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The *callipyge* mutation and other genes that affect muscle hypertrophy in sheep

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Abstract – Genetic strategies to improve the profitability of sheep operations have generally focused on traits for reproduction. However, natural mutations exist in sheep that affect muscle growth and development, and the exploitation of these mutations in breeding strategies has the potential to significantly improve lamb-meat quality. The best-documented mutation for muscle development in sheep is *callipyge* (*CLPG*), which causes a postnatal muscle hypertrophy that is localized to the pelvic limbs and loin. Enhanced skeletal muscle growth is also observed in animals with the *Carwell* (or *rib-eye muscling*) mutation, and a double-muscling phenotype has been documented for animals of the Texel sheep breed. However, the actual mutations responsible for these muscular hypertrophy phenotypes in sheep have yet to be identified, and further characterization of the genetic basis for these phenotypes will provide insight into the biological control of muscle growth and body composition.

sheep / muscle / hypertrophy / callipyge / mutation

1. INTRODUCTION

The trait with the greatest financial impact on sheep production is the number of lambs weaned per ewe [7]. Correspondingly, breeding schemes for improved lamb-meat production have focused on selecting animals with superior reproductive capacities, and studies directed at the genetic improvement of sheep have been primarily concerned with reproductive traits

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(for review, see [45]). From a producer's standpoint, a greater number of lambs result in a higher profit margin. Thus, quantity rather than quality has been the main focus of the sheep community for decades.

Although the importance of improving lamb carcass composition has been recognized since the 1950's (reviewed in [60]), the sheep industry, particularly in the United States, has made little progress toward improving the carcass merit of slaughter lambs. In fact, the only alteration to carcass composition appears to be that from the butcher's knife; Tatum *et al*. [56] reported that extensive fat trimming occurs at the retail level. This excess of fat accumulation is caused by the over-finishing of lambs, which has been widely practiced in recent years to increase profitability. In the United States, the average live weight of slaughter lambs has jumped from 104 pounds in 1975 to 141 pounds in 2002 [28]. However, more than half of all lamb carcasses in the United States currently exceed the recommendations for backfat thickness put forth by the American Sheep Industry Association (ASI), and less than one-third of market lambs meet the criteria for leanness and muscling specified by the ASI's Certified Fresh American Lamb^{TM} program [3].

Recently, efforts to improve carcass merit in sheep have been prompted by studies on consumer acceptability of lambs [18, 27], which indicate that lambs harvested at a younger age are preferred by consumers to over-finished lambs. Health-conscious consumers favor lamb cuts with less fat, but consumers also prefer larger chops, since they look for value in their money. Thus, there is considerable interest in identifying ways to effectively manage sheep-meat operations so as to increase the lean content and decrease the fat content of lamb products.

Unfortunately, producers are generally not financially rewarded for improved carcass quality at the present time. However, strategies to increase lean production and decrease fat deposition result in improved feed conversion efficiency. Among the benefits to the producer of enhanced feed conversion efficiency include lower production costs, higher product yields, less nitrogenous-waste excretion into the environment, and decreased grazing pressure. Achievement of increased feed conversion efficiency can be attained through the use of hormonal growth promoters, transgenic animals, nutritional strategies, choice of terminal sire breed, and marketing lambs at appropriate slaughter weights (reviewed in [3, 52]). However, the banning of hormonal growth promoters by the European Union, the problems with gene expression in transgenic animals, and the limited advance of nutritional studies in sheep have restricted the incorporation of these strategies into production schemes.

The most promising strategy for manipulating and improving carcass composition in sheep, and one that is likely to gain widespread public acceptance, is genetic selection. Within- and between-breed variation in meat coloration, marbling, fatty acid profile, and protein concentration have been documented in sheep (reviewed in [57]). However, until recently, carcass traits could only be measured on dead animals, and this has hampered genetic selection for improved carcass quality. The development of several live-animal measurement techniques (reviewed in [4]) has greatly facilitated the identification of specific carcass traits that respond to genetic selection in sheep. The identification and characterization of major genes and quantitative trait loci (QTL) that influence fat and lean deposition will pave the way for improved lamb-meat products in the future.

