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Effect and mode of action of the Texel Muscling QTL (TM-QTL) on carcass traits in purebred Texel lambs

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Running head: Effect and mode of action of a muscling QTL in Texels

Abstract

TM-QTL is a quantitative trait locus (QTL) on ovine chromosome 18 (OAR18) known to affect loin muscling in Texel sheep. Previous work suggested that its mode of inheritance is consistent with paternal polar overdominance, but this has yet to be formally demonstrated. This study used purebred Texel sheep segregating for TM-QTL to confirm its presence in the chromosomal region in which it was first reported and to determine its pattern of inheritance. To do so, this study used the first available data from a Texel flock, which included homozygote TM-QTL carriers

(TM/TM; n = 34) in addition to homozygote non-carriers (+/+; n=40 and, heterozygote TM-QTL-carriers inheriting TM-QTL from their sire (TM/+; n=53) or their dam (+/TM; n=17). Phenotypes included a wide range of loin muscling, carcass composition and tissue distribution traits. The presence of a QTL affecting ultrasound d muscle depth on OAR18 was confirmed with a paternal QTL effect ranging from +0.54 to +2.82 mm UMD (s.e. 0.37 to 0.57 mm) across the sires segregating for TM-QTL. Loin muscle width, depth and area, loin muscle volume and dissected *M. longissimus lumborum* weight were significantly greater for TM/+ than +/+ lambs (+2.9 to +7.9%; $P<0.05$). There was significant evidence that the effect of TM-QTL on the various loin muscling traits measured was paternally polar overdominant ($P<0.05$). In contrast, there was an additive effect of TM-QTL on both live weight at 20 weeks and carcass weight; TM/TM animals were significantly ($P<0.05$) heavier than +/+ (+11.1% and +7.3%, respectively) and +/TM animals (+11.9% and +11.7%, respectively), with TM/+ intermediate. Weights of the leg, saddle and shoulder region (corrected for carcass weight) were similar in the genotypic groups. There was a tendency for lambs inheriting TM-QTL from their sire to be less fat with slightly more muscle than non-carriers. For example, carcass muscle weight measured by live animal CT-scanning was 2.8% higher in TM/TM than +/+ lambs ($P<0.05$), carcass muscle weight measured by carcass CT-scanning was 1.36% higher in TM/+ than +/+ lambs ($P<0.05$), and weight of fat trimmed from the carcass cuts was significantly lower for TM/+ than +/+ lambs (-11.2%; $P<0.05$). No negative effects of TM-QTL on carcass traits were found. Optimal commercial use of TM-QTL within the sheep industry would require some consideration, due to the apparently different mode of action of the two main effects of TM-QTL (on growth and muscling).

Keywords: genetics, QTL, sheep, Texel, muscling

Implications

There are two contrasting direct effects of TM-QTL: (i) on loin muscling (4-11% increase in highly priced part of the carcass) exhibiting polar overdominance, and (ii) an additive effect on live and carcass weight. This makes TM-QTL an interesting candidate for exploitation within the UK sheep industry and beyond, especially since no major negative impacts on eating quality have been observed. However, there are two main aspects to consider before commercial exploitation is feasible: (i) the development of a commercial genotyping test and (ii) an optimal plan for exploitation in the industry.

Introduction

A quantitative trait locus (QTL) for muscle depth on OAR18, termed the TM-QTL, was first identified in purebred Texel sheep in the UK by Walling *et al.* (2004). In this original study, the effect of carrying a single copy of TM-QTL inherited from the sire was a 1-2mm increase in ultrasound muscle depth (+ 4 to +11%). In a further study using crossbred lambs sired by Texel rams heterozygous for TM-QTL, with Mules or Welsh Mountain ewes as their dams (Macfarlane *et al.*, 2009; Masri *et al.*, 2011) reported a 4 to 11% effect on muscling, specific to the loin region, of carrying a single copy of the QTL. No effect was observed on other carcass traits.

There is evidence that QTL or mutations affecting muscling that lie in the area of OAR18 where TM-QTL is located may show imprinting, specifically polar overdominance in which the QTL effect is expressed only if the QTL is inherited from the sire and not from the dam. This mode of inheritance was first observed for the Callipyge mutation, which has substantial muscling effect in sheep and lies in a similar region of OAR18 to TM-QTL (Cockett *et al.*, 1994b; 1996a; Cockett *et al.*, 1996b, Georges and Cockett, 1996). Additionally, the Carwell QTL (synonymous with LM-QTL, LoinMax) (Nicoll, 2007), which also lies in the region of OAR18 and affects loin muscling to a similar degree as TM-QTL, also appears to have a non-additive mode of inheritance (Jopson *et al.*, 2001, Nicoll, 2007). Lastly, previous work on TM-QTL by Matika *et al.* (2011), examining maternal and paternal variance components for the TM-QTL in commercial Texel lambs, using ultrasound muscle depth as their phenotype, reported results that were also consistent with paternal polar overdominance.

