Effects of Maternal Diets on Fetal Genomic Imprinting in Sheep

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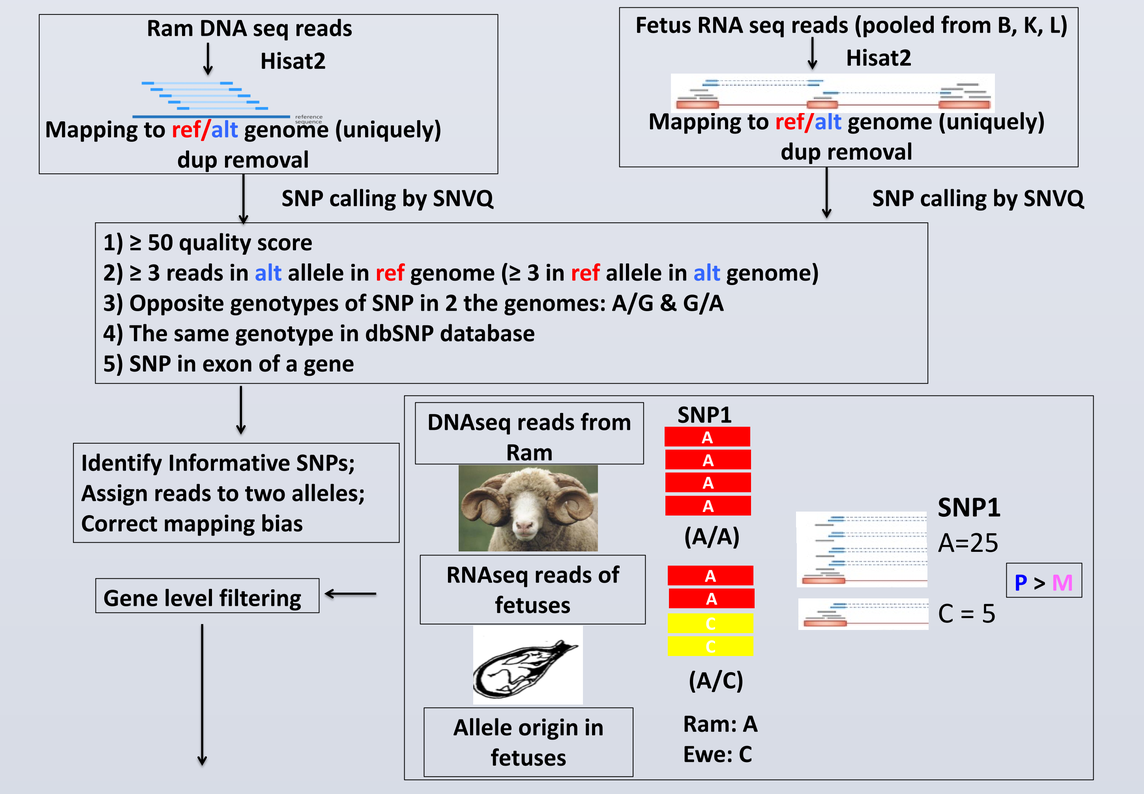
Abstract

Genomic imprinting is an epigenetics phenomenon that causes differential allelic gene expression based on parental origin. To date, 255 imprinted genes have been identified or predicted in all mammals combined. However, imprinting study in sheep lags behind, as only 21 imprinted genes have been described. The current study used DNA/RNA throughput sequencing to identify monoallelically expressed and imprinted genes in day 135 sheep fetal organs, and to assess the influence of maternal nutrition on imprinting. We solved several technical challenges in NGS data analyses including alignment bias of RNA sequencing reads and potential false positives. We identified 80 monoallelically expressed genes among which 18 are likely imprinted, five were previous known imprinted in sheep, and thirteen were known imprinted in other species. Sanger sequencing confirmed four of the imprinted genes identified here: *INPP5F, PLAGL1, CASD1* and *PPP1R9A*. Among the 13 new imprinted genes, five located in the sheep known imprinting clusters of *MEST* domain on chromosome 4, *DLK1/GTL2* domain on chromosome 18 and *KCNQ1* domain on chromosome 21, three were in a novel sheep imprinted cluster on chromosome 4 known in other species as *PEG10/SGCE*. Additionally, we found that *PHLDA2*, *SLC22A18*, *DIRAS3*, and *IGF2* were differentially expressed, but no allele expression reversal was seen among the three maternal nutritional groups. Our results expand the imprinted gene list to 34 in sheep and demonstrate the influence of maternal diet on fetal imprinting in sheep under the conditions studied.