2. LOCI RESPONSIBLE FOR CARCASS QUALITY IN SHEEP

To date, nine distinct loci have been identified that influence carcass composition in sheep (Tab. I). Of these, the three best-known muscle traits in sheep (callipyge, Carwell, and double muscling, respectively) are characterized as a hypertrophy of the myofibers. In contrast to muscle hyperplasia, which is an increase in myocyte number, muscular hypertrophy is an increase in myofiber diameter. However, the actual biological mechanism by which these mutations give rise to muscle enhancement is not yet known. Further characterization of the genes and mutations responsible for muscle hypertrophy in sheep will provide new insight into the control of growth and body composition.

2.1. The *callipyge* (*CLPG*) locus

The callipyge phenotype in sheep is a muscular hypertrophy that is most pronounced in the muscles of the pelvic limb [26,32]. Muscles from callipyge-expressing lambs enlarge to differing degrees, and not all muscles are affected. In an extensive survey of 19 muscles dissected from the right side of carcasses from normal and callipyge individuals [26], the total weight of excised muscles from the pelvic, torso, and thoracic limbs was greater in callipyge lambs by 42%, 50%, and 14%, respectively, than in normally muscled half-sibs. This muscle hypertrophy develops after about three weeks of age [24], so there is no increased risk of dystocia for callipyge lambs.

Callipyge lambs exhibit several desirable production characteristics and meat quality traits. Higher dressing percentages, larger *longissimus* (loin eye) areas, superior lean composition, and higher leg scores have been documented

Table I. Genes and QTL affecting carcass composition in sheep.

Name of phenotype[1]	Ref.	Breed	Chr.	Description
Callipyge	[15]	American Dorset	18	~30% increase in muscle mass ~8% decrease in fat content Localized in the hindquarters
Carwell	[47]	Australian Poll Dorset	18	~10% increase in rib-eye area
	[61]	British Texel	18	Same effect as reported in [47]
Double Muscling	[39]	Australian Texel	n.t.[2]	Generalized muscular hypertrophy
	[36]	Belgian Texel	2	Generalized muscular hypertrophy
	[8]	New Zealand Texel	2	Generalized muscular hypertrophy
Other QTL	[62]	British Suffolk	1	Muscle depth and weight
			3	Fat weight
			18	Weight at 8 weeks of age
	[62]	British Texel	3	Muscle depth and weight
			4	Fat weight
			20	Fat depth

[1]The causative mutations for the Carwell (rib-eye muscling) and double-muscling phenotypes have not been identified. However, QTL at similar chromosomal locations that correspond to each of these phenotypes have been reported in multiple populations. Confirmation that the same genetic mutation is responsible for the similar phenotypes reported in each of these populations will be possible upon identification of the causative mutations.
[2]Not tested. A generalized muscular hypertrophy that is reminiscent of double muscling in Belgian and New Zealand Texels is also segregating in the Australian population.

for callipyge carcasses [25, 32]. These superior carcass traits translate into improved yields of wholesale leg, loin, rack, and shoulder from callipyge animals by 11.8%, 4.7%, 2.5%, and 2.3%, respectively, over normally muscled lambs [9]. In addition, callipyge lambs exhibit superior feed efficiencies and lower daily feed intakes [24], which result in lower production expenses. Therefore, the widespread production of callipyge lamb would have the potential to lower the cost of lamb for consumers and to increase the profitability of the sheep industry. Unfortunately, the stigma associated with callipyge lamb as being unacceptably tough [32, 46, 51] has limited its production in the United States.

