doi:10.1111/j.1365-2052.2008.01734.x

# Two single nucleotide polymorphisms in the *myostatin* (*GDF8*) gene have significant association with muscle depth of commercial Charollais sheep

**G. Hadjipavlou*,†, O. Matika*, A. Clop‡ and S. C. Bishop***

*Roslin Institute and Royal (Dick) School of Veterinary Studies, Roslin BioCentre, Midlothian EH25 9PS, UK. †Institute of Evolutionary Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. ‡Genesis Faraday Partnership, Roslin, Midlothian EH25 9PS, UK

**Summary**

To assess whether the same mutation(s) were responsible for similar phenotypes attributed to ovine chromosome 2 (OAR2) quantitative trait loci (QTL) in different sheep breeds, Suffolk, Texel and Charollais rams from British commercial flocks were genotyped for two single nucleotide polymorphisms (SNPs) located in the *myostatin* (*GDF8*) region of OAR2, previously detected in progeny of Belgian Texel rams exhibiting muscular hypertrophy. The first SNP (g.−2449G>C) was located upstream from the transcription start site and the second SNP (g.+6723G>A) in the 3′ UTR of *GDF8*. The g.−2449C and g.+6723A alleles were absent in the Suffolk sires sampled, almost fixed in the Texel and segregating in the Charollais sires. Mixed model association analyses using SNP data on 338 Charollais lambs from 17 paternal half-sib families and phenotype and pedigree data on 56 500 lambs revealed that both SNPs had a significant association with muscle depth ($P < 0.001$). The SNPs were segregating at intermediate frequencies ($p = 0.3$) and exhibited strong linkage disequilibrium ($r^2 = 0.90$). Animals with the g.+6723AA genotype had significantly greater muscle depth than those with either the g.+6723GG or the g.+6723AG genotypes ($P < 0.002$), with the g.+6723A allele, the likely causative mutation, having an additive effect of 1.20 (±0.30) mm and a dominance effect of −0.73 (±0.36) mm. Based on estimated allelic effects and sample allele frequencies, the g.+6723G>A SNP explained 14% of the additive genetic variance of muscle depth. The maximum genetic variance for the trait (38%) attributed to the SNP would be attained at a g.+6723A allele frequency of 0.7. Our findings indicate that marker-assisted selection using these two *GDF8* SNPs would be beneficial for the Charollais breed.

**Keywords** *GDF8*, genetic markers, marker-assisted selection, muscle depth, myostatin, quantitative trait loci, quantitative trait nucleotides, sheep, single nucleotide polymorphisms.

## Introduction

Fat and lean meat depositions are both selection-responsive traits in sheep. However, routine measurement of carcass composition characteristics (via ultrasonic scanning or computer tomography) remains expensive and difficult. Thus, there is a need to dissect the genetic basis of selection-responsive carcass traits in sheep. This can be facilitated by the detection and characterization of major genes and quantitative trait nucleotides (QTN) affecting muscle and fat traits in sheep.

In the last decade, a large number of experiments have identified chromosomal regions that contain quantitative trait loci (QTL) of commercial benefit in livestock populations. However, identification and verification of the causative variants for the QTL have been successful only in a small number of cases (e.g. Wilson *et al.* 2001; Grisart *et al.* 2002; Van Laere *et al.* 2003; Clop *et al.* 2006). It is particularly important to directly assess the presence, correspondence

Address for correspondence

G. Hadjipavlou, Roslin Institute and Royal (Dick) School of Veterinary Studies, Roslin BioCentre, Midlothian EH25 9PS, UK.
E-mail: georgia.hadjipavlou@roslin.ed.ac.uk

*Accepted for publication 13 February 2008*


 

and significance of identified QTL or QTN across other commercial populations and breeds prior to incorporating them into general breeding programmes for a specific livestock species. Apart from being important for animal breeding, findings from extensive across-breed studies of QTN effects on traits could provide further evidence for the validity of a putative QTN and the means by which the proposed biological function of the causative variant gives rise to phenotypic changes on the traits of interest.

In sheep, intensive studies of major genes connected with sheep muscle and fat composition have focused on the region of ovine chromosome 18 (OAR18) containing the *callipyge* (Cockett *et al.* 1994) and *rib-eye muscling* (*REM* or *Carwell*) loci (Nicoll *et al.* 1998), or on the region of OAR2 containing the *growth differentiation factor 8* (*GDF8*), also known as the *myostatin* (*MSTN)* gene, which is responsible for double muscling in cattle breeds (Kambadur *et al.* 1997; McPherron & Lee 1997; Wiener *et al.* 2002). In sheep, QTL studies showed that a portion of the OAR2 that included *GDF8* had a major effect on muscular development in Belgian Texel (Marcq *et al.* 2002), and on muscling and fat depth in New Zealand Texel sires (Broad *et al.* 2000; Johnson *et al.* 2005) and UK Texel (Walling *et al.* 2004) and Charollais (McRae *et al.* 2005) sheep. Yet, no sequence differences were found between the *GDF8* coding sequence of double-muscled Belgian Texels and normally muscled Romanov controls (Marcq *et al.* 2002). This indicated that the functional polymorphism resided outside the *GDF8* coding segment or in a closely linked gene.

