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Polar overdominance – a putative molecular mechanism and the new examples in mammals*

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ABSTRACT

Polar overdominance is closely related to parental imprinting - a phenomenon where only one (maternal or paternal) allele is expressed in the offspring. In contrast to gametic imprinting, polar overdominance is not very well known and only few examples of this phenomenon have been reported in mammals. One of them is the callipyge phenotype in sheep, which appears in the offspring only when the mutated allele comes from the father and the wild allele comes from the mother. In review, latest concepts on the molecular mechanism underlying the callipyge phenotype are presented and the new examples in mammals are discussed.

KEY WORDS: imprinting, polar overdominance, *DLK1*, *MEG3, PEG11,* callipyge

INTRODUCTION

Polar overdominance is closely related to parental imprinting - a phenomenon where only one – maternal or paternal allele is expressed in the offspring. Gametic imprinting was first discovered in the murine *Igf2* gene (De Chiara et al., 1991) and since then has been studied intensively. To date approximately 80 imprinted genes are known (Moore et al., 2008) in various mammals. One of the most significant studies connected with gametic imprinting was performed by Kono et al. (2006), who used mutant mice with a 13-kilobase deletion in the *H19* gene as non-growing oocyte donors and generated a viable parthenogenetic mouse by ap-

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propriate expression of the *Igf2* and *H19* genes. This experiment has proved that gametic imprinting was a barrier to developing a parthenote. Another important finding was the localization of a causative mutation in the *IGF2* gene, affecting muscularity of pigs (Van Leare et al., 2003). This mutation can be effectively used in the pig industry to improve meatiness of pigs by marker assisted selection.

CALLIPYGE LOCUS – THE FIRST EXAMPLE OF POLAR OVERDOMI-NANCE IN MAMMALS

In contrast to gametic imprinting, polar overdominance is not very well known and only few examples of this phenomenon have been reported in mammals. It was first noticed in a ram called Solid Gold. This sheep was famous for extreme muscularity, especially pronounced in the hindquarters. Surprisingly, Solid Gold passed its unique features only to approximately 15% of its offspring. This suggested that the callipyge phenotype was inherited in a non-Mendelian manner. After a series of crossings it turned out that the callipyge phenotype appears in the offspring only when the mutated allele comes from the father and the wild allele comes from the mother (C^{pat}/N^{mat} genotype) (Cockett et al., 1996). Animals with other genotypes (C^{pat}/C^{mat}, N^{pat}/N^{mat}, N^{pat}/C^{mat}) did not exhibit the characteristic phenotype.

The *CLPG* locus has been localized in the telomeric region of chromosome 18, within a cluster of imprinted genes. After sequencing, a causative mutation has been described (Freking et al., 2002). Single Nucleotide Polymorphism (SNP^{CLPG}) located in an intergenic region, between *DLK1* and *MEG3,* was unique to Solid Gold and its offspring. Since then, substantial efforts have been undertaken to explain the molecular mechanism by which SNP^{CLPG} causes the callipyge phenotype. The causality of SNP^{CLPG} was further supported by the demonstration that Solid Gold was mosaic for this SNP (Smit et al., 2003).

PUTATIVE MOLECULAR MECHANISM UNDERLYING POLAR OVER-DOMI-NANCE IN THE CALLIPYGE LOCUS IN SHEEP.

The region adjacent to SNP^{CLPG} is expressed as a 547-bp RNA transcript containing an open reading frame (ORF) predicting 123 amino acids in which SNP^{CLPG} would alter a serine codon to a proline, but this ORF is not conserved in humans and mice and the likelihood that it produces the corresponding peptide is unknown (Freking et al., 2002). It was supposed that SNP^{CLPG} somehow alters expression of adjacent genes. There are several imprinted genes in the neighborhood of SNP^{CLPG} and all of them could take a part in developing the callipyge phenotype. To date,

paternally expressed *DLK1* (Drosophila Like Homologue 1) is considered as the main candidate gene. *DLK1* codes for signal protein, which regulates differentiation of various cell lines, muscle and adipose tissue. Its expression decreases during differentiation of adipose tissue and thus it is supposed to keep preadipocytes in undifferentiated state.

In this region, there are also three maternally expressed genes which do not code for proteins (*MEG3/GTL2, antiPEG11* and *MEG8*) and paternally expressed *PEG11/Rtl1,* which has an open reading frame and hypothetically codes for proteins, but this has not been identified so far (Cockett et al., 2005).

