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Salahaddin University-Erbil

Characterization of Polar Over Dominance Contributing to the Callipyge Phenotype in Sheep

Research Project

Submitted to the department of animal resources in partial fulfillment of the requirement for the degree of BSc in Animal Resources

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CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the **BSc.** degree in **Agricultural Engineering Sciences – Animal resources** with my approval as a supervisor.

Signature
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Date:

DEDICATION

"Keep your dreams alive. Understand to achieve anything requires faith and belief in yourself, vision, hard work, determination, and dedication. Remember all things are possible for those who believe"

This effort I dedicate to **Allah** Almighty, my lord, my powerful foundation, my source of inspiration, wisdom, knowledge, and understanding. Throughout this project, he was the source of my energy.

Asmaa

ACKNOWLEDGMENTS

To begin with, I thank (Allah) for His blessing, which made me able to complete and perform this study with success, the lord of the universe, blessing, and peace be on Muhammad (Allah's peace and prayers be upon him).

I want to say thanks to my supervisor Mr. Kamaran M Taha for helping me in writing this review article.

Finally, I want to say thanks to all those I forgot them here to mention his/her name, which assisted me even by one useful scientific word directly or indirectly.

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ABSTRACT

A small genetic region near the telomere of ovine chromosome 18 was shown to carry the mutation causing the callipyge muscle hypertrophy phenotype in sheep. Expression of this phenotype is the only known case in mammals of paternal polar over dominance gene action, which appears in the offspring only when the mutated allele comes from the father and the wild allele comes from the mother. A region surrounding two positional candidate genes was sequenced in animals of known genotype. Mutation detection focused on an inbred ram of callipyge phenotype postulated to have inherited chromosome segments identical by descent with exception of the mutated position. Polar over dominance is closely related to parental imprinting a phenomenon where only one (maternal or paternal) allele is expressed in the offspring. These would quickly attract more attention among the scientific community than its potential economic value, especially since the quality of callipyge meat appeared to be mediocre. The objective of this review is to summarize some important information on polar over dominance phenomenon on CLPG gene responsible for the muscular hypertrophy phenotype in sheep.

1. INTRODUCTION

Significant improvement in sheep meat production has been made by using selective breeding and improved animal husbandry. DNA marker-assisted breeding strategies in sheep are now positioned to markedly accelerate the rates of genetic gain for desirable production traits, especially those that are difficult to measure, costly, and only expressed late in life (Tellam *et al*., 2015). The callipyge phenotype (GK *calli*- beautiful + *-pyge* Buttocks) is a generalized muscular hypertrophy described in sheep. It is due to an increase in the size and proportion of fast twitch muscle fibers. It manifests itself only after birth at 1 month of age (Mortimer *et al.,* 2010). It exhibits a rostro-caudal gradient being more pronounced in the muscle of the pelvic limb and torso. It is accompanied by a decrease in all measures of fatness. Affected animals are characterized by an improved feed efficiency and dressing percentage. Quite logically, the callipyge phenotype initially caught the attention of animal breeders because of its potential agronomic value. However, would reveal some remarkable features of the callipyge phenotype, especially its non Mendelian mode of inheritance (Lutz, 2014). These would quickly attract more attention among the scientific community than its potential economic value, especially since the quality of callipyge meat appeared to be mediocre! (Georges *et al.* 2004).

The callipyge mutation in sheep has provided remarkable new insights into genetics, regulation of gene expression and biology. The mutation causes skeletal muscle hypertrophy, but only in paternal heterozygous animals (N^{mat} C^{pat}; N, is the wild type allele and C is the allele carrying the mutation); a characteristic termed polar over-

dominance (Georges *et al.,* 2004), which is closely related to parental imprinting (a phenomenon where only one) maternal or paternal allele is expressed in the offspring (Oczkowicz, 2009). The maternal heterozygote (C^{mat} N^{pat}) and homozygote (C^{mat} C^{pat}) animals show no muscling phenotypes. Specific skeletal muscles in the N^{mat} C^{pat} genotype are increased in size by as much as 35%, although increased meat toughness has prevented commercial exploitation of these animals (Xu, 2017). Live weight is unaffected and carcasses are more compact with a shorter length and greater width at the shoulder and rump not all major muscles are affected by the callipyge mutation (Mortimer *et al.,* 2010). The muscling phenotype is first expressed 1–3 months post-birth and this occurs along a rostro-caudal gradient in the N^{mat} C^{pat} animal, with greatest impact on skeletal muscles innervated through lumbar and sacral roots, e.g., longissimus Dorsi and semimembranosus muscles (Tellam *et al.,* 2015).

