PE1.40 Transcriptomic analysis of Longissimus muscle to select genes correlated to drip loss in pork 267.00

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Water holding capacity is a major quality trait of pork, and is usually evaluated through the drip loss of meat. High drip loss causes a leakage of water together with ions and soluble proteins from meat and generates considerable economic loss in the industry. It is highly and positively correlated with loin eye area, and has on average much higher values in modern pig breeds than in indigenous breads, characterized by a lower lean meat content and higher technological and sensory meat quality. A microarray gene expression analysis in the *Longissimus* muscle (LM) of two pure breeds of pigs, i.e. Large White (LW, conventional) and Basque (B, local breed, low lean meat content, high meat quality) was performed. A total of 50 pigs (LW, n=20 and B, n=30) were used. RNA were extracted from *LM* samples taken 30 minutes after slaughter, labelled and hybridized together with a reference sample to 15K custom Agilent muscle tissue microarray slides. The obtained gene expression profiles were correlated with loin drip loss, in order to find genes whose expression level was highly correlated with drip loss and could therefore be a future marker of this trait. A group of 192 genes of high correlation rate (R^2: 67 to 22%) and a very high statistical significance (<5 10^{-6}) were found. 127 out of 192 genes were identified, This might allow the identification of molecular markers of drip loss and the subsequent development of control tools of pork quality.

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***Index Terms* — correlations, drip loss, pork quality, transcriptomics**

I. Introduction

Water holding capacity, expressed by a differential extend of drip loss, is one of the most important characteristics of pork quality. As it can cause up to 10% loss of mass of the product in meat industry [7] drip loss can lead to considerable economical losses. It negatively correlates with other meat quality traits like intramuscular fat content [10] and sensory traits, i.e. juiciness, flavour, and tenderness [3; 11], but positively with loin eye area [10]. Though, modern pig breeds selected for high growth rate and carcass leanness usually exhibit higher meat drip loss than local, indigenous pig breeds characterized by lower growth rate and lean meat content, but higher eating quality [5]. However, the reason(s) for differential drip loss on genetic level is still not fully known. To study the genetic background of this characteristic, as well as to find relevant markers for it, a microarray experiment was conducted. The aim was to associate gene expression levels with drip loss in *LM* in two pure breeds of pigs exhibiting contrasted mean values for drip loss, i.e. the conventional LW and the local B. Pigs from each breed were raised in different productions systems known to influence meat quality traits and in particular drip loss [6], thereby allowing a wide range of drip loss values among all the pork samples within our study.

II. Materials and Methods

A. Animals

Total of 50 pigs were used for the experiment: 20 LW and 30 B finishing castrated boars. In each breed, 10 pigs were reared in a conventional (slatted floor, 1.0 m²/pig) system, and 10 pigs in an alternative (bedding and outdoor area, 2.4 m²/pig) system, at INRA experimental farm. Moreover, 10 Basque pigs were reared in the extensive (free range) production system of the Basque pigs (south west of France).

B. Slaughtering, muscle sampling and drip loss determination

All animals were slaughtered at the average live weight of 150 kg, according to standard procedures in INRA experimental slaughterhouse. *LM* samples were taken 30 minutes after exsanguination, frozen immediately in liquid nitrogen and stored at -80° C until RNA isolation. The day after slaughter, a

transverse section of *LM* (100 ± 10 g) was taken (keeping the facies around the muscle), weighed and suspended in a plastic bag at 4°C for 2 days. The slices were then gently dried with a paper and weighed for determination of drip loss.

C. *Microarray experiment*

Total RNA was extracted from LM samples [2] and purified using RNeasy MinElute Kit (Qiagen, Hilden, Germany). RNA concentration was evaluated by ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), and RNA quality was assessed using an Agilent Bioanalyser 2100 (Agilent Technologies, Santa-Clara, CA).

