Effect of the Texel muscling QTL (TM-QTL) on spine characteristics in purebred Texel lambs

C. L. Donaldson [a,1], N. R. Lambe [a], C. A. Maltin [b], S. Knott [c], L. Bünger [a]

[a] Scotland's Rural College, King's Buildings, West Mains Road, Edinburgh, EH9 3JG, United Kingdom

[b] Quality Meat Scotland, Rural Centre, Ingliston, Newbridge, EH28 8NZ, United Kingdom

[c] Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Ashworth Laboratories, West Mains Road, Edinburgh, EH9 3JT, United Kingdom

[1] Corresponding author. Animal & Veterinary Sciences Research Group, SRUC, Roslin Institute Building, Easter Bush, Midlothian, United Kingdom, EH25 9RG. Tel: +44 131 6 519325.
Email address: claire.donaldson@sruc.ac.uk (C. L. Donaldson)

Abstract

Previous work showed that the Texel muscling QTL (TM-QTL) results in pronounced hypertrophy in the loin muscle, with the largest phenotypic effects observed in lambs inheriting a single copy of the allele from the sire. As the loin runs parallel to the spinal vertebrae, and the development of muscle and bone are closely linked, the primary aim of this study was to investigate if there were any subsequent associations between TM-QTL inheritance and underlying spine characteristics (vertebrae number, VN; spine region length, SPL; average length of individual vertebrae, VL) of the thoracic, lumbar, and thoracolumbar spine regions. Spine characteristics were measured from x-ray computed tomography (CT) scans for 142 purebred Texel lambs which had been previously genotyped. Least-squares means were significantly different between genotype groups for lumbar and thoracic VN and lumbar SPL. Similarly for these traits, contrasts were shown to be significant for particular modes of gene action but overall were inconclusive. In general, the results showed little evidence that spine trait phenotypes were associated with differences in loin muscling associated with the different TM-QTL genotypes.

Keywords:

Vertebrae, Spine, Texel, Loin, Muscling

1. Introduction

Walling et al. (2004) reported evidence of a quantitative trait locus (QTL) segregating in the United Kingdom's Texel sheep population which significantly increased *longissimus dorsi* (loin) muscle depth (up to +1.15 – +2.00 mm, as measured ultrasonically over the third lumbar vertebra). Observing similar results (QTL effect of +2.57 mm) from an analysis including existing and new Texel family data, the QTL, later termed the Texel muscling QTL (TM-QTL), was further verified by Matika et al. (2006). Located on the distal end of the ovine chromosome 18 (OAR18) (Walling et al., 2004; Matika et al., 2006), the TM-QTL sits in the same region as the *Callipyge* (*CLPG*) and Carwell loci (Cockett et al., 1994; Nicoll et al., 1998) which are also known to affect carcass muscling; the *CLPG* mutation leads to greater muscle mass most pronounced in the hind quarters (loin, pelvis, leg) (Cockett et al., 1994; Koohmaraie et al., 1995; Jackson et al., 1997a, 1997b; Freking et al., 2002), while carriers of Carwell exhibit a larger loin muscle area and weight (Nicoll et al., 1998).

Such QTL are of economic interest as there is the potential to utilise their effects through selection programmes to gain greater carcass value (e.g. reducing fat deposition and increasing lean meat production). In the case of the TM-QTL, the proportion of the high value loin cut may be increased e.g. two-dimensional measurements (estimated from cross-sectional computed tomography (CT) scans, taken at the fifth lumbar vertebra) describing loin depth, width, and area were found to be ~ 0.5 – 11% greater in TM carrier lambs than non-carrier lambs (Macfarlane et al., 2010).

Moreover, taking the QTL's mode of inheritance into consideration allows the opportunity to exploit the TM-QTL more fully and appropriately in a commercial situation. Similar to *CLPG* (Cockett et al., 1994; Freking et al., 1998a), expression of the Texel muscling phenotype has been suggested to follow the complex parent-of-origin-dependent pattern of

inheritance termed polar overdominance (Macfarlane et al., 2010; Matika et al., 2011). This unique type of inheritance is characterised by the instance where heterozygous progeny that inherit a single copy of the allele from the sire exhibit the superior phenotype (Cockett et al., 1996). Indeed, Macfarlane et al. (2010) observed that the largest phenotypic effects of the TM-QTL were particularly apparent in the TM carrier lambs that had inherited a single copy of the TM allele from the sire and the wild type (+) from the dam (genotype $TM^{S}/+^{D}$; where superscripts S (sire) and D (dam) denote the paternal and maternal origin of the alleles, respectively), with loin depth, width, and area measures ~ 2 – 11% greater in these $TM^{S}/+^{D}$ genotype lambs than in the other three genotype groups (homozygote non-carriers, $+^{S}/+^{D}$; heterozygote carriers inheriting TM-QTL from the dam, $+^{S}/TM^{D}$; homozygote carriers, TM^{S}/TM^{D}).