Enlargement of muscles in callipyge-expressing animals is primarily due to myofiber hypertrophy. Histological examination of myofibers [10] in callipyge-responsive *versus* normal muscles showed that the callipyge-responsive muscles exhibit larger average diameters for the fast-twitch

oxidative glycolytic (FOG) and fast-twitch glycolytic (FG) muscle fibers and smaller average diameters for the slow-twitch oxidative (SO) fibers. In addition, the percentage of FG fibers is greater and the percentages of SO and FOG fibers are smaller in callipyge-responsive muscles. Thus, myofiber changes in callipyge animals were strongly associated with the FG fibers, the only fiber type that increase in proportion and diameter in the callipyge-responsive muscles. This hypertrophy was evident in 8-week-old but not in 2-week-old lambs [11], thereby supporting gross phenotypic observations of postnatal development of the callipyge.

The callipyge trait in sheep exhibits a novel mode of inheritance termed "polar overdominance" [15]. The only animals that express the callipyge phenotype are the heterozygous offspring who inherit the *callipyge* (*CLPG*) mutation from their sire (*i.e.* the $+^{M}/CLPG^{P}$ genotype, where the superscripts M and P refer to the maternal or paternal inheritance of the alleles, respectively). The other three genotypes ($+^{M}/+^{P}$, $CLPG^{M}/+^{P}$, and $CLPG^{M}/CLPG^{P}$) are phenotypically normal. Although hybrid dysgenesis in *Drosophila* [30] and polar lethality in mice [58] also exhibit parent-of-origin-dependent and heterozygote-specific phenotypic effects, callipyge in sheep is the only known example of strict polar overdominance reported to date. The polar overdominance model for callipyge in sheep has been confirmed in an independent flock [19], thereby demonstrating that callipyge polar overdominance in sheep represents a truly novel mode of inheritance.

Recently, a decade-long positional cloning effort to identify the *CLPG* mutation came to fruition with the identification of an A-to-G transition that segregates perfectly with the *CLPG* allele [20, 53]. This polymorphism, which is designated as SNP^{CLPG}, lies within a conserved 12-bp motif that is located approximately 33 kb upstream of the *GTL2* gene. The causality of this mutation was strongly supported by our finding that Solid Gold, the founder ram of the callipyge trait, was mosaic for the mutation [53]. We suggested that the *CLPG* mutation (which corresponds to the *G* allele of SNP^{CLPG}) arose during Solid Gold's early embryonic development, thereby rendering him mosaic for SNP^{CLPG} in both somatic and germline tissues.

The SNP^{CLPG} mutation lies within the *DLK1-GTL2* imprinted gene cluster on ovine chromosome 18 (OAR18). This imprinted gene cluster contains several paternally expressed protein-coding genes, including *BEGAIN* [54], *DLK1* [31, 49, 55, 65], *PEG11* [14], and *DIO3* [22, 59, 66], as well as several

maternally expressed non-coding RNA genes, including *GTL2* [44, 49, 55, 65], *antiPEG11* [14], *MEG8* [14], and *MIRG* [50]. The *BEGAIN, DLK1, PEG11, GTL2, antiPEG11*, and *MEG8* genes have been shown to be expressed and subject to genomic imprinting in ovine skeletal muscle tissues [14, 54].

To date, the precise function of SNP^{CLPG} remains elusive. However, a potential function for SNP^{CLPG} has been inferred from studies that examined the expression patterns of genes in the *DLK1-GTL2* cluster in animals of the four *CLPG* genotypes. Northern blot analysis of longissimus muscle-derived RNA has demonstrated that the expression of *DLK1, GTL2, PEG11, antiPEG11,* and *MEG8* is altered in a genotype- and muscle-specific manner [5, 13]. Specifically, the expression levels of these genes are increased in longissimus muscle from 8-week-old individuals when the *CLPG* mutation is inherited in *cis*, yet these genes maintain their exclusive expression from either the paternal or maternal allele [13]. Thus, we have hypothesized that the SNP^{CLPG} position is located within a long-range regulatory element that functions in *cis* to coordinately control gene expression in the region [21]. This regulatory element is also thought to function in an age-dependent manner because the expression of genes in the cluster is normally downregulated postnatally in sheep skeletal muscle [16]. In addition, the expression of these genes appears to be limited to the callipyge-expressing muscles of the hindquarters, because little expression of *DLK1, GTL2, PEG11, antiPEG11,* or *MEG8* is detected in the supraspinatus (shoulder) muscle [6]. Two additional paternally expressed, protein-coding genes flank this core gene cluster (namely, *BEGAIN* on the proximal side and *DIO3* on the distal side), but their expression is not altered by the *CLPG* mutation [54].

