Differential maternal and paternal genome effects on placental and fetal phenotype and gene expression at midgestation

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Declaration

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List of publication/prepared manuscripts

Chapter 2: Novel paternal and maternal genome effects on the placental-fetal system support both conflict-of-interest and maternal-offspring coadaptation

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Chapter 3: Widespread differential maternal and paternal genome effects on fetal bone phenotype at midgestation

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Chapter 4: Maternal and paternal genomes differentially affect myofibre characteristics and muscle weights of bovine fetuses at midgestation

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Abstract

Lifelong development is largely programmed prenatally. Genetic and epigenetic factors, such as mitochondrial (mt) DNA variation and parent-of-origin effects, significantly contribute to variation in important prenatal phenotypes that determine lifetime development, including placenta and fetal musculoskeletal system. Such effects initially impact on transcriptome expression levels and eventually give rise to altered phenotypic traits. However, data regarding the overall magnitude and specificity of maternal and paternal genome effects in mammalian prenatal development is lacking.

The present study aimed to dissect and quantify differential maternal and paternal genome effects on specific placental and fetal traits, and associated transcriptomic events which drive prenatal development. A large bovine fetal resource (n=73), consisting of both purebreds and reciprocal hybrids with *Bos taurus taurus* (Angus) and *Bos taurus indicus* (Brahman) (epi)genetics, was used in this study. We examined 41 gross- and histo-morphological placental and fetal traits, 51 fetal bone weight and geometry parameters, and 22 myofibre characteristics and muscle mass parameters using morphometrical and/or immunohistochemical methods. Expression of the long non-coding RNA H19 in fetal muscle was determined by real time quantitative PCR. Profiles of mRNA and microRNA expression were obtained with microarrays that contained 24,027 and 13,133 mammalian probe sets, respectively, to assess transcript abundances in fetal liver. Phenotypic data were analysed by Analysis of Variance (ANOVA) using general linear models with nested effects and transcriptome data were analysed with microarray ANOVA procedures.

The analyses identified 49 significant placental and fetal traits, including five principal components representing 51 bone parameters, and H19 gene expression levels in muscle, with

ANOVA model significance levels (P) ranging from 3×10^{-2}-9×10^{-17}. We showed that parental genomes contributed to the largest proportion of variation explained by linear models for a majority of placental and fetal traits. Fetal sex was the next most significant factor to explain variation in these traits and non-genetic maternal effects, such as post-conception weight gain and final maternal weight, explained the least amount of variation. Significant effects of the maternal genome ($P<5\times10^{-2}$-5×10^{-13}) predominantly contributed to genetic variation in:

(i) Gross- and histo-morphological placental traits and fetal organ weights (59.6−99.9%,); (ii) most extracted principle components (PCs) representing bone weight and geometry traits, including PC1/bone mass (74%), PC3/limb elongation (73%), PC4/flat bone elongation (74%) and PC5/axial skeletal growth (97%) and (iii) most myofibre characteristics including fast myofibre cross-sectional area (CSA, 93%), total cell CSA (82%), absolute mass of studied muscles (59-88%) and *H19* transcript abundance in fetal muscle (76%). Conversely, significant paternal genome ($P<4\times10^{-2}$-7×10^{-8}) predominantly contributed to genetic variation in:

(i) Fetal fluids weight (73%), umbilical cord weight and length (73%), maternal placenta (70%) and umbilical cord (83%) efficiencies; (ii) PC2/limb ossification (95%) and (iii) Relative mass of studied muscles to fetal weight (54-97%).

Further, using nested effects in ANOVA, we found that maternal genome strongly determined regressions between placental weights and umbilical cord traits ($P<4\times10^{-2}$-2×10^{-6}), whereas paternal genome and/or fetal sex determined regressions between weight of fetus, fetal organ and fetal fluid s and umbilical cord traits ($P<5\times10^{-2}$-10×10^{-8}).

For fetal liver transcription profiles, maternal genome strongly affected expression levels of:

(i) Twenty-four mRNA transcripts (false discovery rate, FDR adjusted $P<4\times10^{-2}$-10×10^{-6}), 13 of which were located in the mt genome and (ii) ten autosomal non-coding RNA transcripts

including mammalian *SNORD113-9*, small nucleolar (sno)RNA, *MIR187* and *MIR1973* microRNA (FDR adjusted $P<5\times10^{-2}$-8×10^{-3}).

Paternal genome moderately affected expression levels of:

(i) Forty-seven autosomal mRNA transcripts (FDR adjusted, $P<5\times10^{-2}$-4×10^{-2}) (ii) *MIR184* microRNA transcripts in five mammalian species (FDR adjusted, $P<5\times10^{-2}$-4×10^{-2}).

Two significant coexpression networks, between 86 significant mRNAs and non-coding RNA transcripts, were also identified for differential maternal and paternal genome effects.

Our results show, for the first time, that a wide range of phenotypic and molecular traits within the placental-fetal system are affected by differential maternal and paternal genome and fetal sex effects. Identified differential maternal and paternal genome effects on specific placental and fetal traits are consistent with expression patterns of parent-of-origin effects predicted by both conflict-of-interest and maternal-offspring coadapdation hypotheses, thereby providing important insights to accommodate both hypotheses that explain the evolutionary basis of genomic imprinting effects. Observed complex, and predominantly maternal genome, effects are suggested to result from interaction between epigenetic factors from nuclear and mt genomes via RNA interference. This is further evidence for complex epigenetic crosstalk and coordination that contributes to mammalian prenatal development. Identified morphological and transcriptional modules within the placental-fetal system help to provide a new level of understanding prenatal development, i.e., systematic integration of omics data. Detailed molecular profiles of all core tissues and organs are now required to elucidate genetic, epigenetic and non-genetic components and interactions that control variation in placental and fetal phenotype. Future studies linking genome and epigenome with phenome data covering the

complete placental-fetal system will provide a new multi-layer picture of understanding coordination for molecular and phenotypic events driving mammalian prenatal development.