Characterization of the imprinting status of porcine loci , preliminary results

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The genetic improvement of prolificacy in pigs has increased the disparity of piglets' birth weight within litters. Low birth weight piglets are therefore more exposed to the risk of stillbirths, with consequences on animal welfare conditions but also economically for the breeder. Interestingly, it is now known that genes subject to genomic imprinting in humans and mice are involved in growth and development processes. Genomic imprinting is an epigenetic phenomenon in which genes are mono allelically expressed, depending on a parent-of-origin (PofO) methylation, resulting in differentially methylated regions (DMR) between the parental allele. Imprinting is important for normal development and growth, and disruptions in imprinting can lead to diseases and disorders. This project is therefore based on the hypothesis that imprinting mechanisms could contribute to the explanation of birth weight disparities in piglets. As genomic imprinting is still poorly studied in pigs, we propose 1) to characterize the imprinting status of regions that are known to be imprinted in humans and mice. 2) to perform linkage/ association studies between the imprinted status and the birthweights phenotypes.

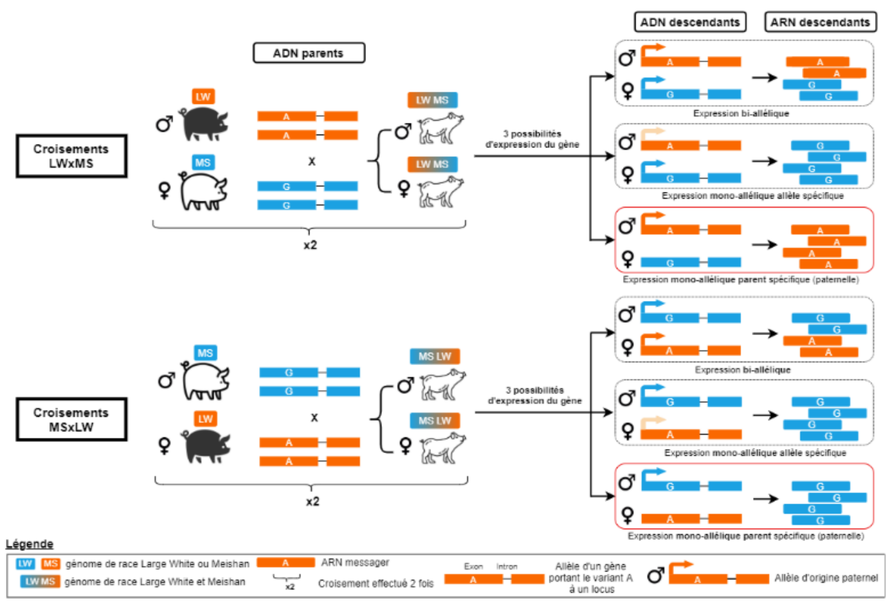

Figure 1. Reciprocal crosses design in order to identify parental mono-allelic expression

Material and Method

Animals and samples

- LW x MS Reciprocal crosses generated at the experimental unit GENESI (INRAE) allowing to **distinguish between parental mono-allelic expression from allele specific mono allelic expression.**
- Blood and tissues (Brain, Muscle) were collected for 10 F1 offsprings (MSp/LWm or LWp/MSm)

Molecular data

- Capture based enzymatic methylation data from blood and muscle (*Iannuccelli et al, 2023*) were generated at the Genotoul facility platform. Methylation data were analyzed through a home made pipeline developped in GenEpi team.
- RNAseq data from muscle and brain were generated at the Genotoul facility platform. Data were analyzed using the open source nfcore RNA-seq pipeline.
- PACE PCR (PCR Allelic Competitive Extension) were performed using the kit PACE ® Genotyping Master Mix.

Promising results on GNAS locus, focus on an example

Porcine map of the different imprinted regions will be constructed combining methylation and expression data. This analysis will be automatized using bioinformatics pipelines. First step has been to check the quality of the result through the characterization of the imprinting status of some regions of interest. Here is present the result of the GNAS locus :