Interestingly, alongside its effect on muscling, TM-QTL appears to have an additive effect on live- and carcass weights; animals carrying 2 copies of TM-QTL were substantially heavier at a fixed age than wildtype animals (Macfarlane *et al.*, 2012). Although no effects have been reported on carcass traits other than in the loin region, given the effect on live and carcass weights seen in homozygote carriers, it is important to know whether overall carcass composition and tissue distribution traits are affected by TM-QTL, and the nature of any effects on these traits.

The aim of this study was to investigate the effect of TM-QTL, in purebred Texel lambs on a range of carcass trait. These included traits measured by ultrasound scanning (muscle and fat depths in the loin region), by x-ray computed tomography (CT) scanning (carcass and joint composition, muscularity) and by commercially relevant butchery (lean meat yields, tissue weight distribution). Critically, for the first time, the experimental group included representatives of all TM-QTL genotypes (wildtype, heterozygotes inheriting TM-QTL from either the sire or the dam, and homozygote carriers), enabling formal testing of the hypothesis that TM-QTL displays polar overdominance for a range of muscling traits. Testing this hypothesis was the third aim of this study.

Materials & Methods

All procedures involving animals were approved by the Scotland's Rural College (SRUC) Animal Ethics Committee and were performed under UK Home Office licence, following the regulations of the Animals (Scientific Procedures) Act 1986.

Position of TM-QTL

A population of Texel sheep located across two farms, one in Wales (IBERS) and one in Scotland (SRUC), was recorded and monitored over 4 years from 2005 to 2009. The SRUC Texel flock had been purchased from The Roslin Institute in 2002 and the presence of TM-QTL, an OAR18 QTL for muscle depth reported by Walling *et al.* (2004), was maintained and its frequency increased in the flock between 2002 and 2005. Sires that had been previously identified as likely carriers of TM-QTL were also mated to existing Texel ewes on the IBERS farm, with some sires used on both farms. All progeny born from 2006 onwards were weighed and ultrasound scanned to measure muscle depth (UMD) at 20 weeks of age.

All animals (sires, dams and lambs) born from 2006 onwards were blood sampled and blood-spotted onto FTA^R cards, and these samples were used for genotyping. In addition blood samples were collected via venepuncture into EDTA-vacutainers and conserved at -40^0; these samples were used if a repeated genotyping test was required. Because the causal mutation responsible for the TM-QTL is still unknown, it was necessary to use markers around the region of interest to classify the likely TM-QTL genotype for each animal. Blood samples were genotyped for five microsatellite markers on OAR18 (MCMA26, CSSM18, OY5, OY3 and OARTMR1) at the Animal Genomics Group, AgResearch Invermay, New Zealand. Marker data collected each year were used along with previously collected data to classify all animals for genotype status for TM-QTL, as described by Macfarlane *et al.* (2009, 2010). The information produced was used each year to plan matings within the flocks, in order to increase the frequency of TM-QTL whilst limiting inbreeding. Between 2005 and 2009, 33 sires were used. Of these, 5 were used across both sites and 7 were used in three or more years. In each year, ewe lambs fit for breeding were retained within the flock and selected ram lambs identified as likely

TM-QTL carriers were also retained. In total, 1731 purebred Texel lambs contributed to this dataset, comprising 759 entire male and 972 female lambs.

Lambs were grazed with the ewes at pasture as either singles (about one third) or twins (about two thirds) up to ultrasound scanning at 20 weeks, except for any hand-reared lambs (n = 42), which were raised indoors until the age of approximately 8 weeks and then grazed and creep-fed up to ultrasound scanning at 20 weeks. All lambs were weighed and ultrasound scanned to measure loin muscle depth (UMD), as described below, at around 20 weeks of age (average age = 138 days, min = 119 days, max = 151 days), before being slaughtered (average age 144 days, min = 126, max = 155).

Microsatellite marker genotypes, UMD and marker map information were used to run single QTL analyses using QTL Express software at http://QTL.cap.ed.ac.uk (Seaton *et al.*, 2002). QTL Express used a multi-marker approach to interval mapping in half sib families (Knott *et al.*, 1996). The probability of a QTL affecting UMD being present was estimated at 1 cM intervals conditional on marker genotypes and recombination fraction/distance from marker. Across families, a test statistic was calculated as an F ratio for every map position obtained using the ratio of mean squares of a model fitting a QTL to not fitting a QTL. Empirical significance thresholds were estimated by using permutation tests (Churchill and Doerge, 1994) involving 10,000 randomisations to estimate the 5 and 1% thresholds. Heterogeneity of QTL position was also explored by estimating the putative position of the QTL indicated by each of the main half-sib families in turn. The models fitted included a

covariate of live weight at scanning and fixed effects of age of dam, rearing rank, farm, sex and year born.