Essentially, muscle hypertrophy from TM allele segregation appears to be localised to the loin muscle (Macfarlane et al., 2010), which is found along the length of the thoracolumbar (thoracic plus lumbar) spine region. Given that development of muscle and bone are closely linked, it is of further interest to investigate, across genotype groups, if the increased loin muscling is associated with any change to characteristics of the underlying spine section i.e. is there a subsequent effect on spine characteristics in relation to the pattern of TM allele inheritance? Freking et al. (1998b), for instance, found that the spinal column was significantly shorter in *CLPG* genotype lambs (-2.5 cm; when all animals compared at the same carcass weight) and the carcasses more compact in skeletal structure in comparison to normal genotype lambs. Given its chromosomal position, it may be a similar condition for the TM-QTL. This is a particularly relevant point to assess in terms of a possible 'trade-off' between increasing loin muscle size (e.g. depth) but, in consequence, shortening the spinal column.

The thoracolumbar spine region, on which the loin is located, encompasses the 'body' (or trunk) vertebrae and the total length of this region (as with any spine region) is a product of the number and length of vertebrae which comprise it. Hence, the difference in body (and carcass) lengths observed from individual to individual is contributed to the variation in these vertebral factors. Recent work has demonstrated that the spine characteristics (vertebrae number, vertebrae length), of the thoracolumbar region, can be reliably measured from CT scans. Using such method, it was also identified that these characteristics exhibit significant intra-breed variation in Texel sheep, for example, thoracolumbar vertebrae number was observed to range from 17 to 21 (Donaldson et al., 2013). Therefore, it may be reasonable to use CT measured spine traits to investigate if any association exists between the pattern of TM allele inheritance and spine characteristics.

2. Materials and Methods

2.1. Animals sampled

The present study used a subset of the 209 available purebred Texel lamb records previously used by Macfarlane et al. (2010) and Lambe et al. (2011). Lambs were sired by seven different rams that were previously identified as carriers of at least one copy of TM-QTL; all 209 lambs were blood-sampled soon after birth (born 2009) in order to classify their TM-QTL genotype (homozygote non-carrier, $+^S/+^D$; heterozygote carrier inheriting TM-QTL from the sire, $TM^S/+^D$; heterozygote carrier inheriting TM-QTL from the dam, $+^S/TM^D$; homozygote carrier, TM^S/TM^D); detailed information on the genotyping of the animals can be found in Macfarlane et al. (2010). However, for a number of animals the genotype could

not be fully classified. These unknowns were excluded from this study's analysis, leaving a total of 142 lamb records in the subset, which divided into the TM-QTL genotype groups as follows: 39 $+^{S}/+^{D}$, 52 $TM^{S}/+^{D}$, 17 $+^{S}/TM^{D}$, 34 TM^{S}/TM^{D}. These 142 lamb records included 59 entire males and 83 female lambs from the purebred population of Texel sheep kept across two sites, one in Scotland and one in Wales, which were reared as either singles (n = 97), twins (n = 34), or artificially (pet; n = 11) (further details on the management of these animals can be found in Macfarlane et al. (2010) and Lambe et al. (2011)).

2.2. Computed tomography (CT) measurements

Lambs were CT scanned at ~ 126 days of age (ranging from 93 to 145 days) and their topogram images (produced from the CT process) used to quantify spine characteristics for each, details provided in Donaldson et al. (2013). In short, spine traits measured directly from the scans included counts of vertebrae in the thoracic and lumbar regions (VN_{THOR} and VN_{LUM} respectively) and length (mm) of the thoracic and lumbar spine region (SPL_{THOR} and SPL_{LUM} respectively). These measures were used to calculate the average length (mm) of individual vertebrae in the thoracic and lumbar regions (VL_{THOR} (SPL_{THOR}/VN_{THOR}) and VL_{LUM} (SPL_{LUM}/VN_{LUM}) respectively). The results for the thoracic and lumbar spine regions were further used to provide the number of thoracolumbar vertebrae (VN_{T+L} ($VN_{THOR}+VN_{LUM}$)), and the length (mm) of the thoracolumbar region (SPL_{T+L} ($SPL_{THOR}+SPL_{LUM}$)). These thoracolumbar spine traits were then used to calculate the average length (mm) of individual vertebrae across the thoracolumbar region (VL_{T+L} (SPL_{T+L}/VN_{T+L})).

For each lamb, the dimensions, width (mm), depth (mm), and area (mm^2), of the *longissimus lumborum* (CT_MLL_W, CT_MLL_D, and CT_MLL_A respectively), were estimated (from

cross-sectional CT scans taken at the fifth lumbar vertebra) by Macfarlane et al. (2010) and included in this study's analysis of the genotypic effect. Essentially, these traits were included to, (i) determine if analysis of the reduced sample of animals shows genotype effects on loin traits similar to that observed for the larger sample, and, (ii) assess, from further analysis of the smaller data set, if the same or similar pattern of TM expression (polar overdominance) can be considered as a source for any genotype differences.