RNA samples and the reference (pool of an equal amount of the 50 LM RNA) were labelled according to Agilent manual with Cy3 and Cy5 dye, respectively. The samples were hybridized to the Agilent custom 15K microarray designed for muscle tissue (Damon and Cherel, in preparation) and washed according to Agilent procedure.

D. *Data analyses and functionnal annotations*

Hybridized microarrays were scanned at 5 µm/pixel resolution on a DNA Microarray scanner (Agilent Technologies, Santa-Clara, CA). Image analyses were performed with Agilent Feature Extraction Software (v9.5). Intensities of selected spots were transformed into log(Cy3/Cy5), and data were normalized by both spot and chip
by the weighted linear regression (LOWESS) method, using
the microarray software package GeneSpring GX 7.3.
After processing, 10279 probe sets were retained for further analysis. Pearson correlation coefficients were calculated between each of these 10279 expression values and drip loss measurements, on the 50 pigs. To account for multiple hypotheses testing, Bonferroni adjusted p-values were calculated with R software [1]. Genes with adjusted p-value ≤ 0.05 were studied further.

Genes showing a significant correlation with drip loss were classified according to their biological process description provided in GO Consortium for Homo sapiens (www.ncbi.nlm.nih.gov/sites/entrez?Db=gene).

III. RESULTS AND DISCUSSION

Boxplots (Figure 1) display the differences in drip loss between breeds and confirm the higher drip loss in the LW compared with the B pig breed ($p = 2.1\ 10^{-6}$).

After Bonferroni correction a list of 192 probes, with both positive (n=100) and negative (n=92) correlations of their transcript abundance with drip loss was obtained. The determination coefficients (R^2) ranged between 22% and 67%. These 192 probes corresponded to 127 identified genes. The 30 genes exhibiting the highest R^2 values are shown in Table 1.

Among these 30 genes, 10 (ARV1, GUP1, TGFBR3, CAV3, SEC23IP, TOR1AIP2, DOC2A, NRG4, CCDC47, VDAC2) appear to be components of cell membrane, taking part in ions, proteins and glycerol binding and transport. Genes responsible for Ca^{++}, metal and anion binding and transport were negatively correlated with drip loss, whereas Caveolin-3 (CAV3) encoding the sodium channel regulator activity protein was positively correlated with drip loss.

Nine of these genes (IRF8, SIRT3, NDN, RPS7, EEF1A1, EIF3G, SNRPD3, EMI5, SRPX) take part in different stages of protein biosynthesis, from transcription regulation stage to translation and one is involved in protein degradation. Three genes (COGA1, SSH2, ACTR1A) appear to have structural molecule activity and are responsible for cell shape and motility.

Distribution of the expression values of the 5 genes (PGM1, ARV1, SMOC2, GUP1, TGFBR3) exhibiting the highest R^2 values with drip loss, according to pig breed, are shown on Figure 2.
The gene with the highest correlation is the phosphoglucomutase 1 (*PGM1)*. This enzyme catalyzes the isomerization of glucose 1-phosphate to glucose 6-phosphate, which can then proceed through glycolysis. One could speculate that this gene expression could affect loin drip loss by an indirect influence on muscle lactate production [9]. *PGM1* expression is weakly correlated with ultimate pH (R^2=15%) but not with muscle pH or lactate content determined 30 min p.m., in our study. However, it was shown that drip loss better correlates to muscle lactate measured 4 h, instead of 45 min or 24 h post-mortem, highlighting the importance of muscle metabolism during the early p.m. hours in the determination of subsequent drip loss [4].

Overall, both *PGM1* and *CAV3* which are both positively correlated to drip loss, are either involved in glycolytic pathway (PGM1) or more expressed in type II glycolytic muscles than in type I oxidative ones [13]. This is in accordance with the negative correlation between drip loss and the oxidative phosphorylation pathway [8].

IV. CONCLUSION

The microarray experiment described in this article revealed numerous genes that have an expression level highly correlated with drip loss in pork. These data are very promising for the

identification of molecular markers of drip loss and the further development of new control tools of this trait, thus allowing an early post-mortem determination of pork quality in the industry.