2009-born animals, their management and genotypes

The population of Texel sheep described above was used to produce a total of 211 purebred Texel lambs in 2009 at SRUC and IBERS which were used for detailed phenotyping. These 211 were out of 181 Texel dams mated to 7 different Texel sires that had previously been identified as carrying at least one copy of TM-QTL. Three of these sires were used on both sites. Of the lambs, 87 were out of dams that had been previously identified as carrying TM-QTL, 65 out of dams not carrying TM-QTL and the remaining 59 out of dams with unknown TM-QTL status. Lambs were either reared as a single (n=126) or a twin (n=73), or hand-reared (n=12), and were either entire male (n=96) or female (n=115). There were 73 lambs at IBERS and 138 at SRUC. Lamb management was as described above, with grazing (supplemented with creep feeding for hand reared lambs) until transportation to slaughter.

Of the 211 lambs used in this study, it was possible to unequivocally assign TM-QTL genotypes to 144: 40 non-carriers (+/+), 17 heterozygote carriers inheriting TM-QTL from the dam (+/TM), 53 heterozygote carriers inheriting TM-QTL from the sire (TM/+) and 34 homozygote carriers (TM/TM). The numbers of lambs of each known genotype from each sire used are shown in Table 1.

Please insert table 1 about here

Pre-slaughter measurements on 2009-born lambs

All lambs were ultrasound scanned at approximately 20 weeks of age (average age = 138 days, max = 151, min = 119) using a Dynamic Imaging Concept MLV ultrasonic scanner with a 3.5 mHz transducer at the third lumbar vertebra to measure muscle depth and fat depth. Muscle depth was measured vertically at the deepest point. Three fat depths were measured on each scan: the first above the boundary between *M. longissimus lumborum* (MLL) and the vertebral spinous process, and the others at progressively lateral intervals of around 2 cm. This resulted in fat depths that, for most animals, spanned the *longissimus* muscle. These fat depths were averaged to provide a single measure of ultrasound fat depth for use in the analyses.

Lambs were CT scanned in three batches. The first batch of lambs (n = 40; all from SRUC) were CT scanned with a Siemens Somatom Esprit CT scanner at the SRUC-BioSS CT Scanning Unit near Edinburgh at approximately 16 weeks of age (average age = 112 days, max = 118, min = 93). Meat from these lambs was due to go for taste panel assessment (results reported by Lambe *et al.*, 2011) so they had to be CT scanned at least 28 days prior to slaughter to allow for a withdrawal period from the sedative used for CT scanning. The other two batches of lambs were CT scanned at approximately 20 weeks of age. The first of these two batches, the IBERS lambs (n = 73), were scanned using a mobile General Electric CT scanner at IBERS (average age = 131 days, max = 141, min = 119). The last batch, the remaining SRUC lambs (n = 98), were scanned with the Siemens Somatom Esprit CT scanner (average age = 136 days, max = 145, min = 121).

All lambs were spiral CT scanned (Navajas *et al.*, 2006; Bunger *et al.*, 2011). Two spiral scans were taken: one from the proximal third of the tibia to the last rib and the

second from the last rib to the fourth to fifth cervical vertebra. These spiral scans were used to provide a series of approximately 60 cross-sectional images through the carcass, each 8mm apart. The cross-sectional images were analysed using STAR software (Mann *et al.*, 2003) to provide total carcass tissue volumes and densities (Hounsfield units) (fat, lean and bone), and tissue volumes and densities (fat, lean and bone) in the leg, saddle and shoulder regions, as well as two-dimensional (2D) and three-dimensional (3D) measurements in the loin region and the leg region. Total tissue weights in the carcass or region of interest were calculated over all images for each tissue in the image by multiplying tissue volume by the weighted mean density of the tissue: (Σ(area x density) / Σarea). For bone, because the density of bone cannot be well estimated from images analysed using STAR, a fixed value of bone density (1.55g/cm^3) was used.

The carcass was virtually split into the leg (equivalent to hind-quarter), saddle and shoulder (equivalent to fore-quarter) regions using in-house algorithms (unpublished data). 2D-CT measurements taken in the loin were depth (D), width (W) and area (A) of the MLL in a cross-sectional scan taken at the fifth lumbar vertebra (Jones *et al.*, 2002). Both left and right sides were measured and the average of these used in analyses. In the leg, the 2D CT measurements were width (W) and length (L) of the hind leg (HL) muscle on a cross-sectional scan taken at the ischium as described by Jones *et al*. (2002). Measurements were made on both right (r) and left (l) legs and the average used in analyses. A 2D gigot shape score was also calculated as 10(HLWr + HLWl)/(HLLr + HLLl). Measurements taken using the 3D capabilities of the CT scanner were loin region muscle volume (LRMV), lumbar spine length (LSL), hind leg muscle volume (HLMV) and femur length (FL). These allowed calculation of

a muscularity index, as described by Navajas *et al.* (2007), for both the loin and hind leg regions. This index relates the weight of muscle in a region (equivalent to muscle volume because muscle density is close to 1 g/cm^3) to the length of the bone in that region and thus provides a dimensionless assessment of muscularity, independent of fatness, at a constant carcass weight. The CT muscularity index for the hind leg (HLMI) was calculated as $10\sqrt{(HLMV/FL^3)}$ and that for the loin region (LRMI) was calculated as $10\sqrt{(LRMV/LSL^3)}$.

