PE1.39 Microarray analysis of differential genes expression patterns in Longissimus muscle of Large White and Basque pigs 266.00

Joanna Wyszynska (1) joanna.wyszynska@rennes.inra.fr, Bénédicte Lebret(1), Marie damon 1,
(1)Institute National de la Recherche Agronomique, UMR SENAH

Along years of pig breeding aiming at improving the production of lean meat, a significant loss of pork quality was observed. To investigate genetic background underlying differences in pork quality from different pig breeds, a microarray experiment was conducted to disclose genes expression profiles in the *Longissimus muscle (LM)* in two pure breeds of pigs, ie. Large White (LW) and Basque (B), differing in muscle and meat characteristics. A total of 50 pigs (LW, n=20 and B, n=30) were used. RNA was extracted from *LM*, labeled and hybridized together with a reference sample, to 15K custom Agilent muscle tissue microarray slides. A total number of 98 genes were found to be differentially expressed, 45 (28 known) being upregulated in the B pigs, and 53 (34 known) upregulated in the LW pigs. Gene ontology analysis showed several functional classes overrepresented by differentially expressed genes, i.e. polysaccharides metabolism and biosynthesis, transcription and metabolism regulation, as well as ubiquitin conjugation.

J. Wyszynska-Koko is with with the Institute National de la Recherche Agronomique, Unité Mixte de Recherches, Systèmes d'Elevage, Nutrition Animale et Humaine, France, 35590 Saint-Gilles
(corresponding author: (0033)223-48-50-80; e-mail: joanna.wyszynska@rennes.inra.fr).
M. Damon is with with the Institute National de la Recherche Agronomique, Unité Mixte de Recherches, Systèmes d'Elevage, Nutrition Animale et Humaine, France, 35590 Saint-Gilles (e-mail: marie.damon@rennes.inra.fr).
B. Lebret is with with the Institute National de la Recherche Agronomique, Unité Mixte de Recherches, Systèmes d'Elevage, Nutrition Animale et Humaine, France, 35590 Saint-Gilles (e-mail: benedicte.lebret@rennes.inra.fr).

***Index Terms*— meat quality, pig breeds, skeletal muscle, transcriptomics**

I. INTRODUCTION

Long time of pig selection focusing on meat production efficiency has resulted in the improvement of parameters like lean meat content, growth rate, and feed conversion ratio, and the decrease in backfat thickness [6]. In the same time however, some stress resistance and meat quality traits were adversely affected [7]. Since around 20 years, in many countries an increasing emphasis has been put on improving pork quality traits [5; 12].

Meat quality is a complex phenotype and depends on the interactive effects of pig genotype and environmental constraints [10; 9]. Moreover, raw meat properties are associated with morphological and physiological characteristics of the skeletal muscle resulting mainly from variability in gene expression.

Ascertaining the transcriptome differences between individuals is an important step to understand how selection and genetic drift may affect gene expression. To that end, divergent livestock breeds offer an ideal genetic material. For this purpose, a microarray experiment was conducted, allowing the comparison between transcriptomic profiles of *LM* from both LW (conventional, high lean meat content) and B (local, low growth performance and lean meat content, high pork quality) pure breeds of pigs.

II. MATERIALS AND METHODS

A. Animals, slaughtering and muscle sampling

Fifty finishing castrated boars were used in the experiment: 20 LW and 30 B [13]. 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.

B. Microarray experiment, data analyses and statistics

Total RNA was extracted from LM samples [Chomczynski and Sacchi 1987] 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 manuals 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.
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.

Transcriptomic data were analyzed using this software and subjected to one way ANOVA to study the breed effect using a 5% false discovery rate [FDR]. The obtained list of genes contained genes of at least 2 times fold change between breeds.

C. Gene ontology analysis

Lists of genes showing a significant difference in gene expression between breeds were subjected to functional classification analysis allowing the data organization in the context of the gene ontology: GoMiner [14] and DAVID [4] on-line softwares were used.

III. RESULTS AND DISCUSSION

Among all the genes from 15K Agilent microarray, 98 probes appeared to be differentially expressed between breeds: 45 were more expressed in the B pigs and 53 in the LW pigs (Table 1). Among the 9265 genes of the array having a UniProt reference number, these probes corresponded to 62 known genes, which were further used for functional classification analysis. The clustering of regulated genes according to biological processes, having an overrepresentation of genes on the slides, is shown on Figure 1.