The presence *versus* absence of the *CLPG* mutation on the maternal versus paternal alleles results in a unique expression profile of genes in the *DLK1-GTL2* cluster for animals of each *CLPG* genotype (Fig. 1). The phenotypic manifestation of callipyge is likely to depend on this unique expression profile. In other words, the callipyge muscle hypertrophy phenotype is postulated to arise exclusively in animals with the $+^{M}/CLPG^{P}$ genotype because they exhibit an overexpression of the paternally expressed *DLK1* and *PEG11* genes but not an overexpression of the maternally expressed *GTL2, antiPEG11,* and *MEG8* genes. In contrast, the other three genotypes ($+^{M}/+^{P}$, $CLPG^{M}/+^{P}$, and $CLPG^{M}/CLPG^{P}$), which exhibit alternative expression profiles of these genes, do not develop the callipyge phenotype.

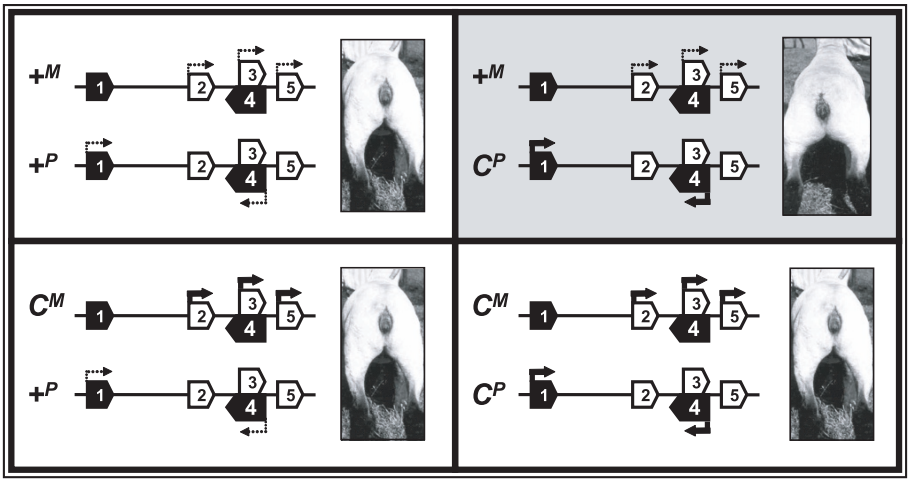

Figure 1. The expression profile of genes in the *DLK1-GTL2* cluster for animals of each of the four *callipyge* genotypes. Each of the five genes whose expression is known to be altered by the *CLPG* mutation is represented (1 = *DLK1*; 2 = *GTL2*; 3 = *antiPEG11*; 4 = *PEG11*; 5 = *MEG8*) by a black or white box to indicate exclusive expression from the paternal (P) or maternal (M) chromosome, respectively. The presence of the *callipyge* (*C*) mutation results in enhanced expression of multiple genes in the cluster, depending on its maternal *versus* paternal inheritance, and this increase in transcript abundance for each gene is depicted by a thick arrow. The only genotype that exhibits the callipyge phenotype is shaded in gray.