Post-slaughter measurements on 2009-born lambs

Mean age at slaughter was 144 days (s.d. 7.5, range 126–155 days) and mean hot carcass weight was 15.2 kg (s.d. 3.1, range 8–25 kg). Post-slaughter, carcasses were chilled for 7-9 days then CT scanned using spiral CT scanning. The CT scanning was similar to that performed on live animals except that thresholds suitable for meat were used (unpublished data), the analysis was simpler as there was no need to edit the images to remove non-carcass parts, and only carcass and regional tissue weights were calculated, not muscularity data. Following CT scanning of the carcasses, they were cut into fore-quarter, saddle and hind-quarter and each of these split into two along the spine. These were weighed and butchered into lean meat yield (LMY), fat trim and bone. Using these data, proportions of LMY, fat trim and bone in the carcass and in each region (fore-quarter, saddle, hind-quarter) were calculated. The proportions of total carcass weight contained in each region were also calculated. During butchery, left and right knuckle muscles were removed from the leg joints and left and right *M. longissimus lumborum* (lamb loin fillet or strip loin) were removed from the loin joint and these muscles weighed individually.

Statistical analyses

General linear models were run in Genstat (GenStat 11 Committee, 2008; linear mixed models, REML) to identify the effect of TM-QTL on the traits described above. The model used included TM-QTL genotype (+/+, +/TM, TM/+, TM/TM or unknown), sex (entire male or female), rearing rank (single, twin or hand-reared), farm (SRUC or IBERS) and dam age (2, 3, 4 years or older) as fixed effects, and sire as a random effect (7 levels, 3 common across farms). A covariate of age at scanning was included to adjust the analyses rams

to an equal age. For all traits, including proportion traits, a covariate of live weight at measurement (for pre-slaughter traits) or carcass weight (for post-slaughter traits) was included. For proportion variables a significant relationship was observed between the proportions and live or carcass weight, and these were used as covariates where applicable.

To partition variation due to TM-QTL genotype effects, after adjusting for all other effects in the model in a GLM analysis, orthogonal contrasts were fitted for +/+, +/TM, TM/+ and TM/TM as defined by Freking et al. (1998) for additive (1, 0, 0 and -1), dominance (-1, 1, 1 and -1) and reciprocal heterozygote (0, 1, -1, and 0) models of gene action. The hypothesis of a paternal polar overdominant action of TM-QTL was tested for (-1, -1, 3, -1) as well as maternal dominance (-1, 2, 0, -1), in a second set of orthogonal contrasts, alongside the additive effect (Freking *et al.*, 1999). The polar overdominance contrast tests whether animals inheriting the QTL from their sire, but not their dam, are significantly different from the mean of the other three genotype categories, whereas the maternal dominance contrast compares the

285 animals inheriting the QTL from their dam, but not their sire, with the average of the
286 two homozygote genotypes.
287
288 **Results**
289 *Position of TM-QTL*
290 Figure 1 shows the F-ratio for the probability from QTL Express of a QTL for UMD
291 being located at each cM along the 23cM segment of OAR18 between MCMA26 and
292 OARTMR1, confirming the presence of a QTL affecting ultrasound muscle depth
293 (adjusted for live weight) in this segment of OAR 18. This interval mapping approach
294 showed that the most likely position of TM-QTL is at 19cM from MCMA26, which is
295 between microsatellite markers OY3 and OARTMR1. However, because relatively
296 few markers define the region tested, no confidence interval for this position is given.
297 There was some variation in the magnitude of the effect of TM-QTL with the effect
298 ranging from 0.54 mm to 2.82 mm UMD (s.e. 0.37 mm to 0.57 mm) across the sires
299 that were segregating for TM-QTL. These analyses assume an additive effect of the
300 QTL and ignore the possibility of paternal polar overdominance.
301
302 **Figure 1 about here**
303
304 *Ultrasound muscle and fat depths and live weight at 20 weeks*
305 Live weight at 20 weeks was significantly higher in TM/TM animals than either +/+
306 (+7.3%) or +/TM animals (+11.7%), with TM/+ animals intermediate (Table 2).
307 Ultrasound muscle depth, when corrected for live weight, was significantly higher in
308 TM/+ than +/+ animals (+6.3%), but when not corrected for live weight, it was similar
309 in TM/TM and TM/+ animals, with both significantly higher than +/+ animals (+8.1%

and +8.4% respectively). Ultrasound fat depth corrected for live weight was highest in TM/TM animals and lowest in TM/+ animals, with these two groups being significantly different from each other (+10.2%), but not from +/+ or +/TM animals. When not corrected for live weight, TM/TM animals had the highest fat depth. The evidence for an additive effect of TM-QTL on live weight was not quite significant (P = 0.08), but there was significant evidence that the effect of TM-QTL on live weight corrected UMD showed paternal polar overdominance (P = 0.05).