The objective of this review is to summarize some information about CLPG responsible for the muscular hypertrophy phenotype in sheep.

2. REVIEW OF LITERATURES

2.1.The Heritability of Muscling Traits in Production Sheep

Sheep muscling traits typically have moderate heritability. Various Merino and Border Leicester crosses have been examined for a variety of carcass and muscling traits (Mortimer *et al.,* 2010). The ranges of heritability for muscle weight, meat yield, and carcass muscle dimensions were 0.22–0.35, 0.24–0.35, and 0.25– 0.34, respectively. For United Kingdom Charollais, heritabilities for muscle depth and muscle depth corrected for live weight were 0.25 and 0.31, respectively, while heritability for muscle depth in Texel, Suffock, and Charollais sheep ranged between 0.38 and 0.54 (Matika *et al*., 2010). A number of QTL affecting muscling traits in various sheep populations have been discovered. Some of the underlying QTL have been identified, especially genetic variants with relatively large effect sizes, e.g., Callipyge mutations. Other QTL, usually of low to moderate effect sizes, have been confirmed in independent sheep populations but only localized to broad chromosomal regions containing many genes (Tellam *et al.,* 2015).

2.2. Imprinted Genes Surrounding the Callipyge Mutation

The causative point mutation (A/G) responsible for the Callipyge muscling phenotype has been located in a 12 bp conserved motif positioned near the telomeric end of OAR 18 between the protein encoding gene DLK1 (delta-like1) and the non-protein encoding gene GTL2 (gene trap locus 2; or MEG3). The ram in which the mutation first arose was a germ line mosaic for the mutation (Alan *et al*., 2015).

The complex interplay between different epigenetic and genetic mechanisms in regulating mammalian imprinted gene expression is aptly illustrated by the callipyge phenotype in sheep, which is responsible for a 30% increase in skeletal muscle (most notably at the hind quarters), a corresponding 8% reduction in fat content and improved feed efficiency (Figure. 1) (Lutz, 2014).

This phenotype is observed only in heterozygous individuals that carry the causative mutation on the paternal chromosome (i.e., mat+/ patC, where 'mat' and 'pat' denote maternal and paternal chromosomes, respectively and superscript'+' and 'C' represent wild-type and callipyge alleles, respectively) a mode of non-Mendelian inheritance termed 'polar over dominance', Polar over-dominance differs from regular over-dominance (also known as heterozygote advantage) where both heterozygote genotypes display a phenotype that has increased fitness regardless of the parent of origin (Alan *et al*., 2015).