Methods

Western white-faced Ewes (n=12) were mated with Dorset Rams (n=4). Pregnancy was confirmed by ultrasound on day 20 of gestation if an ewe was not re-marked by a ram, day 0 represents the initial marking of the ewe by the ram. On day 30 of gestation, pregnant ewes were individually housed and then randomly assigned to control 100% (Con, n=4), restricted 60% (Res, n=4), or overfed 140% (Over, n=4) of NRC total digestible nutrients (TDN). Ewes were euthanized at day 135 of gestation, and a total of 15 fetuses were obtained (Con: n=7; 3 ewes had twins, Res: n=4, Over: n=4). Brain, kidney and lung samples were collected from all fetuses. Tissues were flash frozen in liquid nitrogen and were stored at -80°C until RNA extraction was performed. Whole blood samples were obtained from the four Rams for DNA sequencing.



Results

I. Mapping bias correction

Table 1. Correction of the RNAseq alignment bias in the genome.

	Using Ref genome	Using Alt genome	Averaged counts from 2 genomes
Ref allele percentage	0.55	0.48	0.51
Alt allele percentage	0.45	0.52	0.49

II. Candidate imprinted genes

Table 2. Summary of the candidate imprinted genes in sheep. '-' = no informative SNPs in the tissue. Red and blue: maternally and paternally expressed, respectively. Number= paternal allele reads/(paternal allele reads+ maternal allele reads).

Treatment	Control group			Overfed group			Restricted group		
Tissues	Brain	Kidney	Lung	Brain	Kidney	Lung	Brain	Kidney	Lung
BEGAIN	-	0.91	1.00	-	-	-	-	-	-
BLCAP	-	-	-	0.79	0.81	0.86	-	-	-
CASD1	-	-	-	-	-	-	-	0.29	-
COPG2	0.11	-	0.28	0.18	-	-	0.20	-	-
DIRAS3	-	-	-	-	-	-	-	0.87	-
GATM	0.29	0.29	0.30	-	0.25	-	-	0.28	-
GRB10	-	0.19	0.24	0.29	0.24	0.22	-	0.26	0.18
GTL2	-	-	-	0.00	0.00	0.24	-	-	-
IGF2R	-	0.04	0.16	-	0.09	0.10	-	0.08	0.07
INPP5F	0.97	-	0.70	0.98	-	-	0.97	-	-
KCNQ1	-	-	-	-	-	0.19	-	-	-
PEG3	0.86	0.85	0.86	0.88	0.87	0.86	0.84	0.86	0.88
PHLDA2	-	-	-	-	-	-	-	0.27	-
PLAGL1	-	1.00	0.86	-	-	-	-	-	-
PON3	-	0.73	-	-	-	0.75	-	0.78	0.76
PPP1R9A	-	-	0.24	-	0.23	-	0.28	0.21	0.23
SLC22A18	-	-	0.18	-	0.30	-	-	-	-
WARS	0.74	0.72	-	-	0.74	0.70	0.78	-	-