Table 2 about here

CT measured muscularity and dissected loin muscle weight

Loin muscle width, depth and area, loin muscle volume and dissected *M. longissimus lumborum* weight were significantly greater for TM/+ than +/+ animals (+2.9 to +7.9%), and for depth, area and muscle volume were also significantly greater for TM/+ than +/TM animals (+6.9 to +11.3%) (Table 3). Lumbar spine length was highest for TM/+, significantly higher than +/TM and TM/TM but not significantly different to +/+, but loin muscularity index was not significantly different between groups. There was significant evidence that TM-QTL had a paternal polar overdominant action on CT measured loin muscle area, depth and width and loin region muscle volume and dissected *M. longissimus lumborum* weight. There were no significant effects of TM-QTL on hind leg muscle dimensions or muscularity or femur length (results not shown).

Table 3 about here

Carcass weight and composition

There was an additive effect of TM-QTL on carcass weight with TM/TM being significantly heavier than +/+ (+11.1%) and +/TM animals (+11.9%), with TM/+ intermediate (Table 4). Carcass fat, muscle and bone weights shown in Table 4 are those measured using carcass CT scanning and are adjusted for total carcass weight. TM/+ had higher carcass muscle weights than +/+ (+1.36%) and for this trait the test for paternal polar overdominance was close to significance (P = 0.066). The carcass CT scanning results are shown here as these are believed to be the more accurate reflection of carcass composition. However, the butchery results (shown in supplementary table S1) are the commercially relevant ones. When measured using live animal CT, muscle weight was slightly higher in TM/TM than +/+ animals (+2.8%, P = 0.047). When measured using butchery, weight of lean meat yield was also slightly higher in TM/TM than +/TM animals (+3.0%, P = 0.045). There were no significant effects on CT predicted fat or bone weights in either live animals or carcasses. The butchery results (supplementary table S1) showed no effect on bone weight, but weight of fat trimmed from the carcass cuts was significantly lower for TM/+ than +/+ animals (-11.2%; P = 0.036).

Table 4 about here

Weight and composition of joints

Weights of the leg, saddle and shoulder region and proportion of carcass weight contained in each of these regions did not differ significantly between genotypic groups (data in supplementary table S2). For the carcass CT scanning data, composition of leg, saddle and shoulder regions showed significant differences between genotypic groups for 4 traits (data in supplementary table S3). The leg region had significantly less fat in TM/+ than +/+ lambs (27g; -5.1%; P = 0.04) and

+/TM had significantly less muscle than the other genotypic groups (130g-144g; ~3%; $P < 0.03$). For the saddle region, TM/+ animals were less fat than +/TM animals (84g; -13.5%; $P = 0.049$), and +/+ had significantly less muscle than either +/TM (136g; -7.16%; $P = 0.009$) or TM/+ (86g; -4.53%; $P = 0.016$). For the live CT scanning data, there were no significant differences between genotypic groups for composition of the leg, saddle or shoulder regions (data not shown). In the butchery data (supplementary table S4), +/TM animals had significantly less LMY in the leg region than both TM/+ (77g; -5.70%; $P = 0.019$) or TM/TM animals (78g; -5.77%; $P = 0.026$). TM/TM had significantly less bone in the leg region than the other three groups (-3.24% to -3.99%; $P = 0.008$ to $P = 0.028$), although in real terms this was a difference of only 22.6-28.1g. A significant negative maternal dominance effect was found for LMY in the leg and a significant dominance effect was observed for bone weight in the leg.

Discussion

The work reported here comprises results arising from a comprehensive experiment to evaluate the effect of TM-QTL on carcass traits in purebred Texel lambs. TM-QTL was first reported by Walling *et al.* (2004) on OAR18, located between microsatellite markers MCMA26 and OARTMR1, and the current study has confirmed the presence of TM-QTL on this segment of OAR18. Using the microsatellite markers available to us at the time, it would have been difficult to more accurately position the TM-QTL. There is a possibility that other QTL in this region of OAR18, such as the Carwell QTL, are allelic to TM-QTL. Further work to fine-map this region would be

required to more accurately position TM-QTL and, ultimately, determine whether the other QTL lying in this region are different from or allelic to TM-QTL.

TM-QTL affects loin muscling in Texel sheep. The initial study by Walling *et al.* 2004 showed an effect of +4 to +7% on ultrasound muscle depth, and in a larger population in a follow-on study Matika et al. (2011) showed an effect of +8 to +17% in 6 out of 36 Texel families. This effect was confirmed in Texel sired crossbred lambs out of Mule ewes (Macfarlane *et al.*, 2009) and also out of Welsh Mountain ewes (Masri *et al.*, 2011). Macfarlane *et al.* (2009) also noted that loin muscle (*M. longissimus lumborum*) width, area, volume and weight were also higher in lambs inheriting TM-QTL from their sire.