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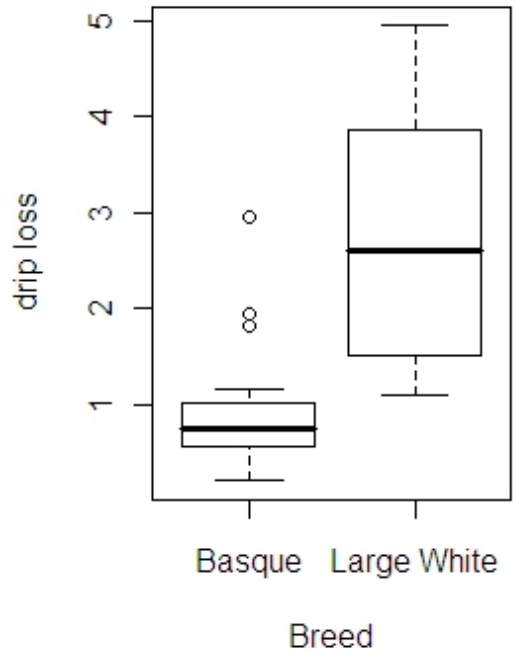

Figure 1. Boxplots of drip loss in Large White and Basque pigs.

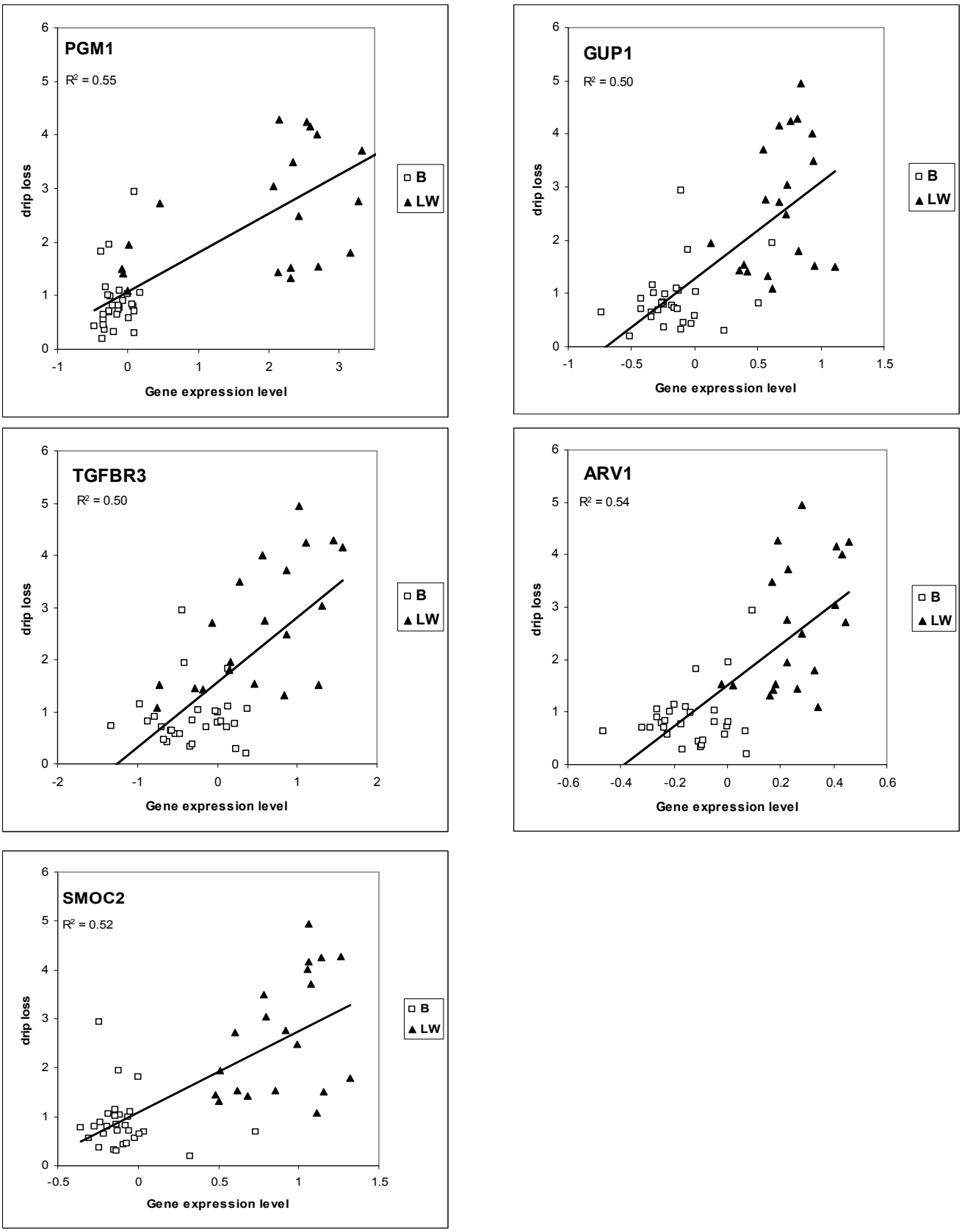

Figure 2. Expression profiles of 5 genes with the highest correlation coefficient with drip loss in the two breeds. X axis – relative expression value, Y – drip loss percentage.

Description	HGNC	r	R^2 (%)
Phosphoglucomutase-1	PGM1	0.74	55.1
Protein ARV1	ARV1	0.74	54.8
SPARC-related modular calcium-binding protein 2 precursor	SMOC2	0.72	52.4
Glycerol uptake/transporter homolog	GUP1	0.71	50.4
TGF-beta receptor type III precursor (Betaglycan)	TGFBR-3	0.71	50.4
Caveolin-3	CAV3	0.71	50.0
Interferon regulatory factor 8	IRF8	0.71	49.7
Collagen alpha-1(XVI) chain precursor	COGA1	0.70	49.4
NAD-dependent deacetylase sirtuin-3, mitochondrial precursor	SIRT3	0.70	49.3
SEC23-interacting protein	SEC23IP	-0.69	48.0
ADP-ribosylation factor-binding protein GGA3	GGA3	-0.69	47.5
Melanoma-associated antigen H1	MAGEH1	0.69	47.2
Necdin	NDN	0.69	47.1
40S ribosomal protein S7	RPS7	0.68	46.8
Torsin-1A-interacting protein 2	TOR1AIP2	-0.68	46.6