In the interpretation, LW pigs were treated as a breed that was subjected to long term selection directed to a high lean meat production, and B pigs as a local - high fatness breed that had preserved genes of high meat quality. According to this, the LW pigs should have a gene expression profile changed in comparison with B pigs.

According to GoMiner results, two main clusters of genes and biological processes whose expression is changed between LW and B pigs can be highlighted. These genes are related to polysaccharides metabolism and biosynthetic process, and to transcription regulation.

These results are in accordance with the higher glycolytic potential, glycogen content and the prevalence of glycolytic muscle fibers in modern compared to indigenous pig breeds [4; 8]. Although not clustered, genes connected with lipid metabolism: LIPI (lipase member 1 precursor) and LIPE (hormone-sensitive lipase) are both downregulated in LW pigs, clearly showing a difference in muscle lipid metabolic profile according to breed. This is in accordance with the higher intramuscular fat content of the B pigs compared with the LW (3.67 vs 2.03%, $p<0.001$), since LIPE is known to be the key enzyme controlling the intracellular triglycerides hydrolysis.

Negative regulation of transcription and metabolic processes, shown to have an overrepresentation of regulated genes in the LW, are in accordance with the higher growth rate, potential of protein deposition and thus metabolic rate in this breed [11].

To complete the information, the functional classification by DAVID software was also performed. The ubiquitin conjugation functional class was downregulated ($p\leq0.01$) in LW pigs, although FDR was not significant. Decreased ubiquitin degradation of regulatory proteins allows many developmental processes to continue and thus to increase growth and metabolism [2].

IV. CONCLUSION

The differences in lean tissue growth and meat quality between the highly selected LW and the indigenous B breeds of pigs are associated with differences expression of genes controlling muscle energy metabolism, transcription regulation and ubiquitin protein degradation. This global gene expression profiling is very promising for a better understanding of the genetic background affecting pork quality.

ACKNOWLEDGEMENT

The authors gratefully acknowledge from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration activities, for the Integrated Project Q-PORKCHAINS FOOD-CT-2007- 036245.

The authors are grateful to Nathalie Bonhomme, Patrick Ecolan and Annie Vincent for their technical assistance.

REFERENCES

[1] Chomczynski, P. & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-

chloroform extraction. Analytical Biochemistry, 162, 156-159.

[2] Hershko, A., & Ciechanover, A. (1998). The ubiquitin system. Annual Review of Biochemistry, 67, 425–79.

[3] Huang, D.W., Sherman, B.T., Tan, Q., Kir, J., Liu, D., Bryant, D., Guo, Y., Stephens, R., Baseler, M.W., Lane H.C. & Lempicki, R.A. (2007). DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Research, 35, W169–W175.

[4] Huang, L.S., Ma, J.W., Ren, J., Ding, N.S., Guo, Y.M., Ai, H.S., Li, L., Zhou, L.H., & Chen, C.Y. (2004). Genetic variations of the porcine PRKAG3 gene in Chinese indigenous pig breeds. Genetics Selection Evolution, 36(4), 481-6.

[5] Hovenier, R., Brascamp, E.W., Kanis, E., van der Werf, J. H., & Wassenberg, A. P. (1993). Economic values of optimum traits: the example of meat quality in pigs. Journal of Animal Science, 71, 1429-1433.

[6] Kovac, M., & Groeneveld, E. (1990). Genetic and environmental trends in German swine herdbook populations. Journal of Animal Science, 68, 3523-3535.

[7] Lebret B. (2004). Rationalization of pig production: consequences on meat quality. Productions Animales, 17(2) 79-91.

[8] Lefaucheur L, Milan D, Ecolan P, & Le Callennec C. (2004) Myosin heavy chain composition of different skeletal muscles in Large White and Meishan pigs. Journal of Animal Science, 82(7), 1931-41.

[9] Rosenvold, K., & Andersen, H.J. (2003). Factors of significance for pork quality – a review. Meat Science 64, 219-237.

[10] Sellier, P., & Monin, G. (1994). Genetics of pig meat quality: a review. Journal of Muscle Foods, 5(2), 187-219.