The first possible explanation of the molecular mechanism underlying polar overdominance at the callipyge locus emerged after examination of the expression of paternally expressed *DLK1, PEG11,* and maternally expressed *MEG3* and *MEG8* genes in the four possible SNP^{CLPG} genotypes (Charlier et al., 2001a). *DLK1* and *PEG11* transcripts were very abundant in skeletal muscle of individuals that have the *CLPG* mutation on their paternal chromosome (genotypes C^{mat}/C^{pat}, N^{mat}/C^{pat}), whereas in skeletal muscle of N^{mat}/N^{pat}, C^{mat}/N^{pat} individuals, they were present at very low concentration or absent. Analogous results were obtained for *MEG3* and *MEG8.* Expression of these genes was higher in skeletal muscles of individuals that have the *CLPG* mutation on their maternal chromosome. Moreover, *CLPG* mutation did not alter imprinting status of analysed genes. The authors concluded that *CLPG* mutation enhanced expression level of *DLK1, MEG3, PEG11* and *MEG8* genes in *cis,* probably by modifying the activity of a common regulatory element. *CLPG* phenotype results from a *DLK1* and/or *PEG11* overexpression in skeletal muscle with simultaneous underexpression of *MEG3* and *MEG8.* The model explaining why callipyge homozygotes do not exhibit the callipyge phenotype was proposed: in C^{mat}/C^{pat} individuals overexpression of *MEG8* and *MEG3* or other maternally expressed genes interfere in trans with *DLK1* and *PEG11* and inhibit expression of *DLK1* and *PEG11* (Charlier et al., 2001a; Georges et al., 2003; Figure 1). The authors proposed that this interference could operate at the transcriptional level (competition for transcription factors), on the stability of the *DLK1/PEG11* mRNAs or even at the protein level. However, new data indicate microRNA interference as a very probable factor (Seitz et al., 2004a; Royo et al., 2008).

FUNCTIONAL STUDIES OF GENES LOCATED IN THE CALLIPYGE REGION

Similarly as in sheep, in the human and mouse genome a cluster of imprinted genes including *DLK1, MEG3/GTL2, PEG11/RTL1, antiPEG11* and *MEG8* is present. Human *DLK1* has been mapped to chromosome 14q32 that is syntenic to mouse distal chromosome 12 and sheep chromosome 18 (Gubina et al., 1999).

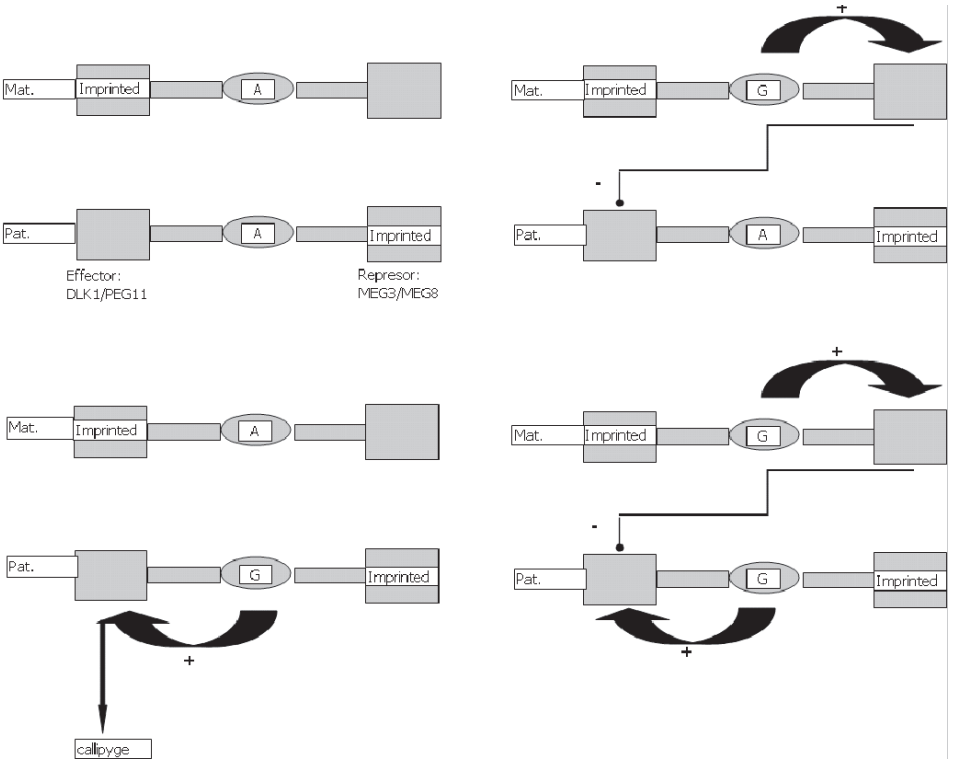

Figure 1. A model for the callipyge polar overdominance according to Georges et al. (2003); description in the text