Protein phosphatase Slingshot homolog 2	SSH2	0.68	46.4
Putative protein tag-73 (Glyoxalase 1)	GLO1	0.68	46.1
Tyrosyl-tRNA synthetase, mitochondrial precursor	YARS2	0.68	46.1
Elongation factor 1-alpha 1	EEF1A1	0.67	45.2
Double C2-like domain-containing protein alpha	DOC2A	-0.67	44.8
Adenylosuccinate lyase	ADSL	0.67	44.8
Eukaryotic translation initiation factor 3 subunit 4	EIF3G	0.67	44.4
Small nuclear ribonucleoprotein Sm D3	SNRPD3	-0.67	44.2
Early meiotic induction protein 5, mitochondrial precursor	EMI5	-0.66	43.3
Alpha-centractin	ACTR1A	-0.66	43.2
Pro-neuregulin-4, membrane-bound isoform	NRG4	0.66	43.0
Proteasome activator complex subunit 2	PSME2	0.66	42.9
Coiled-coil domain-containing protein 47 precursor	CCDC47	-0.65	42.8
Sushi repeat-containing protein SRPX precursor (DRS protein)	SRPX	0.65	42.8
Voltage-dependent anion-selective channel protein 2	VDAC2	-0.65	42.5

Table 1: Coefficients of correlation (r) and of determination (R^2) between drip loss and gene expression level. The table presents the 30 known genes most correlated to drip loss. HGNC, gene name according to Hugo Gene Nomenclature Committee.