Recently, the genetic basis of *GDF8* effects on muscle growth in the Texel sheep appears to have been elucidated. Examination of a 10.5 kb gDNA region spanning *GDF8* (DQ530260) led to the identification of two biallelic SNPs with significantly different allelic frequencies between hyper-muscled Texel and control animals (Clop *et al.* 2006). The first SNP (g.−2449G>C) was located 2.5 kb upstream from the *GDF8* transcription start site. This SNP and its effect on *GDF8* function (and hence on muscle and fat growth) was not studied any further. The second SNP (g.+6723G>A) was found in the 3′ UTR of *GDF8*. Functional studies provided evidence that the presence of the g.+6723A allele creates a miRNA target site. This, in turn, leads to miRNA-mediated translational inhibition of *GDF8* by which the double-muscling phenotype arises (Clop *et al.* 2006). Thus, the *GDF8* g.+6723A allele seems to act as a causative variant of increased muscularity in Texel rams and could be identified as a QTN.

The first objective of this study was to determine whether the above two *GDF8* SNPs were present in the British commercial Texel, Suffolk and Charollais breeds. Subsequently, we performed association analyses of the SNP effects on phenotypes in an extended British commercial population of Charollais sheep in order to further evaluate the SNP contribution to muscle and fat tissue composition, and to quantify and characterize the SNP effects on these traits in this breed.

## Materials and methods

### Genotyping information

Eighteen Suffolk, 38 Texel and 34 Charollais rams sampled across several British commercial flocks were genotyped for the g.+6723G>A and g.−2449G>C SNPs observed in the *GDF8* region of OAR2 (DQ530260) in progeny of Belgian Texel rams displaying muscle hypertrophy (Clop *et al.* 2006). The DNA primers used were described in Clop *et al.* (2006). Because the Charollais sires were segregating for the two SNPs (see the Results section), we then obtained SNP genotypic data on 338 Charollais lambs from 17 paternal half-sib families dispersed in 12 commercial flocks.

### Animals and trait information

Standard records (such as parentage, day of birth, sex, flock, etc.) and phenotypic data on muscle and fat depth ultrasonically scanned at the third lumbar vertebra and on live weight at scanning were provided by Signet (part of the technical division of the British Meat and Livestock Commission) for 56 499 lambs from British commercial Charollais populations that included the 338 genotyped lambs. The 56 499 lambs were born from 1990 to 2006, and the 338 genotyped animals were born from 2002 to 2006. The 56 499 animals were scanned at a mean age of 22.0 weeks (SD = 3.2, range = 6.3–48.0 weeks). The 338 genotyped animals had a mean age at scanning of 21.4 weeks (SD = 1.9, range = 13.7–27.3 weeks). Complete pedigree information was available for all animals with phenotypic records.

### Treatment of data

The distribution of the fat depth measurements was skewed, and, therefore, the data were transformed using a square-root transformation prior to analysis. The live weight at scanning and muscle depth measurements were analysed without the application of any transformation because they were normally distributed. Multiple regression and variance component analyses were performed for each of the traits in order to determine significant fixed effects. All fixed-effect models were fitted using the software package R (R Development Core Team 2006). Significant fixed effects for all traits were sex, litter size at birth, litter size reared, year, flock and age of dam. The age at scanning (in days) was fitted as a covariate for each trait.

### Haplotype reconstruction

The SNP haplotypes for 262 of the 338 genotyped animals were unambiguously reconstructed using R-tools

(Pong-Wong *et al.* 2001). This software utilizes marker genotypic data and pedigree information to determine the gametic haplotypes for each animal. The reconstructed haplotypes were used to estimate the linkage disequilibrium (LD) between the alleles at the two *GDF8* SNPs and perform mixed model association studies (see below) to assess potential effects of the parental origin of SNP alleles on muscle and fat traits.