Moreover, it was proved that in humans and mice, *DLK1* is paternally expressed whereas *MEG3* is maternally expressed (Kobayashi et al., 2000; Wylie et al., 2000). Studies on mouse uniparental disomy for chromosome 12 (UPD12) revealed that both types of UPD (maternal and paternal) result in embryos that are non-viable. Paternal UPD12 conceptuses died in late gestation and exhibited placentomegaly, had costal cartilage defects and hypo-ossification of mesoderm-derived bones. On the other hand, mUPD12 conceptuses survive to term, but die perinatally. Maternal UPD12 conceptuses are growth retarded and both types of UPD12 exhibit skeletal muscle maturation defects (Georgiades et al., 2000). In humans, maternal UPD14 results in scoliosis, hypotonicity, early puberty, obesity and blepharophimosis.

In order to determine the role of *Dlk1* in mouse mUPD12 and human mUPD 14 phenotype, *Dlk1*-null mice were generated. *Dlk1*-null mice displayed growth retardation, eyelid and skeletal abnormalities, hypercholesterolaemia, hyperlipidaemia, enlarged fatty liver and accelerated obesity. Results of the experiment

indicated that *Dlk1* is the gene responsible for most of the imprinting-related phenotypes of mouse mUPD12 and syntenic human mUPD14; however, some of the phenotypes observed in human mUPD14 like hypotonicity and early puberty were not apparent in *Dlk1*-null mice and may be caused by misexpression of another imprinted gene, possibly *Peg11*. In addition, a role of *Dlk1* gene in maintaining homeostasis of lipid metabolism was demonstrated as *Dlk1*-null mice exhibited hypercholesterolaemia and hyperlipidaemia. Furthermore, to determine if heterozygotes with either maternal or paternal inheritance of *Dlk1* knockout allele ($m^-/+$ or $+/p^-$) have phenotypes similar to those of wild-type mice or null mice respectively, male or female heterozygotes (+/-) were mated with wild-type mice. Western blot analysis demonstrated that *Dlk1* protein was expressed exclusively from the paternal allele. Moreover, ($+/p^-$) animals exhibited the same growth retardation and increase in fat pad weight as *Dlk1*-null mice. In contrast, ($m^-/p+$) have the same growth rate and fat pad weight as normal littermates (Moon et al., 2002).

EFFECTS OF CLPG GENOTYPE ON EXPRESSION OF ADJACENT GENES

Since the origin of the model explaining callipyge phenotype (Figure 1), many studies concerning expression of genes within the callipyge cluster have been conducted. Generally, results of these studies are in agreement with the proposed model and indicate new possible factors contributing to the callipyge phenotype.

Bidwell et al. (2004) observed a significant effect of the callipyge genotype on *DLK1* expression in muscles that undergo hyperthrophy (*Gluteus medius*) in 8-week-old lambs. Expression level of this gene was 6-fold higher than in normal animals. In muscle that does not undergo hypertrophy (*Supraspinatus*) *DLK1* transcript abundance was the same in four analysed genotypes. Moreover, Murphy (2005) reported that *DLK1* expression was elevated prenatally, but in adult animals expression decreased threefold. Strikingly, in N^{mat}/C^{pat} animals elevated levels of *DLK1* transcript were maintained. This observation was limited only to hypertrophy responsive muscles. These results were confirmed by Perkins et al. (2006), who observed 16-fold and 5-fold higher expression of *DLK1* in N^{mat}/C^{pat} vs N^{mat}/N^{pat} in 2- and 8-week-old lambs, respectively, in hypertrothy responsive muscle. Similarly to previous results, no differences were observed in *Supraspinatus.*

Contribution of *DLK1* gene to developing the callipyge phenotype was analysed at the protein level as well. Davis et al. (2004) showed by immunohistochemistry that *DLK1* protein is present only in *Longissimus dorsi* of N^{mat}/C^{pat} animals. In muscles of C^{mat}/C^{pat} animals there was no *DLK1* protein despite the presence of *DLK1* mRNA. However, White et al. (2008) detected *DLK1* protein, albeit at a lower level in the C^{mat}/C^{pat} and N^{mat}/C^{pat} animals. The authors suggested that dif-

ferences reflect the sensitivities of the methods used. Nevertheless, observations greatly supported the hypothesis about an important role of *DLK1* in developing the callipyge phenotype.