2.3. Statistical Analysis

Data were analysed using the GLM procedure in SAS (SAS Institute Inc., Cary, NC, USA) to determine the effects of genotype on the collated loin traits and measured spine traits. Fixed effects fitted in the model for loin dimensions (CT_MLL_W, CT_MLL_D, CT_MLL_A), spine length traits (SPL_{THOR}, SPL_{LUM}, SPL_{T+L}, VL_{THOR}, VL_{LUM}, VL_{T+L}) and spine count traits (VN_{THOR}, VN_{LUM}, VN_{T+L}) were site, with two levels (Scotland and Wales), sex, with two levels (male and female), rearing rank, with three levels (single, twin, or pet), and TM-QTL-genotype, with four levels ($+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$ and TM^S/TM^D). The model was run with and without covariate adjustments for live weight (LWT); where any of the traits differed significantly between genotype groups, it was of interest to assess if, by testing the groups at a standard live weight, the differences were removed.

A set of orthogonal contrasts, as described by Freking et al. (1998a), (additive (-1 0 0 1), dominance (-1 1 1 -1), and reciprocal heterozygote (0 1 -1 0)) was fitted to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, TM^S/TM^D genotypes, respectively. The contrasts test for any distinct pattern in the differences amongst the genotype group's least-squares means (for loin and spine traits), from which, a particular model for TM gene action might be suggested. Due to the previous evidence supporting the expression of the TM muscling phenotype through a

polar overdominant mode of inheritance (Macfarlane et al., 2010), if significant differences were found between the heterozygote groups (reciprocal heterozygote test) a further set of orthogonal contrasts was fitted to the genotypes to include a test for the paternally derived polar overdominant mode of inheritance. Again following that from Freking (1998a), this second set of orthogonal contrasts included additive (-1 0 0 1), maternal dominance (-1 0 2 -1), and polar overdominance (-1 3 -1 -1) models of gene action which were fitted to the $+^{S}/+^{D}$, $TM^{S}/+^{D}$, $+^{S}/TM^{D}$, TM^{S}/TM^{D} genotypes, respectively. Contrasts were performed on the spine count data which was not adjusted for LWT and on the loin muscle and spine length data after the adjustment for LWT.

3. Results

In the context of this work it is useful to note that an earlier study (Macfarlane et al., 2012) found that least-squares means for LWT (measured at birth, 5, 10, 15, and 20 weeks of age) and carcass weight for TM^{S}/TM^{D} animals were consistently larger than that measured for $+^{S}/+^{D}$, $+^{S}/TM^{D}$, and $TM^{S}/+^{D}$ genotype lambs (these differences were significant between TM^{S}/TM^{D} and $+^{S}/+^{D}$ lambs for LWT at birth, 5, and 10 weeks of age, and carcass weight). In the present study, statistical models were first run without an adjustment of LWT but, given the above, to remove, as far as possible, any misinterpretation of TM-QTL effects, statistical models were run again with certain traits (loin dimensions, spine length) adjusted for LWT. The following sections discussing these traits will therefore focus only on the LWT adjusted results.

3.1. Loin dimensions

Similar to the findings of Macfarlane et al. (2010), $TM^{S}/+^{D}$ genotype lambs were observed to generally have the largest loin width, depth, and area, on average (Table 1). The differences in loin dimensions between $TM^{S}/+^{D}$ and $+^{S}/+^{D}$ genotype lambs were consistently significant, however, the larger trait averages observed for the $TM^{S}/+^{D}$ group were not all significantly different from those averages observed for the $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotype groups. For example, the $TM^{S}/+^{D}$ group was significantly different from the $+^{S}/TM^{D}$ group in regards to loin area (CT_MLL_A_LWT) but the groups were not significantly different for loin width and depth measures (CT_MLL_W_LWT and CT_MLL_D_LWT, respectively). Further to this, and in contrast to Macfarlane et al. (2010), the $TM^{S}/+^{D}$ group in this smaller data set was not significantly different from TM^{S}/TM^{D} in regards to all three loin dimension traits.

In general, the pattern of results from the analysis of the full data set (Macfarlane et al., 2010) suggested that the effect of the TM allele on these loin dimensions is expressed through a non-additive mode of inheritance (paternal polar overdominance). Analysis of the subset of records suggests a more general paternal TM-QTL effect on the loin with little evidence of a polar overdominance effect.

3.2. Spine length traits

Overall, there was no significant effect of the TM-QTL on the thoracic region length traits (SPL_{THOR}, VL_{THOR}). Nor was there an effect of TM-QTL genotype on the average length of individual lumbar vertebrae (VL_{LUM}), however, associations were shown to exist between TM-QTL genotype groups and length of the lumber region (SPL_{LUM}) (Table 1). Least-squares means showed that, on average, $+^{S}/+^{D}$ and $TM^{S}/+^{D}$ genotype lambs had a longer lumbar length compared to $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotype lambs. However, when

considering the combined length of the thoracic and lumbar regions (SPL_{T+L}), the genotype effect is negligible (Table 1).

3.3. Spine count traits

The segmentation and anatomical regionalisation of the spinal elements (vertebrae) is established in early development (Wellik, 2007; Iimura et al., 2009), hence, it should not be affected by varying LWT. In running the statistical model with the inclusion and omission of a covariate adjustment of LWT, little difference was found between the least-squares means for each model (Table 1), lending support to the previous statement. Therefore, only the results obtained from the model without LWT adjustment will be discussed (results from the model with LWT covariate adjustment are not shown).

