulatory regions. Irrespective of whether it is GDF8, there also is evidence for a second QTL mapping to the region of marker TEXAN2. Given that relatively few genes have been mapped in sheep, identification of further candidate genes is reliant on the synteny that exists between sheep/cattle and humans. This synteny allowed identification of the corresponding region of human DNA. Gene GDF8 maps to human chromosome 2, and a candidate gene search was carried out in this region by lexical analysis of papers relating to the genes identified and also by studying tissue expression profiles for the same genes, as described by Johnson et al. (2003). Of the 123 genes that mapped to the corresponding region (of which only 78 had approved names), GDF8 was the only gene known to have effects on muscle and fat development. New genes are continually being mapped to the region and new functions of existing genes determined, so the procedures described by Johnson et al. (2003) need to be regularly repeated to identify new candidate genes.

Further Considerations

Before the large-scale introgression of the QTL reported here can be used via MAS a number of areas require further research. First, its effects on meat quality must be assessed, as negative associations between meat quality and increased muscling phenotypes have been documented in sheep (Callipyge phenotype in Dorsets; Freking et al., 1999) and pigs (PSE; Fox et al., 1980; Moelich et al., 2003), although positive correlations between the muscle hypertrophy caused by mutations to GDF8 and meat quality have been reported for cattle (Wheeler et al., 2001). Data relating to this have been collected on these animals (P. L.

Johnson, unpublished data). Secondly, the effects of the QTL on other traits of productive importance, including fertility, resistance to diseases, and wool production, need to be evaluated.

The recent results from Laville et al. (2004) have expanded on the results reported by Marcq et al. (2002). Perhaps the most important additional result from this work is reporting of changes in muscle fiber type proportions, with animals carrying the favorable QTL also having an increase in fast contractile type myosin.

The data presented herein show that the QTL detected near myostatin in the Belgian "hypertrophied" Texels (Laville et al., 2004) and the QTL in the New Zealand Texels (imported from Denmark and Finland) are in the same genomic region; however, it is not possible to determine whether they are the same mutation or even whether they are at the same locus. Nonetheless, the magnitude and traits affected are comparable where these have been measured, and the common chromosomal location and historical breed source suggest that the QTL detected here may have a common origin and be allelic to the QTL detected by Laville et al. (2004).

The current experiment did not provide information on whether the QTL is dominant or additive; however, based on the analyses of Marcq et al. (2002) and Laville et al. (2004), it would seem to be additive. This result needs to be confirmed in the current population before successful MAS programs can be developed and implemented.

Implications

The results reported provide evidence for a quantitative trait locus affecting lean meat yield within the New Zealand Texel sheep population. If a suitable marker test can be developed, it would offer breeders the chance to improve rates of genetic gain for lean meat yield without needing to make expensive measurements of carcass traits.

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