Contribution of *PEG11* to developing the callipyge phenotype is more elusive. The *PEG11* gene contains an intronless open reading frame of 1333 amino acids (Charlier et al., 2001b); however, to date the *PEG11* protein has not been identified. *PEG11* mRNA abundance in hypertrophy responsive muscle (*Semimembranosus)* was 33-fold higher in N^{mat}/C^{pat}, compared with other genotypes at 2 weeks of age. Moreover, in another hypertrophy responsive muscle - *Gluteus medius* of N^{mat}/C^{pat} animals expression of *PEG11* was 14-fold higher than expression of *antiPEG11*-transcript transcribed from the plus strand (Charlier et al., 2001b), whereas in the animals of the other three genotypes the situation was just the opposite - *antiPEG11* transcripts were expressed at higher levels than *PEG11* (Bidwell et al., 2004). Perkins et al. (2006) demonstrated that *PEG11* expression was elevated in N^{mat}/C^{pat} 2-week-old lambs in hypertrophy responsive and non-hypertrophy responsive muscles. Compared to prenatal time point *PEG11* expression increased in both muscles by seven- to eight-fold in callipyge animals by 2 weeks of age, but declined in the other three genotypes. Several miRNAs processed from *antiPEG11* have been detected (Davis et al., 2005). The authors demonstrated also the *in vivo* trans-inhibition of the paternally expressed *PEG11* by microRNA processed from maternally expressed *antiPEG11*.

Expression of maternally expressed genes (*MEG3* and *MEG8)* was studied by Murphy et al. (2005) and Perkins et al. (2006). In C^{mat}/N^{pat} and C^{mat}/C^{pat} animals they observed higher expression of *MEG3* and *MEG8* genes than in N^{mat}/N^{pat}, N^{mat}/C^{pat} sheep, but the observations were not restricted to hypertrophy responsive muscles. In humans and mice, microRNA processed from *MEG8* has recently been identified (Cavaille et al., 2002; Seitz et al., 2004b). However, further studies are needed to understand the molecular mechanism by which maternally expressed genes affect the callipyge phenotype.

NEW POSSIBLE MOLECULAR FACTORS CONTRIBUTING TO CALLIPYGE PHENOTYPE

Another factor changing the expression of *DLK1* and *PEG11* was suggested by Murphy et al. (2006), who examined methylation status of the callipyge region and expression of *CLPG1* transcript - noncoding RNA produced from the region covering SNP^{CLPG}, described by Freking et al. (2002). Prenatally, *CLPG1* transcript was expressed biallelically in *Longissimus dorsi* of sheep of all four genotypes, whereas after birth this expression was maintained only in animals carrying the mutation (C^{mat}/C^{pat}, N^{mat}/C^{pat}). Additionally, presence of SNP^{CLPG} changed local

C^{mat}/N^{pat} CpG methylation in adult animals. In callipyge sheep, there was CpG hypomethylation of this region compared with normal sheep, presence of *CLPG1* transcript and high expression of *DLK1*. The authors suggested that SNP^{clpg} may alter the ability of regional chromatin to condense by inhibition of chromatin remodelling complex assembly, inhibition of DNA methylation or histone modification and this affects expression of *DLK1* and *PEG11*.

Studies on influence of SNP^{CLPG} on epigenetic marking were continued by Takeda et al. (2006), who demonstrated that the mutation alters methylation status of *DLK1-GTL2* intergenic region. Because SNP^{CLPG} is able to change expression of a core cluster of neighboring genes it is thought to be located within the locus control region (LCR). Previous reports have shown that such LCRs are DNase-I hypersensitive (Li et al., 2002), therefore the authors decided to check the presence and effect of the *CLPG* mutation on DNase I Hypersensitive Sites (DHS). They detected at least three DHSs in N/N animals and at least two additional DHSs in C/C animals. *CLPG* allele exhibited higher sensitivity to DNase I in skeletal muscle but not in the liver. Moreover, they proved that SNP^{CLPG} strongly enhances biallelic, long-range transcription throughout the *DLK1 - GTL2* IG region. They confirmed the results obtained by Murphy et al. (2006) and demonstrated that the effect of SNP^{CLPG} is not limited to its vicinity but extends to the entire 90-kb *DLK1-GTL2* IG region.