### SNP linkage disequilibrium

Fisher's exact test was used to determine whether the frequencies of the observed haplotypes for the two SNP loci denoted significant LD, i.e. non-random association between alleles at the two SNPs. Haplotype information for the SNP pair was used to determine the extent of linkage disequilibrium (LD) by estimating the correlation, $r$, between alleles at the two SNPs and its square, $r^2$:

$$r_{ij} = D_{ij}/[p_i(1-p_i)p_j(1-p_j)]^{1/2}$$

where $D_{ij} = p_{ij} - p_i p_j$ is the covariance of gametic frequencies, and $p_i$, $p_j$ and $p_{ij}$ are the frequencies of allele $i$ at the first SNP locus, allele $j$ at the second SNP locus and haplotype $ij$ respectively (Hill & Robertson 1968).

### Mixed model association analysis

The significant fixed effects and covariates were included in mixed model association analyses to determine the effects of each of the SNPs on all traits (live weight at scanning, muscle depth and fat depth). In addition to the effects described above, the g.+6723G>A SNP (three classes: 1 = AA, 2 = AG, 3 = GG) and the g.−2449G>C SNP (three classes: 1 = CC, 2 = CG, 3 = GG) genotypes were fitted as fixed effects, separately and simultaneously. Two types of association analyses were performed. In the first analysis, a sire model was fitted using the statistical package R (R Development Core Team 2006) to analyse the trait data from the 338 genotyped animals. In the second analysis, an animal model was fitted using ASREML (Gilmour *et al.* 2002) to analyse all available phenotypic data and pedigree information for 56 499 animals. Detailed information on animals used and data analysed in the two separate mixed models is given in Table 1.

In addition to sire or animal identity (polygenic effects), other random effects were sequentially fitted in each model to determine the model best fitting the trait data, and the nested models were compared using the likelihood ratio test. For the sire model analysis, a model in which flock and dam were also fitted as random effects was chosen. For the animal model analysis, the model in which animal (polygenic effects), dam (maternal genetic) and litter (common environmental effects) were fitted as random effects was always the model best fitting the data. A more complex model, in which rearing dam (permanent environmental effects) was also fitted as a random effect, resulted in a near-zero variance for rearing dam.

A fourth class for the ungenotyped animals was included in the animal model analyses of each SNP genotype effect. Fitting the SNP fixed effect after including a fixed effect with two classes, 'genotyped or not', enabled us to utilize all pedigree and phenotypic information to estimate fixed effects and variance components, whilst assessing the significance of the three SNP genotypes at each locus.

Direct and maternal genetic heritabilities for muscle and fat depth were estimated from the variance components arising from these mixed model analyses. In addition, the proportion of phenotypic variance due to common environmental (litter) effect was estimated.

### Predictions and SNP genotype effects

Predicted trait values for each genotypic class of either SNP, including (co)variances for the predictions and standard error of differences (SED) for contrasts were obtained from the ASREML analyses. The predicted trait values were used to estimate additive and dominance effects on traits for each SNP, and the proportion of additive genetic variance ($V_A$) for each trait accounted for by the SNPs. The equations used were: additive effect, $a = (AA - GG)/2$; dominance effect, $d = AG - [(AA + GG)/2]$; and % $V_A$ due to SNP = $[2pq\,(a + d\,(q - p))^2]/V_A$, where AA, GG and AG were the predicted trait values for each genotype class, $p$ and $q$ were the allelic frequencies at the SNP locus and $V_A$ was the additive genetic variance of the trait obtained from an animal model analysis ignoring the SNP effects. Standard errors of the additive and dominance effects were constructed from the variance–covariance matrix of the

**Table 1** Summary of animals and records used in association analyses of *GDF8* g.+6723G>A and g.−2449G>C SNP effects on carcass traits of commercial Charollais sheep.

| | Numbers of: | | | | | | |
|---|---|---|---|---|---|---|---|
| Model[1] | Flocks | Sires | Dams | Scan weight records | Muscle depth records | Fat depth records | Genotyping records |
| Sire | 12 | 17 | 226 | 338 | 338 | 338 | 338 |
| Animal | 178 | 2019 | 20 100 | 56 499 | 56 499 | 56 496 | 338 |

[1]Sire model refers to the flock/sire/dam model fitted; animal model refers to the animal/litter/dam model fitted.

predicted genotype classes, as were the SED for pairwise contrasts of the SNP genotype classes.

### Parent-of-origin effects

Mixed model association analysis of the parent-of-origin allelic information for each SNP was performed to investigate whether the mode of inheritance of the allele (paternal or maternal origin) had an effect on the traits. Following haplotype reconstruction, the SNP genotype classes were expanded by subdividing the heterozygotes into two classes, according to the parental origin of the g.+6723A or the g.−2449C allele. SNP genotype was then fitted as a fixed effect, first in the sire model and then in the animal model. Because trait data from only the 338 genotyped animals were analysed using the sire model, an overall SNP effect with a total of four genotype classes was included in the sire model association analysis. A fifth SNP genotype class corresponding to the ungenotyped animals was included in the animal model analysis, in which phenotypes from 56 499 animals (including the 338 genotyped ones) were analysed. The parent-of-origin effect was assessed by comparing the predicted trait values of the two classes of heterozygotes for each SNP. Hypothesis testing using a *t*-test was employed.