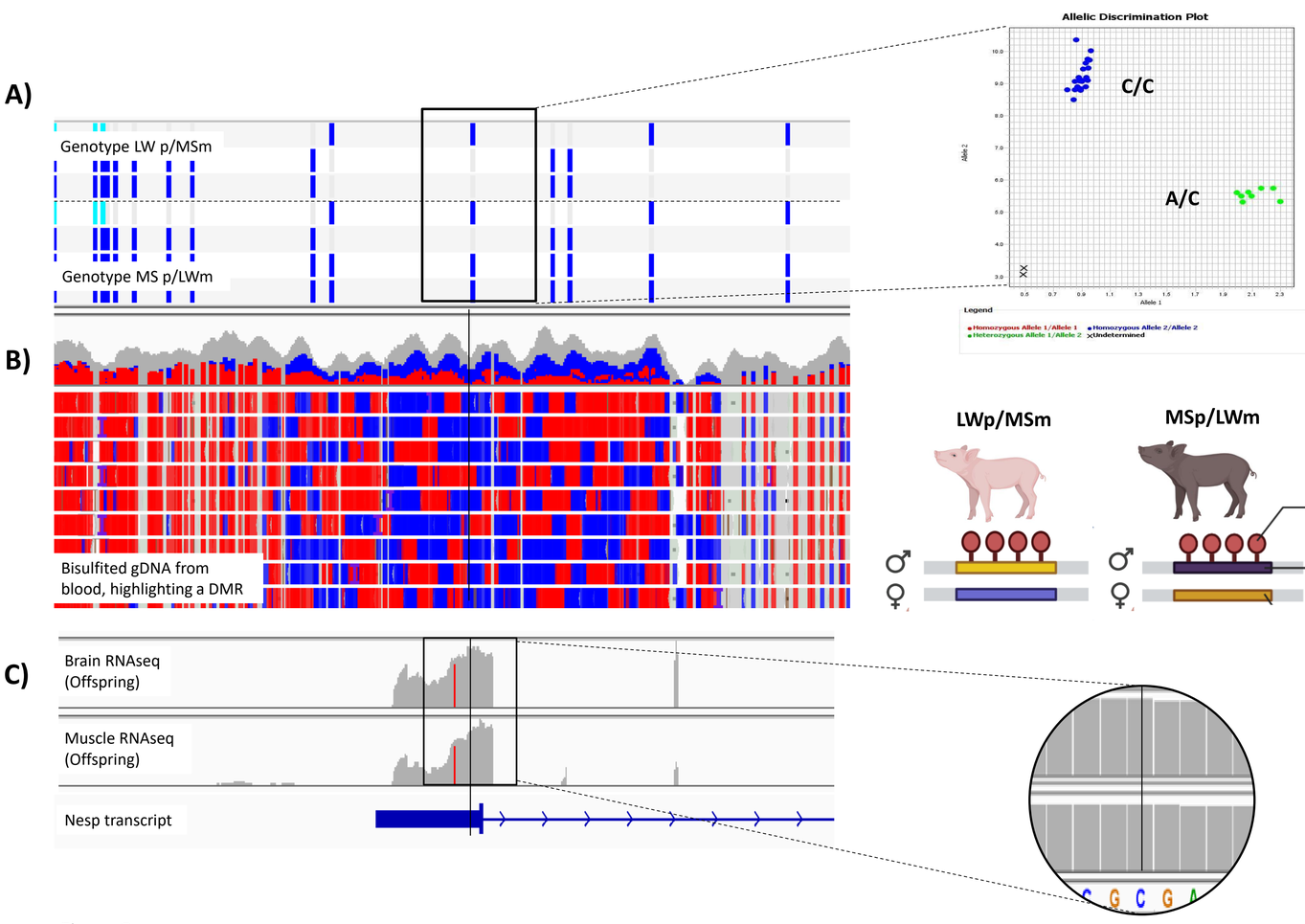

Figure 2

Fig. A Informative SNP determined from bisulfite data analyses were functionnally verified by PACE PCR. In this case, identified PACE genotypes are consistent with GemBS'. (C/C in grey from GemBS profile = C/C in blue from PACE profile; A/C in blue from GemBS profile = A/C in green from PACE profile)

Fig. B The DMR (Strong alternance of red and blue colour pattern) identified from bisulfite data, is tagged by an informative SNP within the first *GNAS* exon (dark vertical line): In this region hypermethylated reads present the paternal allele, the hypomethylated reads, the maternal one. This results suggest that the origin of **this DMR is from paternal origin.**

Fig. C RNAseq analysis demonstrates a **maternal mono-allelic expression** of the allele C from the informative SNP.

Identification and verification of SNP informativeness

Reciprocal crosses allow to get the most informatives variant to track parental origin (PofO) of the two chromosomes.

DMR identification and their parental of origin methylation

From bisulfite data analysis, DMR are identified through the observation of hemi methylation pattern. The PofO methylation is then manually determined.

Identification of a mono-allelic expression

We finally analysed the RNAseq data from brain and muscle to observe a parental mono allelic expression depending on the PofO methylation.

Taken together, our results demonstrate that among the seven potential GNAS DMRs, three are methylated on the maternal allele and one on the paternal allele, two seem to represent allele specific methylation and one that cannot be determined (no informative snp within the hemimetylated region) as shown in the figure 3. In our case, the paternal DMR spanning the Nesp transcript is associated with a maternal allelic expression in the brain and muscle, as shown in the RNA-seq data (Fig. 2C).
Some of these data are consistent according the litterature and new ones will be confirmed with other methods.

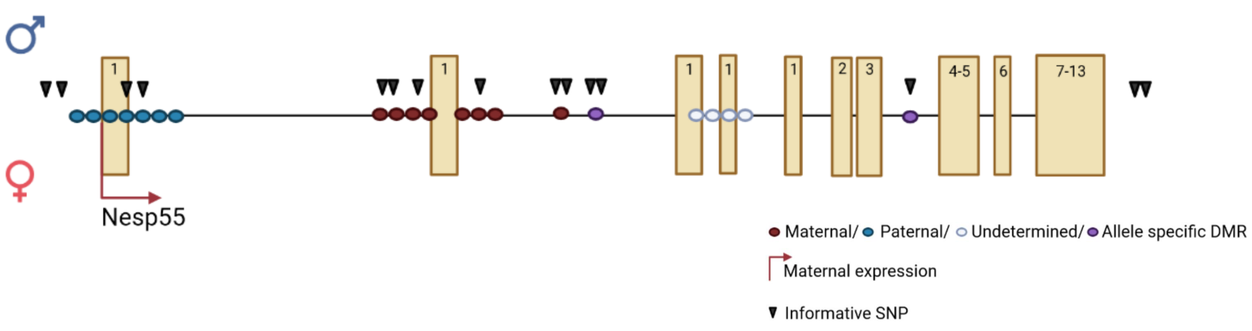

Figure 3 Summary of the results obtained to characterize the imprinting status of the GNAS locus (Exon numerotation are from the *Jinsoo et al*, 2020 publication)

Perspectives

1) Continue the characterization of the imprinting status of some region of interest (IGF2, GNAS, DLK1) will allow to functionally validate the relevance of the bioinformatic tools developped.
2) Map construction of porcine imprinted regions
3) Evaluation of the contribution of imprinting to piglets' birthweight variability.