Regarding vertebrae number in the separate thoracic and lumbar spine regions first (VN_{THOR} and VN_{LUM} respectively), there were some significant differences between the genotype groups, however, the magnitude of these differences was relatively small (Table 1). In more detail, it can be seen from the least-squares means that there is much overlap between the genotype classes with regards to VN_{THOR}. The $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotype lambs had, on average, a greater number of thoracic vertebrae than $+^{S}/+^{D}$ and $TM^{S}/+^{D}$ genotype lambs, however $+^{S}/TM^{D}$ and $+^{S}/+^{D}$ genotype lambs were not significantly different from each other. With regards to VN_{LUM}, the $+^{S}/+^{D}$ and $TM^{S}/+^{D}$ genotype lambs were significantly different from the $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotype lambs. While observed to possess fewer thoracic vertebrae, $+^{S}/+^{D}$ and $TM^{S}/+^{D}$ genotype lambs had a greater number of lumbar vertebrae, on average.

Although significant differences occurred between the genotype groups for the two spine regions when considered separately, when examining the results for the combined thoracic

and lumbar vertebrae number (VN_{T+L}), there were no significant differences between the groups (Table 1).

3.4. Orthogonal contrasts

Previous work on loin dimensions had shown strong evidence that the mode of inheritance for the TM-QTL deviates from a simple additive model (Macfarlane et al., 2010). Although the results obtained in this study's subset of data did not fully provide the same results, there was certainly an indication for superior loin dimensions in TM-QTL carrier lambs, especially in those with a paternal copy of the TM-QTL. Due to this, sets of orthogonal contrasts were fitted to the genotypes to investigate the situation further. These contrasts allowed testing for any particular patterns in the differences among the TM-QTL genotype (least-squares) means, for loin and spine traits, in order to define if certain modes of gene action may be present.

The first set of orthogonal contrasts was fitted to the genotypes to test for additive, dominance, and reciprocal heterozygote models of gene action (Table 2; only traits where TM-QTL genotype had a significant effect are shown).

The additive inheritance model was fitted as -1 0 0 1 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotypes respectively; testing the difference between the means of the homozygote genotypes. Where the contrast value was positive this showed that TM^S/TM^D had a larger mean than $+^S/+^D$ for that particular trait and vice versa if the contrast value was negative. The difference between $+^S/+^D$ and TM^S/TM^D genotype means was significant for all three loin traits CT_MLL_W_LWT, CT_MLL_D_LWT, and CT_MLL_A_LWT, and spine traits, VN_{THOR}, VN_{LUM}, and SPL_{LUM}_LWT. The dominance inheritance model was fitted as -1 1 1 -1 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotypes respectively;

testing the combined means of the heterozygote genotypes ($TM^S/+^D$, $+^S/TM^D$) with the combined means of the homozygote genotypes ($+^S/+^D$, TM^S/TM^D). However, none of the differences between genotype means were significant, providing no evidence of a dominance effect on any of the traits. The reciprocal heterozygote model of gene action was fitted as 0 1 -1 0 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotypes respectively. This contrast tested the difference between the means of the two heterozygote genotypes ($TM^S/+^D$ and $+^S/TM^D$), which were significant for traits CT_MLL_A_LWT, VN_{THOR}, VN_{LUM}, and SPL_{LUM}_LWT.

Freking et al. (1998a) previously commented that in such a case where the reciprocal heterozygote contrast is shown to be significant, the dominance contrast may be misleading i.e. under and over-estimation of heterozygote genotypes, and further analysis required. Therefore, due to this, and with the previous observation of the TM allele's expression through a non-additive mode of inheritance, a further set of orthogonal contrasts, additive, maternal dominance, and polar overdominance, were fitted to the genotypes as (1 0 0 -1) (-1 0 2 -1) (-1 3 -1 -1), respectively. Results for the additive model have been discussed above, and with no significant results for a maternal dominance effect only the results for the polar overdominance model from this set of contrasts were shown (Table 2) and discussed further.

The polar overdominance inheritance model was fitted as -1 3 -1 -1 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotypes respectively and used to test the difference between the mean of the $TM^S/+^D$ group with each of the means calculated for $+^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotype groups. Contrast values for the paternal polar overdominance model are the combined differences between genotype means (condition as defined above) and were shown to be significant for all traits tested (Table 2); with the exception of VN_{THOR}, $TM^S/+^D$ genotype lambs had a larger mean compared with each of the other genotype groups.

4. Discussion

It should be noted that the data set used in the present report was limited in its size, largely due to the restricted availability of sires (identified as TM-QTL carriers) which could be used to produce a study group of lambs. Nonetheless, to date, it is the only available data set which provides detail of the TM-QTL status for a sufficient number of purebred Texel animals, from which, the effects of TM-QTL on carcass, meat quality, and production traits could be assessed.

The analysis in the present report made use of lamb records, where TM-QTL genotype was unambiguously known, to, (i) determine if similar conclusions for loin dimensions could be formulated using only a subset of data in the analysis, repeating, as close as possible, the model described by Macfarlane et al. (2010), (ii) extend this test to determine if there is an effect of TM-QTL on underlying spine characteristics as the loin muscle is located parallel to spinal vertebrae, and, (iii) fit sets of contrasts to the TM-QTL genotype groups in order to determine the inheritance pattern of the TM-QTL.

