ON THE CONTRIBUTION OF IMPRINTED LOCI TO VARIATION IN ANIMAL BODY COMPOSITION

M. Georges¹, F. Baraldi¹, N. Buys², C. Charlier¹, C. Colette¹, E. Davis¹, F. Di Silvestro¹, L. Moreau¹, C. Nezer¹, M. Nguyen¹, K. Segers¹, T. Shay³, M. Smit³ and N. Cockett³

¹Department of Genetics, Faculty of Veterinary Medicine, University of Liège (B43), 20 Bd de Colonster, 4000-Liège, Belgium
²SEGHERSgenetics, 15 Kapelbaan, 9255-Buggenhout, Belgium
³Department of Animal, Dairy and Veterinary Sciences, College of Agriculture, Utah State University, Logan, UT 84322-4700, USA

PARENTAL IMPRINTING OF GENES

Mendel’s first law implies the equivalence of reciprocal crosses, i.e. allelic effects are independent of their parental origin. Since the 1980’s, however, approximately forty genes have been identified that are subject to parental imprinting in eutherian mammals (e.g. Reik and Walter, 2001). Paternal and maternal alleles of imprinted genes are differentially methylated during their passage in the respective germ line resulting in their functional non-equivalence. Typically, one of the alleles – the paternal allele for approximately half the imprinted genes, the maternal allele for the other half – is transcriptionally silent in at least some tissues. Imprinted genes are typically clustered in chromosomal domains containing both paternally and maternally expressed genes that may be under coordinate control of common “imprinting centers” (ICs). Imprinted domains are characterized by a number of idiosyncrasies including the frequent occurrence of non-coding as well as anti-sense RNAs, a high incidence of CpG islands, the presence of tandem repeats, a paucity of SINE sequences, allelic replication asynchrony, and a higher male versus female recombination rate.

The evolutionary significance of imprinting is a matter of active debate. The most publicised hypothesis is the “parental tug-of-war” theory. Selection would favour (i) paternal expression of genes that extract resources from the mother to the benefit of the individual’s fitness but at the expense of future maternal half-sibs, and (ii) maternal expression of genes that tend to conserve resources to divide them among more offspring and to maximize reproductive performance of the female. This theory is supported by the observation that most imprinted genes affect preweaning growth in an antagonistic manner. It may explain why a number of genes influencing growth and body composition exhibit parent-of-origin effects in livestock. Disregulation of imprinted genes is known to be involved in a number of human diseases including Beckwith-Wiedemann, Prader-Willi and Angelman syndromes, as well as Wilms tumour. In addition, the observation of parent-of-origin effects in the inheritance of several disorders including diabetes, autism, bipolar affective disorder, epilepsy, schizophrenia, and Tourette and Turner syndromes supports a more important contribution of imprinted genes in human morbidity.
UNRAVELLING THE MOLECULAR BASIS OF POLAR OVERDOMINANCE AT THE OVINE CALLIPYGE LOCUS

The first evidence that imprinted genes might influence economically important traits in livestock came from the analysis of the callipyge muscular hypertrophy in sheep. This phenotype reflects a “late-onset” (approximately three weeks of age) increase in the proportion and diameter of fast twitch muscle fibers. It was shown to be inherited and under control of a single locus mapping to distal Oar18q (Cockett et al., 1994). It was later realized to be penetrant only in heterozygous individuals having inherited the CLPG mutation from their sire, a unique mode of inheritance referred to as polar overdominance (Cockett et al., 1996). It should be noted that polar overdominance causes a balanced type of polymorphism which precludes the fixation of the CLPG mutation by mass selection. Optimal use of the CLPG mutation could, however, be achieved by crossing wild-type CLPG/CLPG rams with homozygous +/+ ewes to yield 100% offspring expressing the callipyge phenotype.

The map position of the callipyge locus was recently refined to a 400 Kb chromosome segment (Shay et al., 2001; Berghmans et al., 2001). An ovine BAC contig spanning this interval was constructed and has been completely sequenced (Segers et al., 2001; Charlier et al., 2001a and unpublished data). Annotation of the central 250 Kb of sequence lead to the identification of four genes, including DLK1 and GTL2 that were known to be imprinted in man and mice, as well as two novel genes: PEG11 and MEG8 (Charlier et al., 2001a). These four genes were shown to be preferentially expressed in skeletal muscle, and to undergo imprinting in this tissue: DLK1 and PEG11 are expressed exclusively from the paternal allele, GTL2 and MEG8 from the maternal allele. DLK1 and PEG11 are protein encoding genes (an EGF-like homeotic protein and a reverse transcriptase-like protein, respectively), while GTL2 and MEG8 both produce non-coding RNAs. In addition, we found two associated imprinted transcripts referred to as DAT (paternally expressed DLK1 associated transcript) and anti-PEG11 (maternally expressed PEG11 antisense transcript).

By analysing the expression levels and imprinting status of these four genes in skeletal muscle of animals representing the four possible callipyge genotypes, we demonstrated that the CLPG mutation enhances the expression of all of these in cis without altering their imprinting status (Charlier et al., 2001b). Combined with the analysis of the temporal expression profile of GTL2 (Bidwell et al., 2001), these results suggest that the CLPG mutation inhibits a locus control region (LCR) with silencer activity that - in wild type individuals - becomes active in skeletal muscle after birth. Consequently, +/CLPG individuals have a unique expression profile - the overexpression of DLK1 and PEG11 in the absence of an overexpression of GTL2 and MEG8 - which may cause the observed muscular hypertrophy.

