

Scotland's Rural College

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

Donaldson, CL; Lambe, NR; Maltin, CA; Knott, S; Bungler, L

Published in:
Small Ruminant Research

DOI:
[10.1016/j.smallrumres.2013.11.020](https://doi.org/10.1016/j.smallrumres.2013.11.020)

Print publication: 01/01/2014

Document Version
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):

Donaldson, CL., Lambe, NR., Maltin, CA., Knott, S., & Bungler, L. (2014). Effect of the Texel muscling QTL (TM-QTL) on spine characteristics in purebred Texel lambs. *Small Ruminant Research*, 117(1), 34 - 40. <https://doi.org/10.1016/j.smallrumres.2013.11.020>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

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

3 C. L. Donaldson ^{a,1}, N. R. Lambe ^a, C. A. Maltin ^b, S. Knott ^c, L. Bünger ^a

4 ^a Scotland's Rural College, King's Buildings, West Mains Road, Edinburgh, EH9 3JG,
5 United Kingdom

6 ^b Quality Meat Scotland, Rural Centre, Ingliston, Newbridge, EH28 8NZ, United Kingdom

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

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

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)

24 **Abstract**

25

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

40

41 **Keywords:**

42 Vertebrae, Spine, Texel, Loin, Muscling

43

44

45

46

47

48

49 **1. Introduction**

50

51 Walling et al. (2004) reported evidence of a quantitative trait locus (QTL) segregating in the
52 United Kingdom's Texel sheep population which significantly increased *longissimus dorsi*
53 (loin) muscle depth (up to +1.15 – +2.00 mm, as measured ultrasonically over the third
54 lumbar vertebra). Observing similar results (QTL effect of +2.57 mm) from an analysis
55 including existing and new Texel family data, the QTL, later termed the Texel muscling QTL
56 (TM-QTL), was further verified by Matika et al. (2006). Located on the distal end of the
57 ovine chromosome 18 (OAR18) (Walling et al., 2004; Matika et al., 2006), the TM-QTL sits
58 in the same region as the *Callipyge* (*CLPG*) and Carwell loci (Cockett et al., 1994; Nicoll et
59 al., 1998) which are also known to affect carcass muscling; the *CLPG* mutation leads to
60 greater muscle mass most pronounced in the hind quarters (loin, pelvis, leg) (Cockett et al.,
61 1994; Koohmaraie et al., 1995; Jackson et al., 1997a, 1997b; Freking et al., 2002), while
62 carriers of Carwell exhibit a larger loin muscle area and weight (Nicoll et al., 1998).

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

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

74 inheritance termed polar overdominance (Macfarlane et al., 2010; Matika et al., 2011). This
75 unique type of inheritance is characterised by the instance where heterozygous progeny that
76 inherit a single copy of the allele from the sire exhibit the superior phenotype (Cockett et al.,
77 1996). Indeed, Macfarlane et al. (2010) observed that the largest phenotypic effects of the
78 TM-QTL were particularly apparent in the TM carrier lambs that had inherited a single copy
79 of the TM allele from the sire and the wild type (+) from the dam (genotype $TM^S/+^D$; where
80 superscripts S (sire) and D (dam) denote the paternal and maternal origin of the alleles,
81 respectively), with loin depth, width, and area measures ~ 2 – 11% greater in these $TM^S/+^D$
82 genotype lambs than in the other three genotype groups (homozygote non-carriers, $+^S/+^D$;
83 heterozygote carriers inheriting TM-QTL from the dam, $+^S/TM^D$; homozygote carriers,
84 TM^S/TM^D).

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

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

109

110

111 **2. Materials and Methods**

112

113 *2.1. Animals sampled*

114

115 The present study used a subset of the 209 available purebred Texel lamb records previously
116 used by Macfarlane et al. (2010) and Lambe et al. (2011). Lambs were sired by seven
117 different rams that were previously identified as carriers of at least one copy of TM-QTL; all
118 209 lambs were blood-sampled soon after birth (born 2009) in order to classify their TM-
119 QTL genotype (homozygote non-carrier, $+^S/+^D$; heterozygote carrier inheriting TM-QTL
120 from the sire, $TM^S/+^D$; heterozygote carrier inheriting TM-QTL from the dam, $+^S/TM^D$;
121 homozygote carrier, TM^S/TM^D); detailed information on the genotyping of the animals can
122 be found in Macfarlane et al. (2010). However, for a number of animals the genotype could