Because of the observed *CLPG* genotype-dependent expression profile of genes in the *DLK1-GTL2* imprinted domain, we have predicted that the molecular mechanism of polar overdominance at the *CLPG* locus results from (1) a *cis*-effect of the *CLPG* mutation on the expression levels of genes in the *DLK1-GTL2* cluster and (2) a post-transcriptional *trans* interaction between the products of reciprocally imprinted genes [21]. The candidate effector molecules for muscle hypertrophy development in animals of the $+^{M}/CLPG^{P}$ genotype are the protein products of the paternally expressed *DLK1* and *PEG11* genes. The effect of *DLK1* and/or *PEG11* overexpression is effectively silenced in animals of the $CLPG^{M}/CLPG^{P}$ genotype because there is a corresponding overexpression of the maternally expressed genes (*GTL2, antiPEG11*, and *MEG8*). The other two genotypes ($+^{M}/+^{P}$ and $CLPG^{M}/+^{P}$) do not exhibit an overexpression of the putative protein-coding effectors and therefore, do not exhibit the callipyge phenotype. The possibility of a

post-transcriptional *trans* interaction between the products of the paternally expressed effector (*DLK1* or *PEG11*) and the maternally expressed silencer (*GTL2, antiPEG11,* or *MEG8*) is currently being tested.

Involvement of the *DLK1* and/or *PEG11* gene in callipyge muscular hypertrophy is only speculative at this point; neither the *DLK1* nor the *PEG11* genes has been conclusively shown to be involved in muscle growth. The *DLK1* gene encodes a member of the delta-notch family of signalling molecules [33], and the *PEG11* gene encodes for a protein product with similarity to the gag and pol polyproteins of retrotransposons [14]. Two lines of evidence currently point towards the involvement of *DLK1* in muscle development. First, the dlk protein (encoded by *DLK1*) belongs to a family of signaling molecules involved in the process of differentiation during development in multiple cell lineages, and some of these family members have been shown to inhibit myoblast differentiation *in vivo* [17, 23]. Second, relatives of *dlk1* have been implicated in anterior-posterior axis formation during development (reviewed in [48]), and *DLK1* expression is more pronounced in muscles of the hindquarters as compared to shoulder muscles [6]. Studies are ongoing to determine the involvement of *DLK1* and/or *PEG11* in producing the callipyge muscle hypertrophy phenotype.

2.2. The *rib-eye muscling* (*REM*) locus

Interestingly, another locus that affects the longissimus muscle in sheep has been localized to the distal end of OAR18 near *CLPG* [47]. In the late 1980's, Australian Poll Dorset rams possessing unusually large rib-eye (*longissimus dorsi*) areas were identified at the Carwell Stud, New South Wales, Australia [2]. Accordingly, the locus that is responsible for this hypermuscling phenotype is commonly known as "*Carwell,*" although it has been provisionally designated as the *rib-eye muscling (REM)* locus (http://www.thearkdb.org, as consulted on 6 July 2004).

In contrast to *CLPG*, the effect of *Carwell* is limited to the longissimus muscle, with no effect on fat depth, live weight, or hindquarter weight [40]. *Carwell* increases the rib-eye area and weight by approximately 11% and 7%, respectively [41], which translates into a 15% boost in yield for higher-priced cuts [47]. The *Carwell* allele does not affect meat tenderness, does not alter intra-muscular fat deposition, and acts as a completely dominant mutation,

with no parent-of-origin effects on expression [29]. However, *Carwell* appears to exert sire-dependent effects in the progeny, which are presumably caused by epistatic interactions with an unknown modifier locus [47].

The *REM* locus has been mapped to the telomeric side of microsatellite marker *CSSM18* [47], near the *CLPG* map position, but its precise position remains poorly defined. Interestingly, a QTL for muscle growth that corresponds to the position of the *REM* locus on OAR18 was recently reported in British Texel sheep, suggesting that the *Carwell* allele is also segregating in the UK flock [61–63]. Efforts are currently underway to fine-map the region and to identify the causative mutation for the Carwell phenotype [42, 61]. Further characterization of Carwell will allow researchers to examine its relationship, if any, to callipyge.