## Results

Allelic frequencies at the g.+6723G>A SNP locus in the three genotyped sheep breeds are shown in Fig. 1. The g.+6723A allele, associated with muscular hypertrophy, was absent in Suffolk sires that were genotyped and almost fixed in Texel sires. Both the g.+6723A and the g.−2449C alleles were segregating at intermediate frequencies ($p = 0.3$ in this sample) in the sample from the Charollais population. Fisher's exact test showed that the association between the alleles of the two SNP loci was non-random ($P$-value = $10^{-6}$). The correlation between the alleles at the two SNP loci, $r$, and its square, $r^2$, were 0.95 and 0.90, respectively.

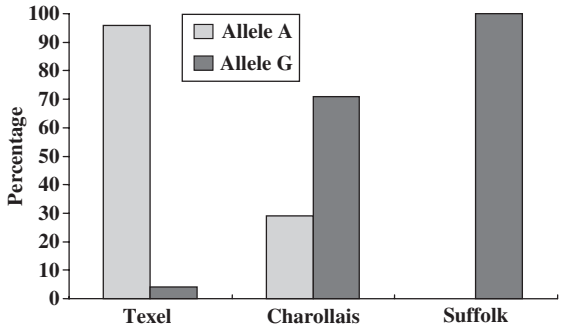


**Figure 1** Allelic frequencies at the *GDF8* g.+6723G>A SNP locus in British commercial sheep. Frequencies are based on locus genotypes of Texel and Suffolk sires and of Charollais lambs.

Means, standard deviations and ranges of the traits studied in the Charollais lambs are shown in Table 2. Direct genetic, maternal genetic heritabilities and the proportion of phenotypic variance due to common environmental effects (litter) for muscle and fat depth are shown in Table 3. The direct genetic heritabilities of muscle and fat depth were moderate, whereas maternal genetic heritabilities for both traits were low. Common environmental contributions to the phenotypic variance for muscle and fat depth were large, yet smaller than those of the direct genetic effects (Table 3).

Mixed model association analyses of the two *GDF8* SNPs were performed for muscle depth, fat depth and live weight. None of the analyses showed significant association of genotype at either SNP locus with live weight. When the genotypic data for either or both SNPs were analysed using a sire model, significant effects were seen for both muscle ($P < 0.01$) and fat depth ($P < 0.05$) (results not shown). When the complete dataset was used, and an animal model was fitted, the significant fat depth effect of the SNP genotypes at either locus disappeared, but the overall SNP genotype effects on muscle depth remained. The overall g.+6723G>A SNP genotype effect on muscle depth

**Table 2** Trait means, ranges and phenotypic standard deviations (SD).

| Trait | Mean | Range | SD[1] |
|---|---|---|---|
| Live weight (kg) | 51.42 | 15.50–97.00 | 6.67 |
| Muscle depth LV3[2] (mm) | 28.31 | 12.00–45.00 | 2.91 |
| Sqrt(Fat depth)[3] LV3[2] ($mm^{1/2}$) | 1.88 | 0.10–3.92 | 0.40 |

[1]Phenotypic standard deviations after adjusting for fixed effects.
[2]LV3 = third lumbar vertebra.
[3]Sqrt(Fat depth) refers to the square-root transformed trait. Raw fat depth data had a mean of 3.78 mm and a range of 0.01–15.33 mm.

**Table 3** Estimated trait variance ratios (variance component/phenotypic variance) for each random effect fitted in the selected REML model.

| Trait | Variance component | Variance ratio ± SE |
|---|---|---|
| Muscle depth | Animal (direct genetic effect, i.e. heritability) | 0.29 ± 0.011 |
| | Dam (maternal genetic effect) | 0.03 ± 0.005 |
| | Litter (common environmental effect) | 0.22 ± 0.007 |
| Sqrt(Fat depth)[1] | Animal (direct genetic effect, i.e. heritability) | 0.31 ± 0.011 |
| | Dam (maternal genetic effect) | 0.04 ± 0.005 |
| | Litter (common environmental effect) | 0.28 ± 0.007 |

[1]Sqrt(Fat depth) refers to the square-root transformed trait.