123 not be fully classified. These unknowns were excluded from this study's analysis, leaving a
124 total of 142 lamb records in the subset, which divided into the TM-QTL genotype groups as
125 follows: 39 $+^S/+^D$, 52 $TM^S/+^D$, 17 $+^S/TM^D$, 34 TM^S/TM^D . These 142 lamb records included
126 59 entire males and 83 female lambs from the purebred population of Texel sheep kept across
127 two sites, one in Scotland and one in Wales, which were reared as either singles (n = 97),
128 twins (n = 34), or artificially (pet; n = 11) (further details on the management of these
129 animals can be found in Macfarlane et al. (2010) and Lambe et al. (2011)).

130

131 2.2. Computed tomography (CT) measurements

132

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

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

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

154

155 2.3. Statistical Analysis

156

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

167 A set of orthogonal contrasts, as described by Freking et al. (1998a), (additive (-1 0 0 1),
168 dominance (-1 1 1 -1), and reciprocal heterozygote (0 1 -1 0)) was fitted to the $+^S/+^D$,
169 $TM^{S/+^D}$, $+^S/TM^D$, TM^S/TM^D genotypes, respectively. The contrasts test for any distinct
170 pattern in the differences amongst the genotype group's least-squares means (for loin and
171 spine traits), from which, a particular model for TM gene action might be suggested. Due to
172 the previous evidence supporting the expression of the TM muscling phenotype through a

173 polar overdominant mode of inheritance (Macfarlane et al., 2010), if significant differences
174 were found between the heterozygote groups (reciprocal heterozygote test) a further set of
175 orthogonal contrasts was fitted to the genotypes to include a test for the paternally derived
176 polar overdominant mode of inheritance. Again following that from Freking (1998a), this
177 second set of orthogonal contrasts included additive (-1 0 0 1), maternal dominance (-1 0 2 -
178 1), and polar overdominance (-1 3 -1 -1) models of gene action which were fitted to the
179 $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, TM^S/TM^D genotypes, respectively. Contrasts were performed on
180 the spine count data which was not adjusted for LWT and on the loin muscle and spine length
181 data after the adjustment for LWT.

182

183

184 **3. Results**

185

186 In the context of this work it is useful to note that an earlier study (Macfarlane et al., 2012)
187 found that least-squares means for LWT (measured at birth, 5, 10, 15, and 20 weeks of age)
188 and carcass weight for TM^S/TM^D animals were consistently larger than that measured for
189 $+^S/+^D$, $+^S/TM^D$, and $TM^S/+^D$ genotype lambs (these differences were significant between
190 TM^S/TM^D and $+^S/+^D$ lambs for LWT at birth, 5, and 10 weeks of age, and carcass weight). In
191 the present study, statistical models were first run without an adjustment of LWT but, given
192 the above, to remove, as far as possible, any misinterpretation of TM-QTL effects, statistical
193 models were run again with certain traits (loin dimensions, spine length) adjusted for LWT.
194 The following sections discussing these traits will therefore focus only on the LWT adjusted
195 results.

196

197 *3.1. Loin dimensions*

198

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

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

214

215 3.2. Spine length traits

216

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

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

225

226 *3.3. Spine count traits*

227

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

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

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

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

250

251 *3.4. Orthogonal contrasts*

252

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

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

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

273 testing the combined means of the heterozygote genotypes ($TM^{S/+^D}$, $+^S/TM^D$) with the
274 combined means of the homozygote genotypes ($+^S/+^D$, TM^S/TM^D). However, none of the
275 differences between genotype means were significant, providing no evidence of a dominance
276 effect on any of the traits. The reciprocal heterozygote model of gene action was fitted as 0 1
277 -1 0 to the $+^S/+^D$, $TM^S/+^D$, $+^S/TM^D$, and TM^S/TM^D genotypes respectively. This contrast
278 tested the difference between the means of the two heterozygote genotypes ($TM^S/+^D$ and
279 $+^S/TM^D$), which were significant for traits CT_MLL_A_LWT, VN_{THOR}, VN_{LUM}, and
280 SPL_{LUM}_LWT.

