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

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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 eutharian 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 for 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 extend 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 offspring) boar families. Comparison of the "Q" bearing (muscle increasing) boar chromosomes revealed a shared chromosome segment in the interval bounded by $p57^{KIP2}$ 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.

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