These earlier studies all used heterozygote carriers of TM-QTL where TM-QTL was inherited from the sire. Based on the maternal and paternal variance components for muscle depth in their data Matika *et al.* (2011) hypothesised that the TM-QTL is characterised by a paternal polar overdominant pattern of expression, although the structure of their study could not provide direct evidence of this form of imprinting. The present study reports, for the first time, the effect of the inheritance of TM-QTL from the dam, either alone or together with TM-QTL from the sire and provides supporting evidence for a polar overdominant pattern of expression for the TM-QTL's effect on loin muscling (ultrasound muscle depth, CT muscle depth, width, area and volume and dissected weight). This mode of inheritance will have an important impact on optimal utilisation of the TM-QTL within the sheep industry, since the TM-QTL phenotype is only expressed in carriers of a single copy of TM-QTL inherited from the sire. Imprinting tends to affect a region of a chromosome and it is therefore

not unexpected that TM-QTL would be imprinted, given its position within the same region as both Carwell (Nicoll, 2007) and Callipyge (Cockett *et al.*, 1994a, Charlier *et al.*, 2001a; Freking *et al.*, 2002) and the cluster of imprinted genes around Callipyge (Charlier *et al.*, 2001b, Cockett *et al.*, 1996b).

The results of Macfarlane *et al.* (2012), showing an apparent additive effect of TM-QTL on live and carcass weights, were replicated here. Of further interest was the effect TM-QTL had on carcass and joint composition. In previous work looking at the effect of a single copy of TM-QTL in crossbred Texel-sired lambs, there did not appear be an effect on other carcass traits (out of Mule ewes, Macfarlane *et al.*, 2009; out of Welsh Mountain ewes, Masri *et al.*, 2011). In the present study, lambs inheriting TM-QTL from their sire (either homozygote or heterozygote carriers), tended to be less fat than wild-type homozygotes and this translated to a commercially relevant significant difference in weight of fat trimmed from the carcass during butchery (-11%) between homozygote wild-types and heterozygotes inheriting TM-QTL from the sire. Furthermore, although the differences were small and not always significant, muscle weight and lean meat yield tended to be higher in lambs inheriting TM-QTL from their sire (either homozygote or heterozygote carriers) than in wild-type homozygotes or lambs inheriting TM-QTL from their dam. This indicates that animals inheriting TM-QTL from their sires are likely to produce carcasses with slightly greater lean meat yield and require less work for fat trimming during butchery, in addition to the greater weight of the high value loin muscle. There did not appear to be any unfavourable effects of TM-QTL on carcass traits and Lambe et al. (2011) has shown that there are no significant effects of TM-QTL on meat quality when meat was conditioned for a period of 7-9 days.

In summary, the direct effects of TM-QTL on loin muscling (4-11% increase in highly priced part of the carcass) and growth make it an interesting candidate for exploitation within the UK sheep industry and beyond, especially since it does not have any major negative impacts on eating quality. However, there are two main aspects to consider before commercial exploitation is feasible: (i) a commercial genotyping test and (ii) a usage plan.

(i) Commercial genotyping test: Exploitation of this QTL will require development of a suitable and affordable DNA test to identify carrier animals, as usage of microsatellite marker panels with family-specific linkage phases is not feasible in practice. This will necessitate further research to fine map and identify closely linked markers or even the specific mutation(s) involved, so that a commercial SNP test can be developed. However, in the case of Parent-of-origin (PofO) effects, such as polar overdominance, the homologous chromosomes exhibit differential gene expression and conventional association studies generally ignore such inheritance patterns, considering maternal and paternal alleles to be equivalent (e.g. Garg *et al.*, 2012). The problems caused by PofO on genome-wide association (GWA) analyses has been discussed in detail by Rowe *et al*. (2012) and it is obvious that this remains challenging, as the recent standard approach for fine mapping using dense SNPs may not work well. Typically, GWA studies regress the phenotype on the number of (minor) alleles present at the locus, however, with polar overdominance and an allele frequency approaching 0.5, the regression of a trait showing polar overdominance on allele count will be close to zero (see Rowe *et al*., 2012). Hence standard GWA analyses miss the

effect. To overcome this problem, one would need phased haplotypes, i.e. knowledge of the PofO, and specifically fit phased-haplotype-derived genotype class in the analysis, as suggested earlier (Rowe *et al.*, 2012).

(ii) Utilisation: Optimal usage in a purebred situation is different from that in a crossbred and it is important to consider if the aim is to exploit the muscling effects or the growth effects of TM-QTL. In a pure-bred scenario, in terms of muscling, one wants to take the QTL to a frequency of ca. 0.5 (although for live weight it should go higher). But for maximum benefit in crossbred progeny (assuming that the dam breed does not carry the QTL) one simply wants all sires to be homozygous, so that their progeny benefit in terms of both liveweight and muscling effects. This implies that for the optimum utilisation strategy for the muscling effects in crossbred lambs, the performance in the purebred population is not at its optimum. In contrast to the muscling effects, the growth effects of TM-QTL seem to show an additive effect, with animals inheriting two copies of TM-QTL showing an increase of 1.5 kg or 9% in carcass weight when slaughtered at a fixed age, and an increase in live weight across a range of ages from birth to slaughter (+4 to +15%), compared to wildtype animals (Macfarlane et al, 2012). Such differences have implications for exploitation within the stratified industry structure typical of the UK. To benefit fully from the effects on growth and carcass weight, the TM-QTL will need to be introgressed into the dam line as well as fixed within terminal sires; however this will lose the benefits for muscling. Exploitation of the effects on loin muscling will require TM-QTL to be absent in the dam line and fixed in a homozygous state within terminal sire breeds to derive maximum commercial benefit.

