Allele-biased DNA methylation is widespread in porcine fetal tissues and reveals novel breed-specific and imprinting gene regulatory regions

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Allele biases in gene regulation and expression are widespread in mammals and can occur in a genotype-dependent and -independent manner. Allele-biased DNA methylation (ABM) is critical in regulating allele-biased gene expression (ABE), but the extent to which ABM contributes to breed-specific and imprinting gene regulation is unknown in the pig. We performed whole-genome bisulfite sequencing to characterize ABM in four pig fetal tissues (brain, liver, loin muscle, placenta) at 30- and 70-days gestation in females derived from Meishan (MS) and White Composite (WC) reciprocal crosses. Breed represented a significant source of allele methylation variance in each tissue (8.2-10.1%), indicative of extensive genotype-dependent ABM. We identified 116,467 ABM regions between MS and WC alleles (meth.diff>10%,FDR<0.01), and tested genes overlapping these ABM regions for tissue-specific enrichment for biological processes. The terms ‘dopamine transport’ and ‘placenta labyrinthine layer development’ were enriched among brain and placenta ABM genes, respectively, in agreement with known behavioral and reproductive differences between MS and European pig breeds. Breed-ABM genes were significantly enriched among breed-ABE genes (p=7.2E-246). *Methionine synthase reductase* (*MTRR*) was consistently hypomethylated and upregulated in MS alleles, suggestive of breed-

specific consequences on *MTRR*-associated developmental processes. We also identified 40,030 genotype-independent ABM regions that were enriched within human-validated imprinted genes (p=2.47E-03) and ABE genes in the same samples (p=1.33E-37). Twenty-three genes exhibited high genotype-independent ABM and ABE and have not previously been reported as imprinted in mammals. This study has identified thousands of putative regulatory regions that may mediate parent-of-origin allele biases in porcine tissues of economic relevance.