[11] Te Pas, M.F.W., Visscher, A.H., & de Greef, K.H. (2004). Molecular genetic and physiologic background of the growth hormone–IGF-I axis in relation to breeding for growth rate and leanness in pigs. Domestic Animal Endocrinology, 27, 287–301

[12] van Wijk, H.J., Arts, D.J., Matthews, J.O., Webster, M., Ducro, B.J., & Knol, E.F. (2005). Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. Journal of Animal Science, 83(2), 324-33.

[13] Wyszynska-Koko, J., Damon, M., & Lebret, B. (2009). Transcriptomic analysis of Longissimus muscle to select genes correlated to drip loss in pork. In Proceedings 55th International congress of meat science and technology (Submitted), 16-21 August, 2009, Copenhagen, Denmark.

[14] Zeeberg, B.A., Feng, W., Wang, G., Wang, M.D., Fojo, A.T., Sunshine, M., Narasimhan, S., Kane, D.W., Reinhold, W.C., Lababidi, S., Bussey, K.J., Riss, J., Barrett, J.C., & Weinstein, J.N. (2003). GoMiner: A Resource for Biological Interpretation of Genomic and Proteomic Data. Genome Biology, 2003 4(4), R2

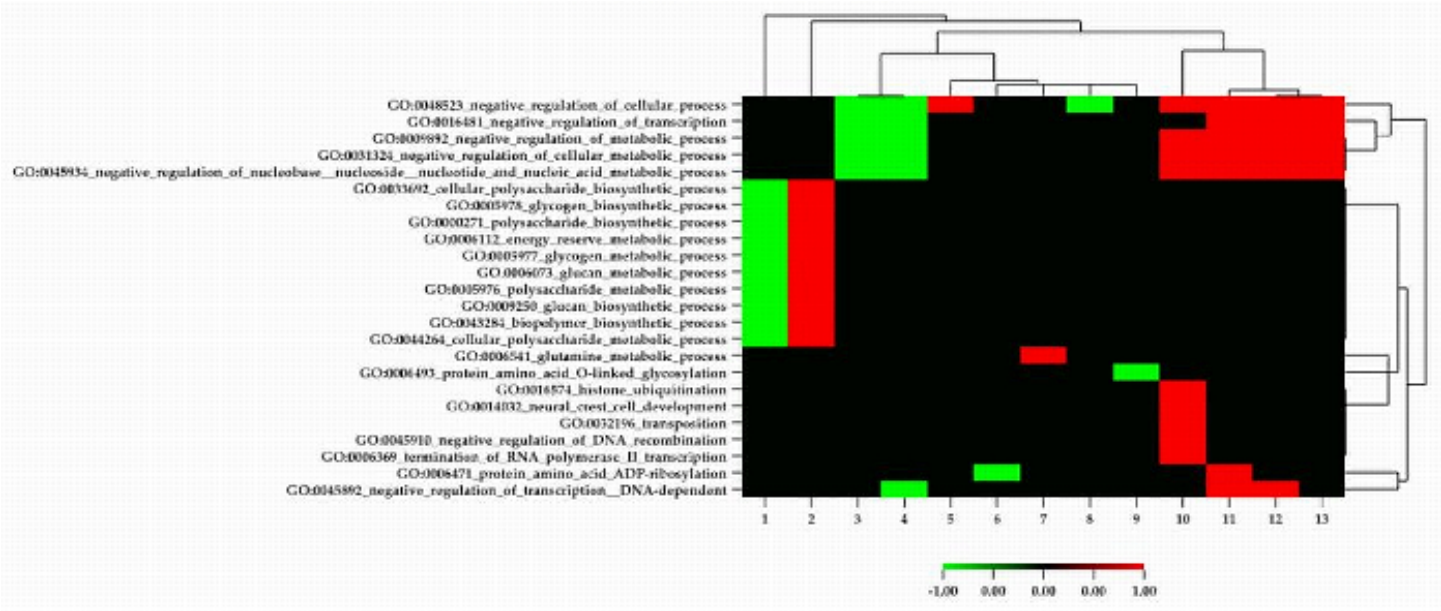

Figure 1.
GoMiner clustering of genes according to biological processes in LW and B pigs. Red means a higher expression in LW pigs, whereas green means a higher expression in B pigs. Numbers on the bottom corresponds to following genes: **1** - GLG1, **2** - PGM1, **3** - ZNF24, **4** - NFX1, **5** - ADAMTS8, **6** - ART5, **7** - ASNS, **8** - FNTB, **9** - GCNT1, **10** - RTF1, **11** - SIRT3, **12** - IRF8, **13** - LRRFIP1.