It should also be noted that the following discussion will continue to refer only to loin dimension and spine length trait results generated from the model where all lamb records were adjusted for LWT.

4.1. TM-QTL and loin dimensions

Regarding the loin dimensions, CT_MLL_W, CT_MLL_D, and CT_MLL_A, the least-squares means for these traits reported by Macfarlane et al. (2010) are in strong agreement with an overdominance mode of expression of the TM allele; there is evidence of both

heterozygote groups lying outside, in this case above, the phenotypic range of the homozygote groups. The results of Macfarlane et al. (2010) even suggested, more specifically, a paternally expressed polar overdominance effect as $TM^{S}/+^{D}$ genotype lambs consistently exceeded $+^{S}/TM^{D}$ genotype lambs in trait means; the difference between heterozygote groups, however, only appeared to be significant for the loin area (CT_MLL_A).

From the present study, the polar overdominance test (Table 2) did show significance, but the pattern of differences between the $TM^{S}/+^{D}$ genotype least-squares means and the least-squares means for $+^{S}/+^{D}$, $+^{S}/TM^{D}$, and TM^{S}/TM^{D} genotype groups (Table 1) conflicts with this outcome and could not support a polar overdominance mode of TM gene action i.e. trait means for $TM^{S}/+^{D}$ genotype lambs did not appear to significantly 'out-perform' over all (or the majority) of the other genotype groups (Table 1). Nonetheless, the pattern of least-squares means did still infer TM expression which could not be explained by simple additive gene action, and though an overdominacne model could not be supported, there was still indication towards some paternal influence of the TM allele; genotype groups which inherited a copy of the TM allele from the sire ($TM^{S}/+^{D}$ and TM^{S}/TM^{D}) were observed to have, on average, larger loin width (CT_MLL_W_LWT), depth (CT_MLL_D_LWT), and area (CT_MLL_A_LWT) measures.

4.2. TM-QTL and spine characteristics

Three out of the nine spine traits (VN_{THOR}, VN_{LUM}, and SPL_{LUM}) were observed to be significantly different amongst the TM-QTL genotype groups (Table 1).

The least-squares means for vertebrae number traits (VN_{THOR}, VN_{LUM}) followed a curious pattern; means for $+^{S}/+^{D}$ and $TM^{S}/+^{D}$ genotype lambs were similar and significantly different,

in most instances, to the means for $+^{S}/TM^{D}$ and TM^{S}/TM^{D} genotype lambs, the latter of which were also similar to each other in their mean values. The $+^{S}/+^{D}$ and $TM^{S}/+^{D}$ groups had, on average, fewer thoracic vertebrae (VN_{THOR}) but more lumbar vertebrae (VN_{LUM}), with the situation reversed for the $+^{S}/TM^{D}$ and TM^{S}/TM^{D} groups. However, the overall number of thoracolumbar vertebrae (VN_{T+L}) across the four genotype groups was not different. It appeared that TM-QTL inheritance patterns had no substantial effect on the total number of thoracolumbar vertebrae but may have some influence on the thoracic-lumbar vertebral arrangement in the spine, but this remains uncertain as the size of difference between genotype groups is small.

Similarly, the total length of the thoracolumbar region (SPL_{T+L}) was not drastically different between the genotype groups. Freking et al. (1998b) observed shorter spinal columns in *CPLG* genotype lambs, and given that TM-QTL falls close to its position on the chromosome, it was an important point to investigate further in connection with TM inheritance. Least-squares means for spine length traits (SPL, VL) from the present study, however, do not suggest any such negative effects of TM-QTL on spine length. The $TM^{S}/+^{D}$ genotype lambs, which express the muscle hypertrophy phenotype, in fact, were observed to have, on average, longer thoracolumbar vertebrae (VL_{T+L}_LWT), and subsequently longer thoracolumbar spine regions (SPL_{T+L}_LWT), but these trait values (23.22mm; 442.0mm respectively) were not significantly different from the other three groups. It is interesting that in the animals which were observed to have the largest loin dimension measures ($TM^{S}/+^{D}$), there is not much of a change to the structure on which it lies.

Given that differences in spine traits are largely non-significant between the genotype groups, and that the overall pattern of least-squares means is indistinct, interpreting the models of gene action should be done with reservation. For example, the contrast tests showed significance for the polar overdominant model of TM gene action on VN_{THOR}, VN_{LUM}, and