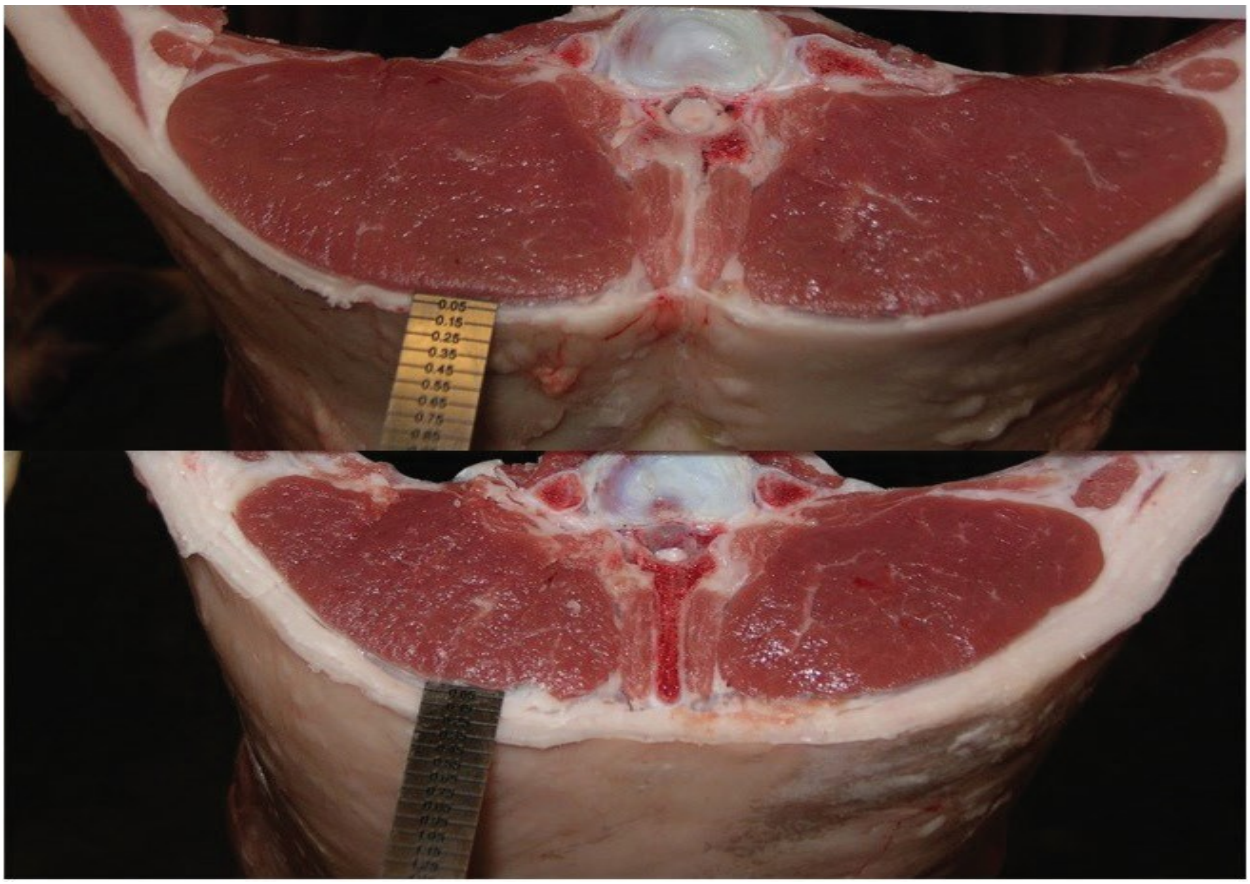

Figure 1. Rib cuts in callipyge lambs.Muscle hypertrophy of the loin and decreased fat in callipyge lambs are shown in two lamb carcasses at 150 days old. The callipyge lamb rib cut is shown on the top, and the normal lamb rib cut is shown on the bottom (Lutz, 2014).

The callipyge phenotype is caused by an A-to-G single nucleotide polymorphism (SNP; i.e., the callipyge mutation) located between the paternally expressed/maternally imprinted DLK1 protein-coding gene and the maternally expressed/paternally imprinted MEG3 long non-coding RNA(ncRNA) gene within the imprinted DLK1- DIO3 gene cluster on ovine chromosome 18 (Figure. 2) (Alan *et al*., 2015). This domain contains the genes whose expression is perturbed upon inheritance of the callipyge mutation (CLPG; an A-to-G SNP).The genes shaded in black represent the expressed imprinted alleles within this domain while white shading indicates the silenced/attenuated