**Table 4** SNP allelic effects and percentage of additive genetic variance explained by *GDF8* SNP genotypes.

| Trait | SNP | $a \pm SE^1$ | $d \pm SE^2$ | Percentage of genetic variance due to SNP[3] |
|---|---|---|---|---|
| Muscle depth (mm) | g.+6723G>A | 1.20 ± 0.30 | −0.73 ± 0.36 | 14 |
| | g.−2449G>C | 1.00 ± 0.25 | −0.45 ± 0.33 | 11 |
| Sqrt(Fat depth)[4] ($mm^{1/2}$) | g.+6723G>A | −0.065 ± 0.040 | 0.040 ± 0.049 | 2.1 |
| | g.−2449G>C | −0.066 ± 0.034 | 0.056 ± 0.044 | 1.5 |

[1]Negative additive genetic effect ($a < 0$) indicates g.+6723A (or g.−2449C) allele decreased the trait.
[2]If $a > 0$ and $d < 0$ (or $a < 0$ and $d > 0$), the g.+6723A (or g.−2449C) allele is partially recessive.
[3]Estimated using allelic frequencies observed in sample ($p = 0.3$ for g.+6723A or g.−2449C allele).
[4]Sqrt(Fat depth) refers to the square-root transformed trait.

had an $F$-ratio of 8.05 ($(2, \sim 303)$; $P < 0.001$) and the g.−2449G>C SNP effect on the trait had an $F$-ratio of 7.78 ($(2, \sim 303)$; $P < 0.001$) (see Table 4 for the estimated allelic effects of each SNP). Genotypic class contrasts for each SNP are discussed below. When either SNP genotype was fitted as a fixed effect after the other one was already included in the model, the second SNP fitted did not have a significant effect on muscle depth.

For each SNP, the significance of pairwise contrasts between the predicted traits values of the three genotypic classes was determined using a two-sample $t$-test (Table 5). Significant differences were found between the AA and GG, and the AA and AG genotype pairs at the g.+6723G>A SNP locus for muscle depth, but not between GG and AG. For the g.−2449G>C SNP locus, significant differences were detected when the CC genotype value was contrasted to either one of the GG and CG genotypes, whereas no significant difference was identified between the GG and CG values. Genotype class effects on square-root-transformed fat depth were generally not significant, except for a marginally significant difference detected between the CC and GG genotypes at the g.−2449G>C SNP ($P \sim 0.05$).

**Table 5** Test statistics for contrasts between SNP genotype class values for muscle and fat depth using the animal model.

| Trait | Effect | Contrast | $t$-statistic[1] | $P$-value |
|---|---|---|---|---|
| Muscle depth | g.+6723G>A | A:A vs. G:A | 3.32 (168 d.f.) | <0.002 |
| | | A:A vs. G:G | 4.01 (193 d.f.) | <0.002 |
| | | G:A vs. G:G | 1.44 (309 d.f.) | <0.20 |
| | g.−2449G>C | C:C vs. G:C | 2.96 (179 d.f.) | <0.01 |
| | | C:C vs. G:G | 3.94 (193 d.f.) | <0.001 |
| | | G:C vs. G:G | 1.72 (298 d.f.) | <0.10 |
| Sqrt(Fat depth)[2] | g.+6723G>A | A:A vs. G:A | 1.35 (168 d.f.) | <0.10 |
| | | A:A vs. G:G | 1.62 (193 d.f.) | <0.10 |
| | | G:A vs. G:G | 0.57 (309 d.f.) | <0.5 |
| | g.−2449G>C | C:C vs. G:C | 1.88 (179 d.f.) | <0.10 |
| | | C:C vs. G:G | 1.95 (193 d.f.) | ∼0.05 |
| | | G:C vs. G:G | 0.23 (298 d.f.) | <0.80 |

[1]The degrees of freedom for the test (in parentheses) are only approximate.
[2]Sqrt(Fat depth) refers to the square-root transformed trait.

Estimations using the predicted trait values for each SNP genotype class showed that the g.+6723A allele had an additive effect of 1.20 (±0.30) mm on muscle depth (Table 4). The dominance effect of the A allele at the locus was negative, *viz.* −0.73 (±0.36) mm. For fat depth, the g.+6723A allele was related to a small but non-significant decrease in fat depth (additive value = −0.065 (±0.040) $mm^{1/2}$). The additive effect of the g.−2449C allele on muscle depth was 1.00 (±0.25) mm, and the dominance effect was −0.45 (±0.33) mm.

Based on the estimated allelic effects and the allelic frequencies observed in the sample at each locus, the proportion of additive genetic variance attributed to the SNP genotype at each SNP locus was determined for both muscle and fat depth (Table 4). The g.+6723G>A SNP genotype explained 14% of the additive genetic variance for muscle depth, and 2.1% for fat depth. The g.−2449G>C SNP genotype accounted for 11% of additive genetic variance for muscle depth and for 1.5% for fat depth.