373 SPL_{LUM} spine traits. These results should, again, be carefully considered alongside least-
374 squares means (Table 1) as, though slightly larger (for VN_{LUM} and SPL_{LUM}), the means for
375 $TM^{S}/+^{D}$ genotype lambs did not significantly 'out-perform' over all other genotype groups
376 for these spine traits. Hence, there was no strong indication that the observed differences in
377 spine trait phenotypes were associated with increased loin muscling specific to TM gene
378 action.
379 What is important to take from the present study is that increased loin muscling, particularly
380 associated with $TM^{S}/+^{D}$ genotype lambs, has been shown to have little associated effect on
381 the underlying spine characteristics. Information on spine characteristics, in general, could
382 potentially be used to improve loin production i.e. through increasing the size and/or number
383 of loin chops (Donaldson et al., 2013). Hence, it would be interesting to investigate further
384 the potential size of increase in loin production from those $TM^{S}/+^{D}$ animals which posses a
385 greater number of thoracolumbar vertebrae.
386
387
388 **5. Conclusion**
389
390 Given the results from the present study, it was evident that some effect of the TM allele on
391 loin dimension phenotypes was linked to a paternal genetic influence, but, with a weaker data
392 set (67 fewer records) this study could not provide further evidence for a specific polar
393 overdominance inheritance pattern. With regards to spine characteristics, in general terms,
394 the analysis of the subset of data did not reveal any obvious (advantageous or
395 disadvantageous) associations with TM-QTL inheritance. There did not appear to be any
396 effect on spine/vertebrae length and detailing how, or if, the TM allele interacts in the
397 vertebral patterning process (given the thoracic-lumbar vertebral combinations across

genotype groups) would require analysing a substantially larger data set than what was available at present.

Acknowledgements

The authors gratefully acknowledge funding from BBSRC and Defra under the Sustainable Livestock Production LINK programme. We thank our industry sponsors and project partners: EBLEX, HCC, QMS, LMCNI, Pfizer Animal Genetics, Innovis Genetics Ltd, Vion Food Group, E+V, ASDA and SAMW, and are grateful for contributions of colleagues, especially from the CT unit providing the topograms. C. L. Donaldson is funded by a BBSRC CASE Studentship award to the University of Edinburgh with contributions from ASDA and QMS.

Reference List

Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Snowder, G.D., Nielsen, D.M., Georges, M., 1996. Polar overdominance at the Ovine callipyge locus. Science 273, 236-238.

Cockett, N.E., Jackson, S.P., Shay, T.L., Nielsen, D., Moore, S.S., Steele, M.R., Barendse, W., Green, R.D., Georges, M., 1994. Chromosomal Localization of the Callipyge Gene in Sheep (Ovis-Aries) Using Bovine Dna Markers. Proceedings of the National Academy of Sciences of the United States of America 91, 3019-3023.

Donaldson, C.L., Lambe, N.R., Maltin, C.A., Knott, S., Bunger, L., 2013. Between- and within-breed variations of spine characteristics in sheep. Journal of Animal Science 91, 995-1004.

Freking, B.A., Keele, J.W., Beattie, C.W., Kappes, S.M., Smith, T.P.L., Sonstegard, T.S., Nielsen, M.K., Leymaster, K.A., 1998a. Evaluation of the ovine Callipyge locus: I. Relative chromosomal position and gene action. Journal of Animal Science 76, 2062-2071.

Freking, B.A., Keele, J.W., Nielsen, M.K., Leymaster, K.A., 1998b. Evaluation of the ovine Callipyge locus: II. Genotypic effects on growth, slaughter, and carcass traits. Journal of Animal Science 76, 2549-2559.

Freking, B.A., Murphy, S.K., Wylie, A.A., Rhodes, S.J., Keele, J.W., Leymaster, K.A., Jirtle, R.L., Smith, T.P.L., 2002. Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. Genome Research 12, 1496-1506.

Iimura, T., Denans, N., Pourquie, O., 2009. Establishment of Hox Vertebral Identities in the Embryonic Spine Precursors. Current Topics in Developmental Biology 88, 201-234.

Jackson, S.P., Miller, M.F., Green, R.D., 1997a. Phenotypic characterization of Rambouillet sheep expressing the Callipyge gene .2. Carcass characteristics and retail yield. Journal of Animal Science 75, 125-132.

Jackson, S.P., Miller, M.F., Green, R.D., 1997b. Phenotypic characterization of Rambouillet sheep expressing the Callipyge gene .3. Muscle weights and muscle weight distribution. Journal of Animal Science 75, 133-138.

Koohmaraie, M., Shackelford, S.D., Wheeler, T.L., Lonergan, S.M., Doumit, M.E., 1995. A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. Journal of Animal Science 73, 3596-3607.

Lambe, N.R., Richardson, R.I., Macfarlane, J.M., Nevison, I., Haresign, W., Matika, O., Bunger, L., 2011. Genotypic effects of the Texel Muscling QTL (TM-QTL) on meat quality in purebred Texel lambs. Meat Science 89, 125-132.

Macfarlane, J.M., Lambe, N.R., Haresign, W., Bunger, L., 2012. The effect of the Texel Muscling QTL on live and carcass weight in Texel lambs. Small Ruminant Research 105, 117-121.

Macfarlane, J.M., Lambe, N.R., Matika, O., Mclean, K.A., Masri, A.Y., Wolf, B.T., Haresign, W., Bishop, S.C., Bunger, L., 2010. Texel loin muscling QTL (TM-QTL) located on ovine chromosome 18 appears to exhibit imprinting and polar overdominance. Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 1-6, 2010 comm. no. 199.

Matika, O., Pong-Wong, R., Woolliams, J.A., Low, J., Nieuwhof, G.J., Boon, S., Bishop, S.C., 2006. Verifying quantitative trait loci for muscle depth in commercial terminal sire sheep. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil, August 13-18, 2006, 22-10.