281 Freking et al. (1998a) previously commented that in such a case where the reciprocal
282 heterozygote contrast is shown to be significant, the dominance contrast may be misleading
283 i.e. under and over-estimation of heterozygote genotypes, and further analysis required.
284 Therefore, due to this, and with the previous observation of the TM allele's expression
285 through a non-additive mode of inheritance, a further set of orthogonal contrasts, **additive**,
286 **maternal dominance**, and **polar overdominance**, were fitted to the genotypes as (1 0 0 -1) (-1
287 0 2 -1) (-1 3 -1 -1), respectively. Results for the additive model have been discussed above,
288 and with no significant results for a maternal dominance effect only the results for the polar
289 overdominance model from this set of contrasts were shown (Table 2) and discussed further.

290 The polar overdominance inheritance model was fitted as -1 3 -1 -1 to the $+^S/+^D$, $TM^S/+^D$,
291 $+^S/TM^D$, and TM^S/TM^D genotypes respectively and used to test the difference between the
292 mean of the $TM^S/+^D$ group with each of the means calculated for $+^S/+^D$, $+^S/TM^D$, and
293 TM^S/TM^D genotype groups. Contrast values for the paternal polar overdominance model are
294 the combined differences between genotype means (condition as defined above) and were
295 shown to be significant for all traits tested (Table 2); with the exception of VN_{THOR}, $TM^S/+^D$
296 genotype lambs had a larger mean compared with each of the other genotype groups.

297

298

299 **4. Discussion**

300

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

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

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

317

318 *4.1. TM-QTL and loin dimensions*

319

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

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

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

341

342 4.2. TM-QTL and spine characteristics

343

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

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

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

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

369 Given that differences in spine traits are largely non-significant between the genotype groups,
370 and that the overall pattern of least-squares means is indistinct, interpreting the models of
371 gene action should be done with reservation. For example, the contrast tests showed
372 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 possess 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

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

400

401

402 **Acknowledgements**

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

410

411

412

413

414

415

416

417

418

419 Reference List

420

421 Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Snowder, G.D., Nielsen,
422 D.M., Georges, M., 1996. Polar overdominance at the Ovine callipyge locus. *Science*
423 273, 236-238.

- 424 Cockett, N.E., Jackson, S.P., Shay, T.L., Nielsen, D., Moore, S.S., Steele, M.R., Barendse,
425 W., Green, R.D., Georges, M., 1994. Chromosomal Localization of the Callipyge
426 Gene in Sheep (*Ovis-Aries*) Using Bovine Dna Markers. *Proceedings of the National*
427 *Academy of Sciences of the United States of America* 91, 3019-3023.
- 428 Donaldson, C.L., Lambe, N.R., Maltin, C.A., Knott, S., Bunger, L., 2013. Between- and
429 within-breed variations of spine characteristics in sheep. *Journal of Animal Science*
430 91, 995-1004.
- 431 Freking, B.A., Keele, J.W., Beattie, C.W., Kappes, S.M., Smith, T.P.L., Sonstegard, T.S.,
432 Nielsen, M.K., Leymaster, K.A., 1998a. Evaluation of the ovine Callipyge locus: I.
433 Relative chromosomal position and gene action. *Journal of Animal Science* 76, 2062-
434 2071.
- 435 Freking, B.A., Keele, J.W., Nielsen, M.K., Leymaster, K.A., 1998b. Evaluation of the ovine
436 Callipyge locus: II. Genotypic effects on growth, slaughter, and carcass traits. *Journal*
437 *of Animal Science* 76, 2549-2559.
- 438 Freking, B.A., Murphy, S.K., Wylie, A.A., Rhodes, S.J., Keele, J.W., Leymaster, K.A., Jirtle,
439 R.L., Smith, T.P.L., 2002. Identification of the single base change causing the
440 callipyge muscle hypertrophy phenotype, the only known example of polar
441 overdominance in mammals. *Genome Research* 12, 1496-1506.
- 442 Imura, T., Denans, N., Pourquie, O., 2009. Establishment of Hox Vertebral Identities in the
443 Embryonic Spine Precursors. *Current Topics in Developmental Biology* 88, 201-234.
- 444 Jackson, S.P., Miller, M.F., Green, R.D., 1997a. Phenotypic characterization of Rambouillet
445 sheep expressing the Callipyge gene .2. Carcass characteristics and retail yield.
446 *Journal of Animal Science* 75, 125-132.
- 447 Jackson, S.P., Miller, M.F., Green, R.D., 1997b. Phenotypic characterization of Rambouillet
448 sheep expressing the Callipyge gene .3. Muscle weights and muscle weight
449 distribution. *Journal of Animal Science* 75, 133-138.
- 450 Koohmaraie, M., Shackelford, S.D., Wheeler, T.L., Lonergan, S.M., Doumit, M.E., 1995. A
451 muscle hypertrophy condition in lamb (callipyge): Characterization of effects on
452 muscle growth and meat quality traits. *Journal of Animal Science* 73, 3596-3607.
- 453 Lambe, N.R., Richardson, R.I., Macfarlane, J.M., Nevison, I., Haresign, W., Matika, O.,
454 Bunger, L., 2011. Genotypic effects of the Texel Muscling QTL (TM-QTL) on meat
455 quality in purebred Texel lambs. *Meat Science* 89, 125-132.
- 456 Macfarlane, J.M., Lambe, N.R., Haresign, W., Bunger, L., 2012. The effect of the Texel
457 Muscling QTL on live and carcass weight in Texel lambs. *Small Ruminant Research*
458 105, 117-121.
- 459 Macfarlane, J.M., Lambe, N.R., Matika, O., Mclean, K.A., Masri, A.Y., Wolf, B.T.,
460 Haresign, W., Bishop, S.C., Bunger, L., 2010. Texel loin muscling QTL (TM-QTL)
461 located on ovine chromosome 18 appears to exhibit imprinting and polar
462 overdominance. *Proceedings of the 9th World Congress on Genetics Applied to*
463 *Livestock Production, Leipzig, Germany, August 1-6, 2010 comm. no. 199.*