Parent-of-origin effects, tested by contrasting the two heterozygote classes at each SNP locus, were generally not significant. An effect that approached significance was detected with the sire model association analysis of each SNP haplotype with muscle depth (heterozygote contrast = 2.82 (±1.53) mm, $P$-value = 0.07). However, our analyses were limited by the fact that in our sample only five heterozygous animals for the g.+6723G>A SNP and only 10 for the g.−2449G>C SNP had inherited the minor allele from their dam.

## Discussion

This study examined the presence of two *GDF8* SNPs in British commercial sheep breeds. It is, to our knowledge, the first published report of significant association of these polymorphisms with muscle depth in commercial Charollais sheep. In addition, an extensive study of the allelic and genotypic effects on muscle depth was performed, and the mode of SNP action was determined for this breed, with the

effect appearing to be partially recessive, at least for the trait measured. Finally, in accordance with the strategy outlined by Ron & Weller (2007), our analyses provided further statistical validation for the g.+6723G>A SNP being a QTN for muscularity in sheep, as previously proposed by Clop *et al.* (2006).

The first objective of our study was to examine whether the same genetic mutation(s) were responsible for the similar phenotypes attributed to OAR2 QTL in different breeds (Walling *et al.* 2004; McRae *et al.* 2005). More specifically, we sought to determine whether the g.+6723A and g.−2449C alleles of the two *GDF8* SNPs associated with increased muscularity in Belgian Texel rams were present in British terminal sire breeds. Because the favourable alleles were absent in the Suffolk animals sampled and nearly fixed in the Texel breed (Fig. 1 for the g.+6723G>A SNP results), other OAR2 loci must be responsible for the QTL detected for muscle and fat traits in these breeds. Fixation of the A allele at the 3′-UTR g.+6723G>A SNP locus was previously found for Belgian Texel rams (Clop *et al.* 2006). The Charollais is the only breed, apart from the Australian White Suffolk (Kijas *et al.* 2007), in which these *GDF8* SNPs have been found to segregate with the favourable allele of each SNP having an intermediate frequency ($p = 0.3$) in the genotyped animals.

A mixed model in which animal, dam and litter were fitted as random effects revealed highly significant association of SNP genotype at both *GDF8* SNP loci with muscle depth in our lamb population. Estimates for the direct genetic, maternal genetic heritabilities and the proportion of variance due to common environmental effects (litter) were consistent with published values of these genetic parameters for these traits in Charollais and other sheep breeds (Ap Dewi *et al.* 2002; Jones *et al.* 2004; Safari *et al.* 2005). In these analyses, the age at scanning (in days) was fitted as a covariate for each trait to adjust for systematic trends in trait values associated with age *per se*. Alternatively, fat and muscle depths may be corrected for live weight at scanning or live weight together with age at scanning, to remove differences in these traits attributable to animal size. When these analyses were performed, i.e. fitting live weight as a covariate or live weight and age as covariates, the SNP genotype association results and the magnitude and direction of the estimated additive and dominance effects were essentially the same as those seen when the data were corrected for age at scanning. Including both live weight and age as covariates in the analysis of muscle depth resulted in the predicted mean trait value for the heterozygote at each SNP locus being significantly different from the trait means of both homozygotes. Prior to the association analysis, the fat depth data were subjected to square-root transformation to ensure that a normally distributed trait would be analysed. All results have been presented on this transformed scale. Back transformation of the genotype values yielded predicted mean fat depths of 2.80, 3.16 and 3.25 mm for the g.+6723AA, g.+6723AG and g.+6723GG genotypes, respectively.

The two SNPs, which are about 9 kb apart in the *GDF8* region (Clop *et al.* 2006), exhibit substantial LD ($r^2 = 0.90$). Thus, if the real functional effect on muscle depth arises from the g.+6723A allele, as proposed by Clop *et al.* (2006), then the detected effect of the g.−2449C allele is probably due to its LD with the causative variant. Additive and dominance values for the g.−2449G>C SNP were comparable in size and in sign with those for the g.+6723G>A SNP, as would be expected from the strong LD between the two SNPs. The opposite signs of the allelic effects (positive additive and negative dominance value) supported a partially recessive nature of the causative g.+6723A allele for muscle depth. This is in accordance with the previously detected partially recessive mode of action of the OAR2 QTL (later mapped to the g.+6723A allele) on various traits related to muscularity in an $F_2$ population of Belgian Texel × Romanov animals (Clop *et al.* 2006).