imprinted allele on either the maternal(MAT) or paternal (PAT) chromosomes. The arrowhead denotes the direction of transcription of each gene (Xu, 2017). The core imprinted genes that have been shown to play a role in the callipyge phenotype occur within a 340 kb region. The expression of the core genes for each of the four possible callipyge genotype sat the CLPG SNP and the observed is summarized in the accompanying Table 1, (Bidwell *et al* 2014). The relative RNA transcript abundance for the paternally (DLK1, PEG11) and maternally (PEG11AS, MEG3, MEG8, and MIRG) expressed genes are shown for each callipyge genotype (Xu, 2017). Callipyge animals (mat+/patC) exhibit over expression of DLK1 and PEG11 and an absence of MEG3 and MEG8 over expression suggesting that DLK1 and/or PEG11 encodes the primary effect or of the callipyge phenotype. Overexpression of the maternal non-coding RNA genes and the absence of muscle hypertrophy in matC/patC animals suggest that these transcripts exert their effect via post-transcriptional suppression of the effect or the micro RNAs encoded by MIRG have been postulated to also play a role in post-transcriptional suppression of the paternally expressed effector (Alan *et al*., 2015).

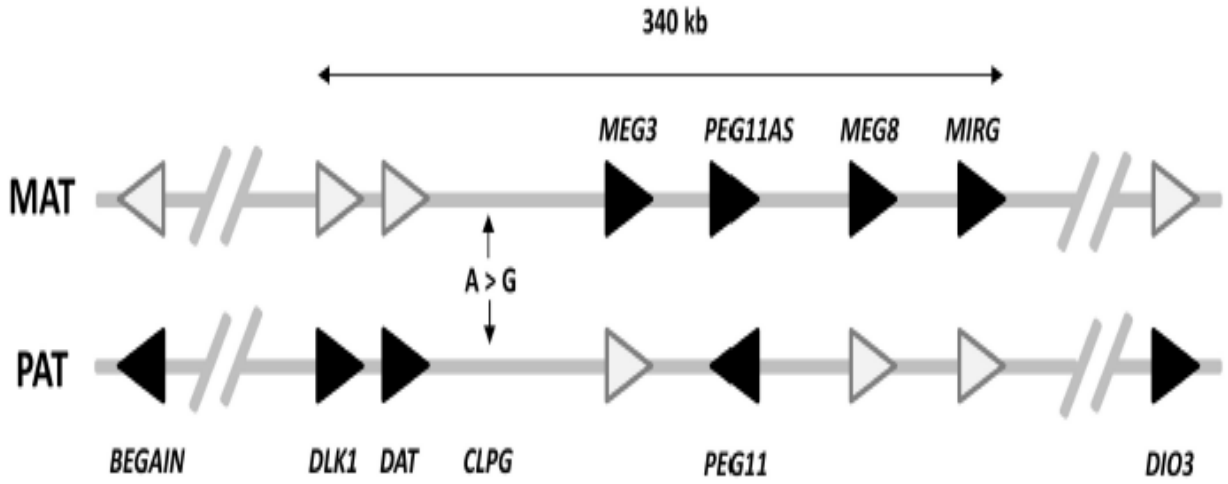

Figure 2. The DLK1-DIO3 imprinting domain on ovine chromosome 18 (Alan *et al*., 2015).

Table 1. The expression of the core genes for each of the four possible callipyge genotype

Genotype	SNP genotype	*DLK1* (PAT)	*PEG11* (PAT)	*PEG11AS* (MAT)	*MEG3* (MAT)	*MEG8* (MAT)	Phenotype
mat^{+}/pat^{+}	mat^{A}/pat^{A}	+	+	+	+	+	Wild-type
mat^{C}/pat^{+}	mat^{G}/pat^{A}	+	+	+	+++	++	Wild-type
mat^{C}/pat^{C}	mat^{G}/pat^{G}	++	++	+	++	++	Wild-type
mat^{+}/pat^{C}	mat^{A}/pat^{G}	+++	+++	+	+	+	Callipyge

(Alan *et al*., 2015).