- 464 Matika, O., Pong-Wong, R., Woolliams, J.A., Low, J., Nieuwhof, G.J., Boon, S., Bishop,
465 S.C., 2006. Verifying quantitative trait loci for muscle depth in commercial terminal
466 sire sheep. Proceedings of the 8th World Congress on Genetics Applied to Livestock
467 Production, Belo Horizonte, Brazil, August 13-18, 2006, 22-10.
- 468 Matika, O., Sechi, S., Pong-Wong, R., Houston, R.D., Clop, A., Woolliams, J.A., Bishop,
469 S.C., 2011. Characterization of OAR1 and OAR18 QTL associated with muscle depth
470 in British commercial terminal sire sheep. *Animal Genetics* 42, 172-180.
- 471 Nicoll, G.B., Burkin, H.R., Broad, T.E., Jopson, N.B., Greer, G.J., Bain, W.E., Wright, C.S.,
472 Dodds, K.G., Fennessy, P.F., Mcewan, J.C., 1998. Genetic linkage of microsatellite
473 markers to the Carwell locus for rib-eye muscling in sheep. Proceedings of the 6th
474 World Congress on Genetics Applied to Livestock Production, Armidale, Australia,
475 January 11-16, 1998 26, 529-532.
- 476 Walling, G.A., Visscher, P.M., Wilson, A.D., Mcteir, B.L., Simm, G., Bishop, S.C., 2004.
477 Mapping of quantitative trait loci for growth and carcass traits in commercial sheep
478 populations. *Journal of Animal Science* 82, 2234-2245.
- 479 Wellik, D.M., 2007. Hox patterning of the vertebrate axial skeleton. *Developmental*
480 *Dynamics* 236, 2454-2463.
481

482 **Table 1.** Least-squares means (and standard errors) for **live weight**, loin dimensions¹ and spine traits² for Texel lambs of each TM-QTL
 483 genotype³

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

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

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

486 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* (mm²)

487 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
488 (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);
489 VL_{LUM} = average length of individual lumbar vertebrae (mm); VL_{T+L} = average length of individual thoracolumbar vertebrae (mm)
490 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 =
491 homozygote carrier

492

493

494

495

496

497

498

499

500

501

502

503

504 **Table 2.** Estimates of TM-QTL genotype contrasts (and standard errors) and significance levels (*P-value*) for additive, dominance, reciprocal
505 heterozygote, and paternally derived polar overdominant effects on loin dimensions¹ and spine traits²

Trait	Additive			Dominance			Reciprocal heterozygote			Polar overdominance		
	Contrast		<i>P-value</i>	Contrast		<i>P-value</i>	Contrast		<i>P-value</i>	Contrast		<i>P-value</i>
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

506 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
507 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},
508 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
509 previously reported with first set of contrasts and the maternal dominance test was not significant for any of the traits.

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

511 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* (mm²)

512 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
513 (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);

514 VL_{LUM} = average length of individual lumbar vertebrae (mm); VL_{T+L} = average length of individual thoracolumbar vertebrae (mm)

515

516