III. Visualization of sheep imprinted genes and imprinting clusters in the genome

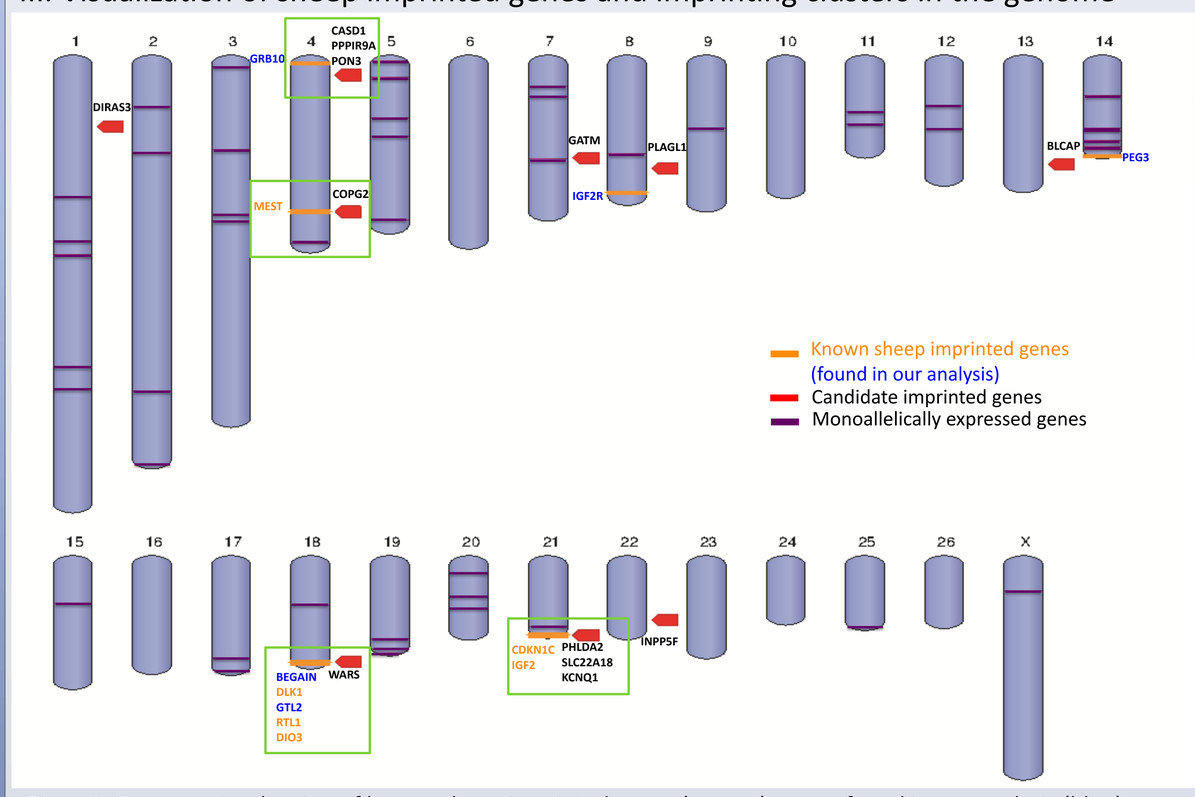


Figure1. Genome visualization of known sheep imprinted genes (orange), genes found in our analysis (blue), candidates imprinted genes (read mark), and monoallelically expressed genes (purple lines). Green box indicates imprinting clusters.

IV. Validation of candidate imprinting genes

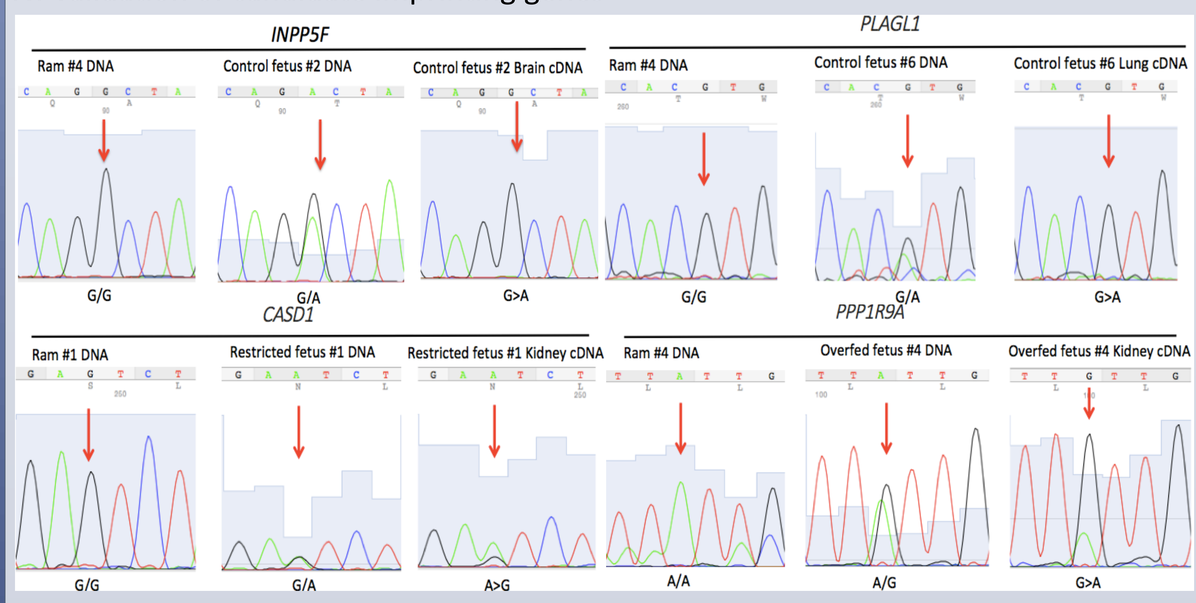


Figure2. Validation of four candidate imprinted genes identified by RNAseq. Red arrows indicate the informative SNP locations.

Conclusion

We used the NGS technology to expand the imprinted gene list to 34 in the sheep, and identified four imprinted clusters on chromosome 4, 18 and 21. Additionally, the levels of four imprinted genes were significantly affected by maternal diets with no allele expression reversal.

Acknowledgements

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