2.3. From the Phenotype to the Genotype

At the beginning of the 1990s, a long and fruitful collaboration began. Michel Georges, who was in Genmark in the US at that time, was approached by Dr. Noelle Cockett of Utah State University who enlisted his help in finding the gene responsible for the callipyge phenotype. Since then, Michel Georges has returned to Belgium but the collaboration has continued to this day (Lutz, 2014). The reason for this collaboration: identifying the genetic mechanisms behind the callipyge phenomenon is no mean feat. First of all, the mutation which is responsible is discretely located in a large non-coding section of the genome. This mutation is called CLPG and was independently highlighted in 2002 by an American team as well as Michel George's team. But, in addition to the difficulty of identifying this mutation, the callipyge phenomenon has a very singular particularity: its unique mode of heredity transmission. This is what makes it so interesting in the eyes of geneticists (Bidwell *et al.,* 2014).

In order to properly understand this particularity, we first need to return to the common way genetic transmission takes place, known as the Mendelian laws of inheritance (Lutz, 2014). We can greatly simplify Mendel's laws as follows: each individual possesses two versions or alleles of each gene: the maternal version and the paternal version. According to whether or not the parents are carriers of a gene mutation, four possible types of scenarios exist for their offspring. Imagine a gene "A" which we will call "a" in its mutated version. Four combinations (or genotypes) are possible: "AA", "Aa", "aA" or "aa". In "Aa" individuals (Figure. 3), the mutated allele "a" is

transmitted by the mother; in "aA" individuals, the mutated allele has been transmitted by the father. The mutation is therefore absent in individuals who are carriers of the "AA" genotype and present in the three other cases. If the mutation 'a' is dominant, the individuals of the "Aa", "aA" and "AA" genotypes will express the associated phenotype, while If the mutation "a" is recessive, only the "aa" individuals will express the phenotype (Xu, 2017).