Matika, O., Sechi, S., Pong-Wong, R., Houston, R.D., Clop, A., Woolliams, J.A., Bishop, S.C., 2011. Characterization of OAR1 and OAR18 QTL associated with muscle depth in British commercial terminal sire sheep. Animal Genetics 42, 172-180.

Nicoll, G.B., Burkin, H.R., Broad, T.E., Jopson, N.B., Greer, G.J., Bain, W.E., Wright, C.S., Dodds, K.G., Fennessy, P.F., Mcewan, J.C., 1998. Genetic linkage of microsatellite markers to the Carwell locus for rib-eye muscling in sheep. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia, January 11-16, 1998 26, 529-532.

Walling, G.A., Visscher, P.M., Wilson, A.D., Mcteir, B.L., Simm, G., Bishop, S.C., 2004. Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations. Journal of Animal Science 82, 2234-2245.

Wellik, D.M., 2007. Hox patterning of the vertebrate axial skeleton. Developmental Dynamics 236, 2454-2463.

Table 1. Least-squares means (and standard errors) for live weight, loin dimensions[1] and spine traits[2] for Texel lambs of each TM-QTL genotype[3]

Trait	Genotype								Site	Sex	Rearing rank	TM-QTL genotype	Live weight	R^2
	$+^S/+^D$ n = 39*		$TM^S/+^D$ n = 52*		$+^S/TM^D$ n = 17		TM^S/TM^D n = 34							
Live Weight	30.53	(1.004)	31.78	(0.855)	30.59	(1.402)	31.45	(1.094)	**< .001**	0.104	**< .001**	0.672	.	0.253
CT_MLL_W	66.27[b]	(1.034)	69.49[a]	(0.881)	67.12[a,b]	(1.444)	68.82[a]	(1.127)	0.084	0.069	0.199	**0.034**	.	0.126
CT_MLL_D	28.45[b]	(0.779)	30.96[a]	(0.663)	29.28[a,b]	(1.087)	30.44[a]	(0.848)	0.234	0.411	**0.001**	**0.029**	.	0.163
CT_MLL_A	1684[c]	(64.94)	1883[a]	(55.33)	1689[b,c]	(90.68)	1851[a,b]	(70.76)	0.385	0.633	**< .001**	**0.021**	.	0.157
CT_MLL_W_LWT	66.27[b]	(0.591)	68.43[a]	(0.507)	67.06[a,b]	(0.825)	68.04[a]	(0.646)	**< .001**	0.400	0.059	**0.001**	**< .001**	0.716
CT_MLL_D_LWT	28.45[b]	(0.560)	30.28[a]	(0.481)	29.25[a,b]	(0.782)	29.94[a]	(0.612)	**0.007**	**0.008**	0.148	**0.029**	**< .001**	0.570
CT_MLL_A_LWT	1684[c]	(37.68)	1817[a]	(32.36)	1685[b,c]	(52.63)	1803[a,b]	(41.17)	**< .001**	**0.002**	**0.353**	**0.004**	**< .001**	0.718
VN_{THOR}	12.69[b,c]	(0.064)	12.65[c]	(0.055)	12.89[a,b]	(0.090)	12.88[a]	(0.070)	**0.026**	0.350	**0.044**	**0.006**	.	0.174
VN_{LUM}	6.356[a]	(0.074)	6.387[a]	(0.063)	6.111[b]	(0.104)	6.143[b]	(0.081)	0.092	0.427	0.061	**0.009**	.	0.149
VN_{T+L}	19.05	(0.063)	19.04	(0.054)	19.00	(0.088)	19.02	(0.069)	0.759	0.981	0.645	0.967	.	0.011
SPL_{THOR}	255.6	(3.941)	260.7	(3.358)	257.9	(5.504)	262.5	(4.295)	**0.003**	0.429	**0.034**	0.503	.	0.122
SPL_{LUM}	184.4[a,b]	(2.411)	186.8[a]	(2.054)	176.6[c]	(3.366)	180.3[b,c]	(2.627)	0.208	0.742	**0.029**	**0.018**	.	0.125
SPL_{T+L}	440.0	(5.138)	447.5	(4.378)	434.5	(7.176)	442.8	(5.599)	**0.004**	0.447	**0.015**	0.329	.	0.128
VL_{THOR}	20.13	(0.276)	20.60	(0.235)	20.00	(0.386)	20.38	(0.301)	**0.009**	0.246	**0.007**	0.352	.	0.133
VL_{LUM}	29.03	(0.292)	29.29	(0.249)	28.91	(0.408)	29.36	(0.319)	**< .001**	0.702	**0.045**	0.657	.	0.124
VL_{T+L}	23.10	(0.265)	23.51	(0.226)	22.86	(0.370)	23.28	(0.289)	**0.004**	0.443	**0.007**	0.332	.	0.135
SPL_{THOR}_LWT	255.6	(2.495)	256.9	(2.143)	257.7	(3.485)	259.7	(2.726)	0.379	0.456	0.088	0.610	**< .001**	0.650
SPL_{LUM}_LWT	184.4[a]	(2.033)	185.2[a]	(1.746)	176.5[b]	(2.839)	179.1[b]	(2.221)	0.151	0.513	0.333	**0.007**	**< .001**	0.382
SPL_{T+L}_LWT	440.0	(2.720)	442.0	(2.336)	434.2	(3.798)	438.8	(2.971)	0.061	0.241	0.575	0.289	**< .001**	0.758
VL_{THOR}_LWT	20.13	(0.160)	20.32	(0.138)	19.99	(0.224)	20.18	(0.175)	0.051	0.772	0.595	0.531	**< .001**	0.710
VL_{LUM}_LWT	29.03	(0.147)	28.97	(0.126)	28.89	(0.205)	29.13	(0.160)	0.350	**< .001**	**0.011**	0.721	**< .001**	0.781
VL_{T+L}_LWT	23.10	(0.130)	23.22	(0.112)	22.85	(0.182)	23.07	(0.142)	**0.023**	0.185	0.488	0.297	**< .001**	0.793