The proportion of additive genetic variance for muscle depth accounted for by SNP genotype at each *GDF8* SNP locus depends on the allelic frequencies. The values presented in Table 4 for the g.+6723G>A and the g.−2449G>C SNPs (14% and 11%) increase to 29% and 21% of the additive genetic variance, respectively, if allelic frequencies of 0.5 are assumed. Due to the partially recessive mode of the g.+6723A allele for muscle depth, the breeding and genotypic values for each genotype are also strongly affected by the allelic frequencies at the locus. Further, it can be shown that the additive and total genetic variance explained by the SNP genotype will maximize when the frequencies of the g.+6723A allele are 0.70 and 0.68 respectively. The maximum additive genetic variance that can be attributed to the SNP genotype at this frequency corresponds to 38% of the total additive variance of muscle depth. Thus, based on both the estimated magnitude of the SNP effect on muscle depth and the amount of genetic variance of the trait that it explains, genetic selection for the g.+6723G>A QTN is of particular economic importance for the Charollais breed.

The detection of a partially recessive action of the g.+6723A allele on the muscle phenotype raises the issue whether a recessive mode of allelic expression is indeed plausible. The proposed molecular mechanism of miRNA-mediated translational inhibition of myostatin by which the g.+6723A allele leads to increased muscle (Clop *et al.* 2006) does accommodate a partially recessive action of the mutation on myostatin expression. According to this mechanism, in a heterozygous animal, translational inhibition of the mutant mRNA would lead to about one-third of the normal amount of myostatin produced (Clop *et al.* 2006), whereas the wild-type *GDF8* mRNA would produce normal amounts of protein. Thus, it is likely that enough active myostatin would be produced to facilitate myostatin-mediated regulation of muscle development. This

would result in the observed partially recessive effect on the phenotype.

It should be noted that the overall mode of action and magnitude of the observed effect of the g.+6723A allele on phenotype would probably depend on the genetic background of a particular animal population. Indeed, the influence of background genetics on the effects of *GDF8* mutations on phenotypes has been shown in cattle (Short *et al.* 2002) and mice (Bünger *et al.* 2004; Rehfeldt *et al.* 2005). Additionally, the effect of overexpression of other molecules interacting with myostatin and other growth factors on muscle development was recently shown in mice (Lee 2007). The magnitude and perhaps even the mode of action of the *GDF8* mutational effects on phenotype are probably affected by the genetic background of the breed in which the mutation is seen.

In Great Britain, the frequency of the favourable A allele of the g.+6723G>A SNP in the Charollais breed has probably increased somewhat due to mass selection on muscle traits. Yet, because of its partially recessive action on muscle phenotype, the rate of genetic progress for the trait depends heavily not just on the allelic frequency but also on the proportion of homozygote animals for the A allele in the population. Consequently, marker-assisted selection (MAS) for this SNP could be of substantial benefit. In fact, our findings indicate that it actually may be more advantageous to apply MAS for a (partially) recessive, rather than a dominant, allele when the dominant allele is unfavourable for the trait. Overall, for maximum gain from MAS, a comprehensive strategy for nucleotide-assisted selection that takes into account a SNP's mode of action on the traits of interest, and the effects of the genetic background of the breed or population, should be employed.

## Acknowledgements

This work was conducted as part of the GENACT Project, funded by the Marie Curie Host Fellowships for Early Stage Research Training, as part of the Sixth Framework Programme of the European Commission. We would also like to thank LINK, Defra, SEERAD, EBLEX and HCC for funding and Charollais Sires (the group breeding scheme for the Charollais breed) for assistance.

## References

Ap Dewi I., Saatci M. & Ulutas Z. (2002) Genetic parameters of weights, ultrasonic muscle and fat depths, maternal effects and reproductive traits in Welsh Mountain sheep. *Animal Science* **74,** 399–408.

Broad T.E., Glass B.C., Greer G.J., Robertson T.M., Bain W.E., Lord E.A. & McEwan J.C. (2000) Search for a locus near to *myostatin* that increases muscling in Texel sheep in New Zealand. *Proceedings of New Zealand Society of Animal Production* **60,** 110–2.

Bünger L., Ott G., Varga L., Schlote W., Rehfeldt C., Renne U., Williams J.L. & Hill W.G. (2004) Marker assisted introgression of the *Compact* mutant *myostatin* allele $Mstn^{Cmpt\text{-}dl1Abc}$ into a mouse line with extreme growth effects on body composition and muscularity. *Genetical Research* **84,** 161–73.

Clop A., Marcq F., Takeda H. *et al.* (2006) A mutation creating a potential illegitimate microRNA target site in the *myostatin* gene affects muscularity in sheep. *Nature Genetics* **38,** 813–8.

