Texel loin muscling QTL (TM-QTL) located on ovine chromosome 18 appears to exhibit imprinting and polar overdominance

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Introduction

Increasing numbers of potentially exploitable genes and quantitative trait loci (QTL) are being discovered that have identifiable effects on production traits and could be of value to livestock industries. A cluster of genes/QTL affecting muscling have been found on ovine chromosome 18; *Callipyge*, Carwell QTL, TM-QTL. The *Callipyge* mutation displays a complex mode of inheritance with imprinting and polar overdominance (Georges & Cockett, (1996)). Animals inheriting a single, paternal copy of *Callipyge* have increased pelvic and torso muscle weights (up to 46%), reduced back fat depth (26%), increased *M. longissimus dorsi* area (30%) and increased leg score (Jackson et al. (1997a); Jackson et al. (1997b)), although the meat is significantly less tender (Duckett et al. (2000)). The mutation underlying *Callipyge* is a single A to G transition approximately 32 kb upstream of the *GTL2* gene, and appears to affect the expression of a closely-linked cluster of imprinted genes (Freking et al. (2002); Smit et al. (2003)). The Carwell QTL, identified in Australian Poll Dorsets, has a more moderate effect restricted to the loin region; a single copy increasing *M. longissimus dorsi* weight and area by 7% to 11%, respectively (Banks (1997); Nicoll et al. (1998)). Carwell is reported to be between 2 and 6 cM telomeric of microsatellite marker *CSSM18* (Nicoll et al. (1998)). There is evidence that Carwell does not display an additive mode of inheritance (Jopson et al. (2001)) and a suggestion that it is imprinted (paternally expressed) (Nicoll (2007)). The TM-QTL was identified in purebred UK Texel sheep (Walling et al. (2004)) and its effect is also restricted to the loin (Macfarlane et al. (2009)), a single copy inherited from the sire leads to a 4 to 7% increase in loin muscle depth (Walling et al. (2004); Macfarlane et al. (2009)). The position for TM-QTL reported by Walling et al. (2004) indicates that TM-QTL is likely to be close to, or allelic to, Carwell but the *Callipyge* mutation is not causal for TM-QTL (unpublished results). Given the evidence for imprinting in this region, if TM-QTL is to be used in commercial sheep breeding programmes, it is important to investigate its effect in homozygotes and both paternal and maternal heterozygotes, identify the mode of inheritance and quantify any evidence for imprinting. This study investigates the inheritance pattern of TM-QTL and examines its direct effects on loin muscling using a variety of methods: ultrasound, X-ray computed tomography (CT) scanning, dissected *M. longissimus lumborum* and meat yield of the loin primal cut.

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Material and methods

Animals and their management. A population of Texel sheep across two farms, one in Wales and one in Scotland, was used to produce 211 Texel lambs in 2009. The population of Texel sheep had previously been managed to increase the frequency of heterozygote TM-QTL carriers and produce some homozygote TM-QTL carriers. The 211 lambs used in this study 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 dams, 46 had been previously identified as carrying TM-QTL, 86 as not carrying TM-QTL and the remaining 49 had unknown TM-QTL status. Lambs were either reared as a single (n=140) or a twin (n=59), or hand-reared (n=12), and were either entire male (n=96) or female (n=115). There were 73 lambs in Wales and 138 in Scotland. At both sites, lambs were grazed at pasture with the ewes until being transported to slaughter, except for the hand-reared lambs, which were raised indoors until the age of approximately 8 weeks and then grazed and creep-fed a barley-based concentrate mix until transported to slaughter.

Measurement of loin muscling. All lambs were CT scanned, ultrasound scanned and slaughtered and the carcasses butchered. At around 19 weeks of age (average age = 134 days) lambs were CT scanned in cross-section at the 5^{th} lumbar vertebra and the CT images analysed to provide two-dimensional (2D) measurements: *M. longissimus lumborum* (MLL) depth (CT_MLL_D), width (CT_MLL_W) and area (CT_MLL_A) (Jones *et al.*, 2002). Both left and right side MLL were measured and the average of these used in analyses. At around 19 weeks of age (average age = 138 days) all lambs were also weighed and ultrasound scanned at the third lumbar vertebra, to measure MLL depth at the deepest point (UMD). At around 20 weeks of age (average age = 144 days) the lambs were slaughtered and the carcasses chilled and split into primal cuts. The left loin primal cut was butchered into lean meat, fat and bone to measure lean meat yield (LMY_LOIN), and MLL from both sides were removed, weighed and the average used in analyses (MLL_WT).

Genotyping All lambs born in 2009 were blood-sampled shortly after birth, using a nose-prick, and blood spotted onto FTA® cards. Because the causal mutation responsible for the TM-QTL is as yet unknown, it is 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 chromosome 18 (*MCMA26*, *CSSM18*, *OY5*, *OY3* and *OARTMR1*) at AgResearch, New Zealand. Marker data from these lambs, along with all marker data collected on the population previously, were edited for marker inheritance inconsistencies. Marker data were used to identify the paternal and maternal haplotypes for each animal in the population. Marker and UMD data from all animals in the population, along with the pedigree, were then subjected to variance component estimation procedures to calculate estimated breeding values (EBVs) for UMD. The proportions of genes identical-by-descent (IBD) between all individuals at the QTL location were then estimated using a deterministic method (Pong-Wong et al. (2001)). Combining phenotypic information and marker data, EBVs were partitioned into background (polygenic) and QTL components inherited from the sire and from the dam using MA-BLUP (Fernando and Grossman (1989)). Within sire families, animals carrying the haplotypes previously associated with the favourable allele of TM-QTL were identified and these checked to see that they matched with the higher sire QTL-EBVs. This enabled classification of all animals into carrier (TM^S) or non-carrier ($+^S$) or unknown ($?^S$) status (for recombinant haplotypes or missing marker information, or, for older animals in the pedigree, unknown parentage) for TM-QTL