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Tables

Table 1. Distribution of TM-QTL genotype status of lambs across the seven different sires used

TM-QTL	Sire							Total
Genotype	**1***	2	3	4	5	**6**	**7**	
+/+		1	2	11	3	14	9	40
+/TM		3			7		7	17
TM/+	21	1	2	5	4	14	6	53
TM/TM	22	1			5		6	34
Unknown	11	4	7	2	4	11	28	67

*Note: Sire 1 was homozygous and sires 2 to 7 all heterozygous

The sires in bold have been used on both farms

Table 2. Least squares means[†] for live weight at 20 weeks (US LW) and ultrasound muscle depth and fat depth, both adjusted (UMD_LW, UFD_LW) for live weight and unadjusted for live weight (UMD, UFD) for the four TM-QTL genotype groups and the p-values for the tests of different modes of action for the QTL

Genotype	US LW[1]	UMD[2]	UMD_LW[3]	UFD[4]	UFD_LW[5]
+/+[$]	35.8[b]	23.5[b]	22.5[b]	3.04[b]	2.87[ab]
+/TM	34.4[b]	23.7[ab]	23.5[ab]	2.91[b]	2.88[ab]
TM/+	36.9[ab]	25.5[a]	23.9[a]	3.08[b]	2.81[b]
TM/TM	38.4[a]	25.4[a]	23.3[ab]	3.46[a]	3.10[a]
average s.e.d.	1.43	0.940	0.593	0.182	0.155
minimum s.e.d.	1.13	0.71	0.480	0.145	0.122
maximum s.e.d.	1.67	1.12	0.700	0.213	0.182
P values for:					
Additive effect	0.08	0.05	0.32	0.03	0.19
Dominance effect	0.56	0.41	0.10	0.20	0.23
Reciprocal heterozygote effect	0.70	0.38	0.40	0.80	0.51
Maternal dominance effect	0.55	0.92	0.50	0.46	0.64
Paternal polar overdominance	0.99	0.14	0.045	0.25	0.13

[†] LS means with common letters in their superscripts, within column, are not significantly different (P > 0.05), where differences were significant, p-values are shown in the numbered footnote corresponding to that trait. Average, minimum and maximum s.e.d. are shown for information.

[$]TM-QTL genetic groups: +/+ homozygous for the wild-type allele; TM/+ and +/TM heterozygote carriers of paternal and maternal origin of allele , respectively and TM/TM homozygous for the TM-QTL allele

1 US LW: TM/TM vs. +/+ = 0.049, TM/TM vs. +/TM = 0.017

2 UMD: TM/TM vs. +/+ = 0.020, TM/TM vs. +/+ = 0.050

3 UMD_LW: TM/+ vs. +/+ = 0.004

4 UFD: TM/TM vs. +/+ = 0.018, TM/TM vs. +/TM = 0.010, TM/TM vs. TM/+ = 0.013

5 UFD_LW: TM/+ vs. TM/TM = 0.025

Table 3. Least squares means[†] for live weight adjusted CT measured loin muscle area, depth and width (MLLA; mm^2, MLLD; mm, MLLW; mm), loin muscularity index (LRMI), loin muscle volume (LRMV; cm^3) and lumbar spine length (LSL; cm) and dissected loin muscle weight (MLL wt; g) for the four TM-QTL genotype groups and the p-values for the tests of different modes of action of the QTL on these traits

	MLLA[1]	MLLD[2]	MLLW[3]	LRMI	LRMV[4]	LSL[5]	MLL wt[6]
+/+	1739^{bc}	29.13^{b}	67.74^{b}	2.953	548.5^{bc}	18.3^{ab}	806^{b}
+/TM	1699^{c}	29.12^{b}	68.61^{ab}	2.966	519.4^{c}	17.8^{b}	801^{ab}
TM/+	1877^{a}	31.13^{a}	69.71^{a}	2.99	577.9^{a}	18.7^{a}	837^{a}
TM/TM	1838^{ab}	30.35^{ab}	69.17^{ab}	3.041	567.4^{ab}	18.2^{b}	817^{ab}
ave s.e.d.	57.45	0.858	0.853	0.085	18.6	0.338	20.9
min s.e.d.	45.6	0.68	0.69	0.069	14.9	0.260	16.5
max s.e.d.	66.9	1.00	1.00	0.099	21.8	0.430	24.4
P values for							
Additive effect	0.08	0.091	0.031	0.35	0.62	0.73	0.83
Dominance effect	0.91	0.26	0.58	0.52	0.39	0.69	0.78
Reciprocal Heterozygote effect	0.004	0.09	0.022	0.66	0.01	0.08	0.02
Maternal dominance effect	0.15	0.90	0.41	0.51	0.06	0.24	0.32
Paternal polar overdominance	0.002	0.01	0.01	0.99	0.04	0.12	0.01

[†] LS means with common letters in their superscripts, within column, are not significantly different (P > 0.05), where differences were significant, p-values are shown in the numbered footnote corresponding to that trait. Average, minimum and maximum s.e.d. are shown for information.