[a-c] Within a row, means with common letters, or no letters, in their superscript are not significantly different ($P > 0.05$)

LWT = live weight (kg) fitted as a covariate in model

[1] CT_MLL_W = width of *M. longissimus lumborum* (mm); CT_MLL_D = depth of *M. longissimus lumborum* (mm); CT_MLL_A = area of *M. longissimus lumborum* (mm^2)

[2] VN_{THOR} = number of thoracic vertebrae; VN_{LUM} = number of lumbar vertebrae; VN_{T+L} = number of thoracolumbar vertebrae; SPL_{THOR} = length of thoracic spine region (mm); SPL_{LUM} = length of lumbar spine region (mm); SPL_{T+L} = length of thoracolumbar spine region (mm); VL_{THOR} = average length of individual thoracic vertebrae (mm); VL_{LUM} = average length of individual lumbar vertebrae (mm); VL_{T+L} = average length of individual thoracolumbar vertebrae (mm)

[3] $+^S/+^D$ = homozygote non-carrier; $TM^S/+^D$ = heterozygote carrier inheriting TM-QTL from sire; $+^S/TM^D$ = heterozygote carrier inheriting TM-QTL from dam; TM^S/TM^D = homozygote carrier

Table 2. Estimates of TM-QTL genotype contrasts (and standard errors) and significance levels (*P-value*) for additive, dominance, reciprocal heterozygote, and paternally derived polar overdominant effects on loin dimensions[1] and spine traits[2]

	Additive			Dominance			Reciprocal heterozygote			Polar overdominance		
Trait	Contrast		*P-value*	Contrast		*P-value*	Contrast		*P-value*	Contrast		*P-value*
CT_MLL_W_LWT	1.767	(0.736)	**0.017**	1.184	(1.151)	0.305	1.368	(0.893)	0.128	3.919	(1.698)	**0.023**
CT_MLL_D_LWT	1.496	(0.697)	**0.034**	1.142	(1.090)	0.297	1.032	(0.847)	0.225	3.207	(1.609)	**0.048**
CT_MLL_A_LWT	118.9	(46.92)	**0.013**	15.90	(73.38)	0.829	131.5	(56.98)	**0.023**	279.0	(108.3)	**0.011**
VN_{THOR}	0.186	(0.080)	**0.022**	-0.032	(0.126)	0.800	-0.243	(0.097)	**0.014**	-0.518	(0.185)	**0.006**
VN_{LUM}	-0.213	(0.092)	**0.022**	-0.001	(0.144)	0.994	0.275	(0.112)	**0.015**	0.550	(0.212)	**0.012**
SPL_{LUM}_LWT	-5.321	(2.531)	**0.037**	-1.857	(3.958)	0.640	8.675	(3.073)	**0.006**	15.49	(5.840)	**0.009**

First set of orthogonal contrasts was fitted as -1 0 0 1(additive), -1 1 1 -1(dominance), and 0 1 -1 0(reciprocal heterozygote) to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotypes, respectively. Second set of orthogonal contrasts was fitted as -1 0 0 1(additive), -1 0 2 -1(maternal dominance), and -1 3 -1 -1(polar overdominance) to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotypes, respectively. From the second set of contrasts, only the results from the polar overdominance test are shown; additive results previously reported with first set of contrasts and the maternal dominance test was not significant for any of the traits.

LWT = live weight (kg) fitted as a covariate in model

[1] CT_MLL_W = width of *M. longissimus lumborum* (mm); CT_MLL_D = depth of *M. longissimus lumborum* (mm); CT_MLL_A = area of *M. longissimus lumborum* (mm^2)

[2] VN_{THOR} = number of thoracic vertebrae; VN_{LUM} = number of lumbar vertebrae; VN_{T+L} = number of thoracolumbar vertebrae; SPL_{THOR} = length of thoracic spine region (mm); SPL_{LUM} = length of lumbar spine region (mm); SPL_{T+L} = length of thoracolumbar spin region (mm); VL_{THOR} = average length of individual thoracic vertebrae (mm); VL_{LUM} = average length of individual lumbar vertebrae (mm); VL_{T+L} = average length of individual thoracolumbar vertebrae (mm)