inherited from the sire. Animals whose dam had been classified as TM^S were then examined to identify those which carried the maternal grand sire's TM-QTL-associated marker haplotype to classify animals based on TM-QTL inherited from the dam in a similar way to that described for classification of TM-QTL inherited from the sire. Of the 211 lambs born in 2009 which had live-animal and carcass measurements, 41 were classed as homozygote non-carriers of TM-QTL ($+^S/+^D$), 53 were classed as heterozygote carriers with TM-QTL coming from the sire ($TM^S/+^D$), 17 classed as heterozygote carriers with TM-QTL coming from the dam ($+^S/TM^D$), and 34 were classed as homozygotes for TM-QTL (TM^S/TM^D). Of the remainder, 26 were $+^S/?^D$, 30 were $TM^S/?^D$, 5 were $?^S/+^D$, 2 were $?^S/TM^D$, and 3 were $?^S/?^D$.

Statistical analyses. General linear model (GLM) analyses were performed in Genstat to identify the effect of TM-QTL genotype on UMD, CT_MLL_D, CT_MLL_W, CT_MLL_A, MLL_WT and LMY_LOIN. Data from all lambs were used in the analyses, including those with unknown genotype status, to enable better estimation of the other fixed effects, but only results for the four known genotype classes are reported; homozygote carrier, homozygote non-carrier, heterozygote carrier with QTL inherited from sire and heterozygote carrier with QTL inherited from dam. Fixed effects fitted included genotype, rearing rank, sex and farm. A covariate of live weight at scanning was included for UMD and the CT measurements, and a covariate of hot carcass weight included for MLL_WT and LMY_LOIN. Sire was included as a random effect. Dam age as a fixed effect and a covariate of age scanning were both tested but they were not significant and thus were not included in the final model.

Results and discussion

Least square (LS) means for the four genotype classes for each of the loin muscling traits measured are shown in Table 1. There is clear evidence that $TM^S/+^D$ lambs show greater loin muscling (4 to 11%) than $+^S/+^D$ lambs ($P = 0.04$ to $P < 0.001$). However, $+^S/TM^D$ lambs show no significant difference in loin muscling compared to $+^S/+^D$ lambs. Homozygote TM^S/TM^D lambs also do not show any significant difference in loin muscling compared to homozygote $+^S/+^D$ lambs. For CT_MLL_D, CT_MLL_W and CT_MLL_A, which are the most accurate measurements of loin muscling available to us, homozygote TM^S/TM^D lambs in fact showed significantly lower loin muscling (4 to 8%) than $TM^S/+^D$ lambs ($P = 0.02$ to $P = 0.002$) and were no different to non-carrier homozygotes ($+^S/+^D$).

Table 1: LS means[†] for loin muscling traits for the four different TM-QTL genotype classes

	$+^S/+^D$	$TM^S/+^D$	$+^S/TM^D$	TM^S/TM^D	Av. s.e.d. [‡]
UMD (mm)	23.02^b	24.65^a	$23.59^{a,b}$	$23.66^{a,b}$	0.574
CT_MLL_D (mm)	28.51^b	30.96^a	$29.61^{a,b}$	29.26^b	0.749
CT_MLL_W (mm)	66.62^b	69.40^a	$68.23^{a,b}$	66.95^b	0.859
CT_MLL_A (mm^2)	1691^b	1876^a	1744^b	1734^b	49.1
MLL_WT (g)	395^b	412^a	391^b	$400^{a,b}$	10.2
LMY_LOIN (g)	1063^b	1115^a	$1105^{a,b}$	$1102^{a,b}$	28.2

[†] LS means with common letters in their superscripts are not significantly different ($P > 0.05$)

[‡] Av. s.e.d is average of the s.e.d. for the comparisons of only the 4 genotype classes shown

The magnitude of the effect of one copy of TM-QTL inherited from the sire here is similar to that reported previously (Walling et al. (2004); Macfarlane et al. (2009)). The pattern of effects seen here suggests that TM-QTL exhibits a polar overdominant mode of inheritance and imprinting (paternally expressed), similar to that described for *Callipyge* (Georges and Cockett (1996)). Nicoll (2007) noted that there is some evidence that the Carwell QTL might also be imprinted. As imprinting tends to affect a region of a chromosome, it is not unexpected that TM-QTL may be imprinted, given its position within the same region as Carwell and *Callipyge* and the cluster of imprinted genes around *Callipyge*. Further work to evaluate TM-QTL's effect on meat quality is ongoing. Fine mapping of this region would be of interest to reveal whether Carwell and TM-QTL are indeed allelic.

Conclusion

This study has shown that TM-QTL, a QTL located on ovine chromosome 18 and affecting loin muscling, appears to be imprinted (paternally expressed) and to exhibit a polar overdominant mode of inheritance. This information will be valuable if TM-QTL is to be utilised in sheep breeding programmes to increase muscling of the highly priced loin region. Knowledge of the action of TM-QTL on meat quality and health and welfare traits is also required before utilisation of TM-QTL in sheep breeding programmes can be confidently recommended, to ensure there are no negative effects of the QTL.

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