[1]MLL_A: +/+ vs. TM/+ = 0.003, +/TM vs. TM/+ = 0.005, +/TM vs. TM/TM = 0.039

[2] MLL_D: +/+ vs. TM/+ = 0.004, +/TM vs. TM/+ = 0.034; [3] MLL_W: +/+ vs. TM/+ = 0.005

[4] LRMV: +/+ vs. TM/+ = 0.049, +/TM vs. TM/+ = 0.004, +/TM vs. TM/TM = 0.029

[5] LSL: TM/+ vs. +/TM = 0.035, TM/+ vs. TM/TM = 0.047; [6] MLL wt: +/+ vs. TM/+ = 0.047

Table 4. Least squares means[†] for cold carcass weight (kg) and carcass fat, muscle and bone weights (all adjusted for carcass weight) measured using post-slaughter carcass CT scanning for the four TM-QTL genotype groups and the p-values for the tests of different modes of action for the QTL on these traits

	Carcass wt (kg)[1]	Fat wt (g)	Muscle wt (g)[2]	Bone wt (g)
+/+	14.6[b]	2037	9390[b]	2138
+/TM	14.5[b]	2025	9428[ab]	2103
TM/+	15.2[ab]	1922	9518[a]	2141
TM/TM	16.2[a]	1980	9488[ab]	2096
ave s.e.d.	0.715	80.9	72.6	39.2
min s.e.d.	0.567	65.0	58.2	31.2
max s.e.d.	0.836	95.5	85.8	46.3
P values for:				
Additive effect	0.03	0.22	0.06	0.46
Dominance effect	0.69	0.12	0.27	0.22
Reciprocal heterozygote effect	0.93	0.64	0.30	0.41
Maternal dominance effect	0.75	0.42	0.84	0.21
Paternal polar overdominance	0.85	0.12	0.07	0.99

[†] LS means with common letters in their superscripts, within column, are not significantly different (P > 0.05), where differences were significant, p-values are shown in the numbered footnote corresponding to that trait. Average, minimum and maximum s.e.d. are shown for information.

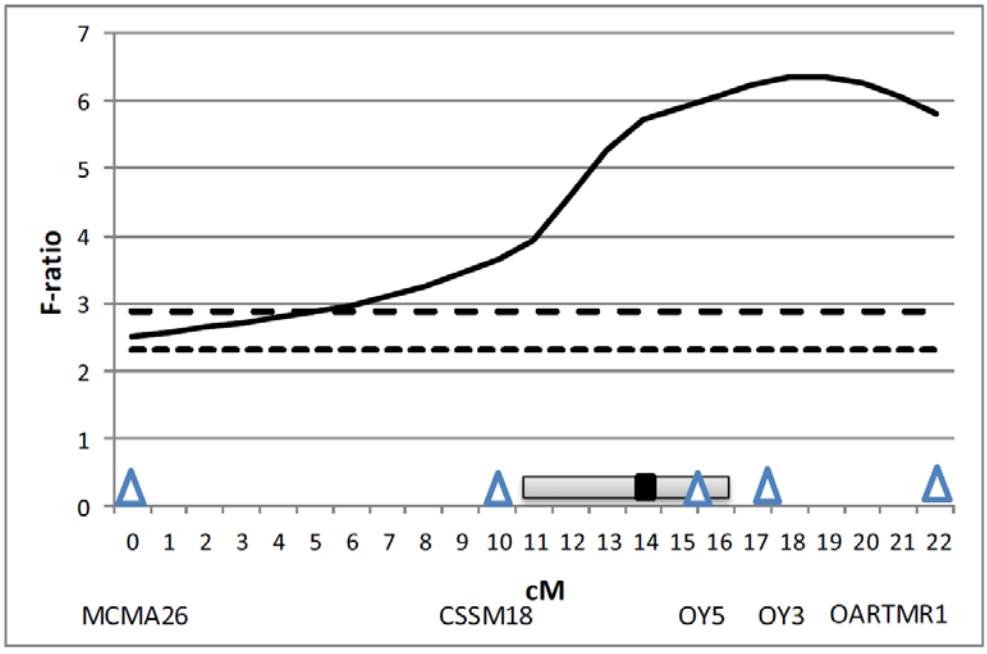
[1] Carcass weight: TM/TM vs. +/+ P = 0.021, TM/TM vs. +/TM P = 0.040

[2] Muscle weight: TM/+ vs. +/+ P = 0.029

Caption for the Figure

Figure 1. Statistical evidence for a QTL affecting ultrasound muscle depth (shown as solid line), expressed as an F ratio, at each cM along a segment of ovine chromosome 18 between MCMA26 and OARTMR1, using an interval mapping approach. Significance thresholds are shown by horizontal lines (P = 0.01 - - - ; P = 0.05 -----). Also included are the position of the Callipyge mutation (■; Freking *et al.*, 2002) and the approximate region thought to be associated with the Carwell QTL (iiiiiiiii iiiiiii; McLaren *et al.*, 2003).

8 Figures



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