Although primary effectors of a muscle hypertrophy phenotype are known, several attempts to identify new genes that may play a role in the expression of the callipyge phenotype have been undertaken. Vuocolo et al. (2006) used microarrays (bovine GeneChip) and identified 159 differentially expressed genes in *Longissimus dorsi* of normal and callipyge sheep. Eight of them, including *DLK1* and *MEG3* were differentially expressed at birth and at 12 weeks of age. These were: *HDAC9* - histone deacetylase 9, activating transcription factor-3 (*ATF3*), Ras protein dexamethasone-induced1 (*RSAD 1*), leucine-rich repeat-containing protein 29 (*LRRC2*), *FOS* transcript mapping to 3` UTR of the maternally expressed *SLC22A3* gene. Several of them are transcription factors - positive or negative regulators of myogenesis. The authors proposed that overexpression of *DLK1* results in inhibiton of the activity of *Notch 1* - a known negative regulator of myogenesis and eventually alters expression of *FOS, ATF 3* and *HDAC 9* (Vuocolo et al., 2006).

A similar study was performed by Fleming-Wadell et al. (2007), who analysed gene expression in *Longissimus dorsi* of normal and callipyge sheep at 10, 20 and 30 days of age. After combining data from Affymetrix microarray and Real-Time PCR, they chose several differentially expressed genes (*PFKM, PDE7A, LOC513822, DNTTIP1*) to be the most likely candidates for direct involvement in *DLK1* and/or *PEG11*-mediated muscle growth.

NEW EXAMPLES OF POLAR OVERDOMINANCE IN MAMMALS

The considerable effect of SNP^{CLPG} on muscularity of sheep has made it a very interesting marker for marker assisted selection in other farm animals. However, SNP^{CLPG} appeared to be a private allele, encountered exclusively in the calipyge flock (Smit et al., 2003). Nevertheless, Kim et al. (2004) identified *DLK1* polymorphism which is associated with growth, fatness and body composition in pigs. In addition, the authors proved a polar overdominant inheritance of this polymorphism. These results were confirmed by QTL (Quantitative Trait Locus) analyses (Li et al., 2008). Additionally, paternal expression of *DLK1* and maternal expression of *MEG3* in porcine muscle and adipose tissues was shown (Li et al., 2008).

In humans a SNP located only 93bp from the polymorphism described by Kim (2004) in pigs has been identified (Wermter et al., 2008). It was shown that this polymorphism is associated with child and adolescent obesity. The study was performed on 1025 trio families and analysis of the allelic transmission pattern indicated the existence of polar overdominance in this locus. The biological function of this silent polymorphism is not known. Sequencing of human *DLK1-MEG3* region did not reveal additional polymorphism, so further molecular analyses are needed to identify the biological mechanism underlying polar overdominant effects of *DLK1* gene in pigs and humans.

Recently, Wolf et al. (2008) performed a genome-wide scan for imprinted QTL affecting growth in mice. The results suggested that imprinting patterns may be more diverse than previously assumed. Not only new patterns of imprinting (bipolar dominance and polar underdominance) but also changes in the pattern of imprinting during development have been described. Bipolar dominance is the expression pattern where the two heterozygotes differ from each other but the two homozygotes have similar phenotypes. In polar underdominance expression pattern is the same as in polar overdominance, but one of the heterozygotes exhibits lower intensity of the trait than the other three genotypes. In Wolf's study six loci were paternally expressed and only one maternally expressed. Additionally, three loci showed bipolar expression pattern, one - polar underdominance and three - polar overdominance. What is more, expression pattern of some loci shifted during development, for example the *Wti2.1* locus showed bipolar dominance early in the development and changed to maternal expression by week 7. The loci identified by the authors are probably novel imprinted genes as no currently known imprinted genes are located within the confidence intervals of appropriate chromosomes (Wolf et al., 2008).

CONCLUSIONS

Knowledge about the mechanism underlying polar overdominance is continuously increasing. 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. Finally, examination of new examples and patterns of expression in mammals may shed a new light on the molecular mechanism underlying this phenomenon.

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