2.3. QTL on OAR2 for Texel double muscling

Some animals of the Texel breed [64] are characterized by a generalized muscular hyper-development that is reminiscent of the double muscling phenotype in cattle; thus, this Texel hyper-muscling phenotype is commonly known as "Texel double muscling." Studies on Texel sheep in Belgium [12], Australia [39], and New Zealand [8] have indicated the presence of major genes for increased muscling segregating in these flocks. On a histological level, muscles from Belgian Texel double-muscled animals have larger fiber diameters with higher frequencies of type-II fibers [12], indicating that Texel hyper-muscling is due to myocyte hypertrophy. Texels are utilized extensively as a terminal sire breed because of their exceptional conformation and potential to produce higher-yielding carcasses with increased lean and decreased fat content [34].

Mutations in the *myostatin (MSTN)* gene are known to be responsible for double muscling in cattle (for review, see [1]). Because of the phenotypic similarity between cattle double muscling and Texel double muscling, studies have primarily focused on the possible involvement of *MSTN* in the generalized muscular hypertrophy that is characteristic of the Texel sheep breed. The entire coding sequence of the ovine *MSTN* gene has been determined for both double-muscled Belgian Texel animals and normally muscled Romanov controls, but no sequence differences have been found [36]. However, the involvement of *MSTN* in Texel hyper-muscling cannot be ruled out because a

functional polymorphism could reside outside of the coding segments of the *MSTN* gene.

Preliminary results of a whole-genome scan to identify QTL underlying Belgian Texel double muscling showed a major effect of a portion of OAR2 that includes *MSTN* on muscular development [36–38]. In a separate study, New Zealand scientists reported that the chromosomal region spanning the ovine *MSTN* locus influenced muscling and fat depth in four of 12 New Zealand Texel sires tested [8]. In addition, UK workers have recently defined two QTL on OAR2, one (at approximately 60 cM) that affects muscle growth and another (at about 170 cM) that influences fat growth in UK Texel sheep [62]. The QTL for fat growth on OAR2 corresponds to the region containing *MSTN*. The involvement of *MSTN* in fat deposition is plausible given the reduction in adipose tissue observed in *myostatin*-deficient mice [35, 43]. However, despite these reports of association of the *MSTN* locus with the Texel double muscling phenotype, the causative mutation that influences muscle and fat growth has yet to be identified, and it may lie outside the coding segments of the *MSTN* gene or in a closely linked gene.

2.4. Other QTL for carcass composition in sheep

The UK Sheep Genome Mapping Project (http://www.projects.roslin.ac.uk/sheepmap, as consulted on 6 July 2004) was initiated by the Roslin Institute (Edinburgh, Scotland) to detect QTL and identify genes that underlie growth and carcass composition in sheep and to utilize those findings for the benefit of the UK sheep industry. As part of this project, a candidate region approach was undertaken to detect QTL segregating in the UK Texel and Suffolk populations [62]. In addition to the significant effects described above on OAR2 and OAR18 for Texel double muscling and Carwell, respectively, several other chromosomal regions were identified with significant effects on muscling or fat (Tab. I). While the effects of OAR2 and OAR18 were supported across multiple sires, the other effects were only detected in individual sires. These effects included QTL on OAR1 (near the *transferrin* gene) and OAR3 (spanning the *IGF1* locus) for muscle and fat growth, respectively, in the Suffolk breed. QTL on OAR4 (spanning the *leptin* gene) and OAR20 (encompassing the *MHC* locus) were detected for fat growth in the Texel breed. Further characterization of these effects is ongoing [62].

3. CONCLUSIONS

In summary, nine genes or QTL have been identified as being associated with carcass traits in sheep (Tab. I). The only causative genetic mutation with a large effect on carcass composition that has been characterized to date is the callipyge (*CLPG*) mutation. Despite the detection of other QTL affecting muscle growth in sheep (namely, Carwell and Texel double muscling), the underlying genes responsible for these phenotypes have not been identified. Further characterization of mutations responsible for muscle hypertrophy in sheep will not only allow producers to incorporate gene-assisted selection (GAS) into their breeding programs, but it will also contribute to our basic understanding of muscle formation.

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