To identify the actual CLPG mutation, we have resequenced more than 180 Kb spanning the DLK1-GTL2-PEG11-MEG8 gene cluster from a CLPG allele as well as a phylogenetically closely related wild-type allele. A single A to G substitution was identified in an evolutionary footprint located 30.5 Kb upstream of GTL2. The possible role of this mutation in the determinism of the callipyge phenotype is being examined.

Work in progress directed towards unravelling the molecular basis of polar overdominance will be presented and includes: (i) in silico annotation and molecular analysis of the remainder of the 400 Kb interval in order to determine the boundaries of the imprinted domain as well as the extent of the effect of the CLPG mutation, (ii) the identification of possible chromosomal rearrangements associated with the CLPG mutation by dynamic molecular combing, (iii)
analysis of the role of DLK1 overexpression in the determinism of the callipyge phenotype via the production of transgenic mice that constitutively overexpress DLK1 in skeletal muscle, and
(iv) analysis of the epigenetic effects of the CLPG mutation including methylation status and chromatin configuration.

MOLECULAR DISSECTION OF AN IMPRINTED QTL INFLUENCING MUSCULARITY AND FAT DEPOSITION ON PROXIMAL SSC2

The second evidence in favour of a significant contribution of imprinted loci to the variation for animal body composition was the identification of an imprinted QTL with major effect on muscle mass and fat deposition on the centromeric end of porcine chromosome SSC2. While performing a whole genome scan to identify QTL influencing growth and carcass traits in a Piétrain x Large White intercross, we identified a QTL with major effect on muscularity and fat deposition towards the centromeric end of SSC2 (Nezer et al., 2001). Comparative mapping information pointed towards HSA11 as the human orthologue, and thereby to IGF2 and MyoD as potential positional candidates. Mapping these genes with respect to the porcine marker map showed that IGF2 co-localized with the QTL. Assuming that IGF2 was indeed responsible for the QTL effect and that the IGF2 gene would be imprinted in the pig as it is known to be in the human and mice, we made the prediction that in our F2 population a significant substitution effect would be found between the paternally inherited Piétrain versus Large White alleles, but not between the corresponding maternally inherited QTL alleles. We demonstrated that IGF2 is indeed imprinted in the pig, and that only the QTL alleles inherited from the boar influenced the phenotype, thereby supporting our hypothesis (Nezer et al., 2001). Similar results were simultaneously obtained in a Wild Boar x Large White intercross by Jeon et al. (2001).

Despite the strong candidacy of IGF2 (given its known role in myogenesis), our results did not formally prove that this gene caused the observed QTL effect. IGF2 indeed maps to an imprinted domain containing other putative candidates. To refine the map position of the QTL, we (i) constructed a BAC contig spanning the TSSC5-H19 interval containing the KVLQT1 and IGF2 imprinted domains and from it developed a high density microsatellite and SNP-based marker map of proximal SSC2, and (ii) identified six distinct “Q” and “q” chromosomes by marker assisted segregation analysis performed in 32 large (> 100 ofspring) boar families. Comparison of the “Q” bearing (muscle increasing) boar chromosomes revealed a shared chromosome segment in the interval bounded by p57kip2 and the 3’ UTR of IGF2, thereby mapping the QTL to this interval. As INS and IGF2 are the only known paternally expressed genes in this well-characterized interval, both remained good causative candidates. We therefore resequenced 28 Kb corresponding to the 3’ TH-3’ IGF2 interval for the shared “Q” haplotype as well as the six “q” chromosomes. Corresponding results will be presented.

EVIDENCE FOR THE CONTRIBUTION OF ADDITIONAL IMPRINTED QTL TO VARIATION IN BODY COMPOSITION

The results obtained on Oar18 and SSC2 spurred researchers to systematically test for imprinting when mapping QTL. Recently, de Koning et al. (2000) reported the detection of additional imprinted QTL influencing back-fat thickness on SSC2, intramuscular fat on SSC6 (two QTL) and muscle depth on SSC7, while Hirooka et al. (2001) reported evidence for two imprinted QTL influencing teat number on SSC2 and SSC12, respectively. Such a high incidence of imprinted QTL was not observed by other groups performing similar studies in the
pig, including Knott et al. (1998) and Nezer et al. (2002). The reasons for these discrepancies remain unknown. It should be noted that de Koning et al. (2002) showed that when mapping QTL in experimental crosses, the non-fixation of alternate QTL alleles in the parental lines may generate spurious evidence for imprinted QTL.

CONCLUSIONS
Despite the fact that parental imprinting only concerns a minor fraction of the eutharian genome, QTL mapping experiments targeting body composition in livestock are revealing an unexpectedly high incidence of parent-of-origin effects shown to involve imprinted genes in at least two instances. This may reflect the fact that body composition is influenced by genes that affect the allocation of maternal resources to offspring, genes which are thought to be prime targets for imprinting in polyandrous eutharian mammals. The occurrence of parent-of-origin effects should be considered when estimating breeding values and opens new avenues for improved marker assisted breeding schemes.

REFERENCES