Gene description	HGNC	FC
Beta-1.3-galactosyl-O-glycosyl-glycoprotein beta-1.6-N-acetylglucosaminyltransferase	GCNT1	0.3
Cofilin-1	CFL1	0.4
Lipase member I precursor	LIPI	0.4
ATP synthase gamma chain. mitochondrial precursor	ATP5C1	0.4
Proto-oncogene protein c-fos	FOS	0.4
Probable RNA-binding protein orb2		0.4
Blood vessel epicardial substance	BVES	0.4
Protein C12orf4 homolog		0.4
Circulating cathodic antigen		0.4
DNA double-strand break repair rad50 ATPase		0.4
Golgi apparatus protein 1 precursor	GLG1	0.4
Zinc finger protein 410	ZNF410	0.4
Transcriptional repressor NF-X1	NFX1	0.4
Putative nicotinamide N-methyltransferase		0.4
F-box only protein 32 - Sus scrofa	FBXO32	0.4
Prostaglandin-F synthase 1		0.5
Protein MICAL-2 - Homo sapiens	MICAL2	0.5
SH3-domain kinase-binding protein 1	SH3KBP1	0.5
Acid sphingomyelinase-like phosphodiesterase 3a precursor	SMPDL3A	0.5
Hypothetical protein C02F5.13		0.5
Armadillo repeat-containing protein 6	ARMC6	0.5
Hormone-sensitive lipase	LIPE	0.5
Protein farnesyltransferase subunit beta	FNTB	0.5
Zinc finger protein 24	ZNF24	0.5
Hypothetical protein C1D4.03c in chromosome I		0.5
Hormone-sensitive lipase	LIPE	0.5
Transmembrane protein 51		0.5
Ecto-ADP-ribosyltransferase 5 precursor	ART5	0.5
SPARC-related modular calcium-binding protein 2 precursor	SMOC2	2.0
Glutathione peroxidase 7 precursor	GPX7	2.0
Ras-related protein Ral-B	RALB	2.1
Thyrotroph embryonic factor	TEF	2.1
Interleukin-10 receptor beta chain precursor	IL10RB	2.1
Aldose reductase	AKR1B1	2.1
Casein kinase I isoform gamma-2	CSNK1G2	2.2
Armadillo repeat-containing X-linked protein 2	ARMCX2	2.2
Asparagine synthetase [glutamine-hydrolyzing]	ASNS	2.2
Glyoxylate reductase/hydroxypyruvate reductase	GRHPR	2.2
Interferon regulatory factor 8	IRF8	2.2
Ribosomal protein L7-like 1	RPL7L1	2.2
NAD-dependent deacetylase sirtuin-3. mitochondrial precursor	SIRT3	2.2
DNA replication complex GINS protein PSF2	GINS2	2.2
Ankyrin repeat domain-containing protein 1	ANKRD1	2.3
Low affinity sodium-glucose cotransporter	SLC5A4	2.3
Chemokine C-C motif receptor-like 2	CCRL2	2.3
Putative protein tag-73	GLO1	2.4
Neuritin precursor	NRN1	2.4
Apolipoprotein R precursor	APOR	2.4
Protein BEX4	BEX4	2.5
Leucine-rich repeat flightless-interacting protein 1	LRRFIP1	2.5
Ras-related protein R-Ras2	RRAS2	2.5
Chloride channel protein 2	CLCN2	2.5
Thymosin beta-4	TMSB4X	2.6
Emopamil-binding protein-like	EBPL	2.6
Dynactin subunit 3	DCTN3	2.8
Polyhomeotic-like protein 2	PHC2	2.8
SPARC precursor (osteonectin)	SPARC	2.9
RNA polymerase-associated protein RTF1 homolog	RTF1	3.0
U6 snRNA-associated Sm-like protein LSm3	LSM3	4.1
ADAMTS-8 precursor	ADAMTS8	4.3
Phosphoglucomutase-1	PGM1	4.5
Zinc finger protein 7	ZNF7	4.9

Table 1.
62 up- or down-regulated genes between Large White (LW) and Basque (B) breeds in pig *Longissimus* muscle. FC, fold change represents the expression ratio of LW to B samples; HGNC, Gene name from Hugo Gene Nomenclature Committee