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 markers. *Proceedings of the National Academy of Sciences of the United States of America* **91,** 3019–23.

Gilmour A.R., Gogel B.J., Cullis B.R., Welham S.J. & Thompson R. (2002) *ASREML User Guide Release 1.0*. VSN International Ltd, Hemel Hempstead, UK.

Grisart B., Coppieters W., Farnir F. *et al.* (2002) Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Research* **12,** 222–31.

Hill W.G. & Robertson A. (1968) Linkage disequilibrium in finite populations. *Theoretical and Applied Genetics* **38,** 226–31.

Johnson P.L., McEwan J.C., Dodds K.G., Purchas R.W. & Blair H.T. (2005) A directed search in the region of *GDF8* for quantitative trait loci affecting carcass traits in Texel sheep. *Journal of Animal Science* **83,** 1988–2000.

Jones H.E., Lewis R.M., Young M.J. & Simm G. (2004) Genetic parameters for carcass composition and muscularity in sheep measured by X-ray computer tomography, ultrasound and dissection. *Livestock Production Science* **90,** 167–79.

Kambadur R., Sharma M., Smith T.P.L. & Bass J.J. (1997) Mutations in *myostatin* (*GDF8*) in double-muscled Belgian Blue and Piedmontese cattle. *Genome Research* **7,** 910–5.

Kijas J.M., McCulloch R., Hocking Edwards J.E., Oddy V.H., Lee S.H. & van der Werf J. (2007) Evidence for multiple alleles effecting muscling and fatness at the ovine *GDF8* locus. *BMC Genetics* 8:80. URL: http://www.biomedcentral.com/1471-2156/8/80.

Lee S.-J. (2007) Quadrupling muscle mass in mice by targeting TGF-$\beta$ signaling pathways. *PLoS ONE* **2,** e789.

Marcq F., Larzul C., Marot V., Bouix J., Eychenne F., Laville E., Bibé B., Leroy P.L., Georges M. & Elsen J.M. (2002) Preliminary results of a whole-genome scan targeting QTL for carcass traits in a Texel × Romanov intercross. *Proceedings of the 7th World Congress on Applied Livestock Production*, Montpellier, 19–23 August, France, Abstract 02–14.

McPherron A.C. & Lee S.J. (1997) Double muscling in cattle due to mutations in the *myostatin* gene. *Proceedings of the National Academy of Sciences of the United States of America* **94,** 12457–61.

McRae A.F., Bishop S.C., Walling G.A., Wilson A.D. & Visscher P.M. (2005) Mapping of multiple quantitative trait loci for growth and carcass traits in a complex commercial sheep pedigree. *Animal Science* **80,** 135–41.

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* **26,** 529–32.

Pong-Wong R., George A.W., Woolliams J.A. & Haley C.S. (2001) A simple and rapid method for calculating identity-by-descent

matrices using multiple markers. *Genetics Selection Evolution* **33,** 453–71.

R Development Core Team (2006) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Rehfeldt C., Ott G., Gerrard D.E., Varga L., Schlote W., Williams J.L., Renne U. & Bünger L. (2005) Effects of the *Compact* mutant myostatin allele $Mstn^{Cmpt\text{-}dl1Abc}$ introgressed into a high growth mouse line on skeletal muscle cellularity. *Journal of Muscle Research and Cell Motility* **26,** 103–12.

Ron M. & Weller J.I. (2007) From QTL to QTN identification in livestock – winning by points rather than knock-out: a review. *Animal Genetics* **38,** 429–39.

Safari E., Fogarty N.M. & Gilmour A.R. (2005) A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. *Livestock Reproduction Science* **92,** 271–89.

Short R.E., MacNeil M.D., Grosz M.D., Gerrard D.E. & Grings E.E. (2002) Pleiotropic effects in Hereford, Limousin and Piedmontese F2-crossbred calves of genes controlling muscularity including the Piedmontese *myostatin* allele. *Journal of Animal Science* **80,** 1–11.

Van Laere A.S., Nguyen M., Braunschweig M. *et al.* (2003) A regulatory mutation in *IGF2* causes a major QTL effect on muscle growth in the pig. *Nature* **425,** 832–6.

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,** 2234–45.

Wiener P., Smith J.A., Lewis A.M., Wooliams J.A. & Williams J.L. (2002) Muscle-related traits in cattle: the role of the *myostatin* gene in the South Devon breed. *Genetics Selection Evolution* **24,** 221–32.

Wilson T., Wu X.Y., Juengel J.L. *et al.* (2001) Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of *bone morphogenetic protein IB receptor* (*ALK-6*) that is expressed in both oocytes and granulosa cells. *Biology of Reproduction* **64,** 1225–35.