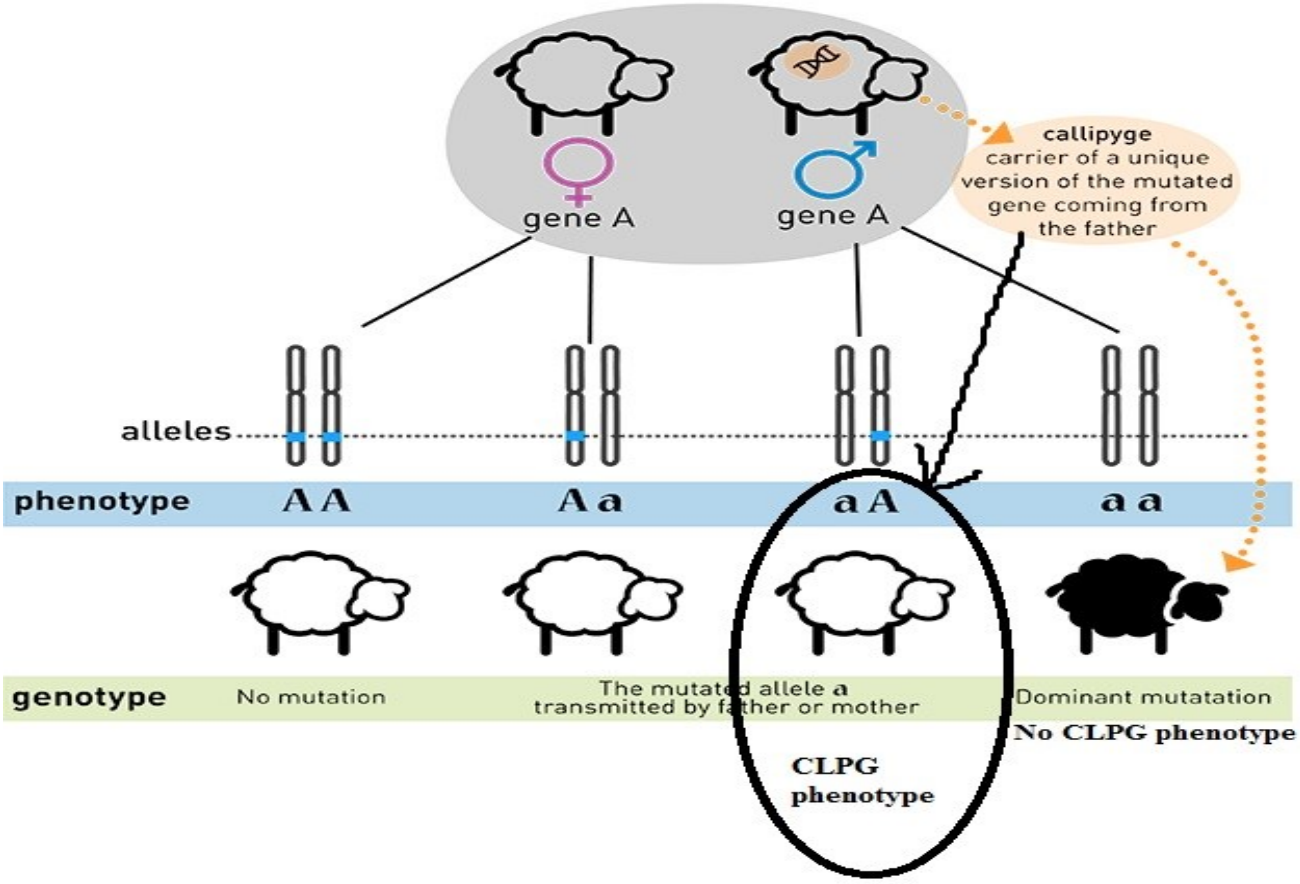

Figure 3. Four combinations (or genotypes) are possible (Xu, 2017).

CONCLUSION

The study of the callipyge phenomenon has provided some unique opportunities to probe the novel epigenetic mechanisms that underlie its unusual mode of inheritance polar over dominance. Expression studies revealed that DLK1 gene is probably the primary effector and that RNA interference plays a key role in developing the callipyge phenotype. New genes that may contribute to this unusual phenotype in a response to changes in DLK1 expression have been identified and need further studies.

REFERENCES

Alan M. O'Doherty, David E. MacHugh, Charles Spillane and David A. M., 2015. Genomic imprinting effects on complex traits in domesticated animal species. *Frontiers in genetics*, 6:156.

Bidwell, C.A., Waddell, J.N., Taxis, T.M., Yu, H., Tellam, R.L., Neary, M.K., 2014. New insights into polar over dominance in callipyge sheep. Animal Genetics. 45(Suppl.1):51–61.

Georges, M., Charlier, C., Smit, M., Davis, E., Shay, T., Tordoir, X., Takeda, H., Caiment, F. and Cockett, N., 2004, January. Toward molecular understanding of polar overdominance at the ovine callipyge locus. In *Cold Spring Harbor Symposia on Quantitative Biology*, 69: 477-484.

Lutz, K. 2014. Determining the role of *DNTTIP1*: Piecing together the callipyge sheep muscle hypertrophy pathway. *Journal of Purdue Undergraduate Research, 4:*16–21.

Mortimer, S.I., Van der Werf, J.H.J., Jacob, R.H., Pethick, D.W., Pearce, K.L., Warner, R.D., Geesink, G.H., Edwards, J.H., Gardner, G.E., Ponnampalam, E.N. and Kitessa, S.M. 2010. Preliminary estimates of genetic parameters for carcass and meat quality traits in Australian sheep. *Animal Production Science*, *50*(12):1135-1144.

Matika, O., Sechi, S., Pong-Wong, R., Houston, R.D., Clop, A., Woolliams, J. A., and Bishop, S. C. 2010. Characterization of OAR 1 and OAR 18 QTL associated with muscle depth in British commercial terminalsire sheep. *Animal Genetics,* 42:172–180.

Oczkowicz, M. 2009. Polar overdominance–a putative molecular mechanism and the new examples in mammals. *Journal of Animal and Feed Sciences*, *18*(1):17-27.

Tellam, R.L., Cockett, N.E., Vuocolo, T. and Bidwell, C. A. 2015. Genes contributing to genetic variation of muscling in sheep. *Frontiers in genetics*, *3*:164.

Xu X, Ectors F, Davis EE, Pirottin D, Cheng H, Farnir F. 2017. Ectopic Expression of Retrotransposon-Derived PEG11/RTL1 Contributes to the Callipyge Muscular Hypertrophy. *PLoS ONE,* 10(10): 0140-0594.