GENES DIFFERENTIALLY EXPRESSED IN LITTERMATE DUROC AND DUROC-PIETRAIN PIGS DIFFERING IN INTRAMUSCULAR FAT CONTENT

J.Estany* and M. Tor
jestany@prodan.udl.cat
Department of Animal Production, University of Lleida, 25198 Lleida, Spain

Key Words: Pig, intramuscular fat, microarray, candidate genes

Introduction

There is an increasing interest in manipulating intramuscular fat (IMF) content and composition in pigs. IMF content has been associated to meat eating quality, particularly for production of high quality cured products, while IMF composition has been associated with organoleptic characteristics and human health. Variation among breeds in IMF content has been the main genetic resource used by the industry for varying IMF grades. Attempts for direct selection of IMF conflict with the fact that there is still no easy procedure to obtain live measurements. This has prompted the research to identify genetic polymorphisms associated to IMF variation. Several genetic markers associated to IMF content and composition, including single genes (Gerbens et al. 2001) or quantitative trait loci (Pérez-Enciso et al., 2000), as well as some physiological biomarkers (Suzuki et al, 2004), have been recently reported. However, their functional role remains mostly unknown. Microarray and analytical mass spectrometry technologies are powerful tools for screening both the genome and the proteome and for providing new insight into the genetic and physiological mechanisms underlying target traits (Mullen et al., 2006). In this work we present an experiment aimed at comparing the genetic and the proteomic profiling of two pig breeds differing for IMF content. In particular, here we report the first results concerning genes differentially expressed in Duroc and Duroc x Pietrain dam half-sibs.

Materials and Methods

Experimental design and animals. Duroc sows from a line with high IMF content were inseminated with a pool of semen from one Duroc and one Pietrain boar in order to produce DU and DUx PI dam half sibs. The three DU and three DUxPI littermate barrows used in this experiment were sampled from three litters showing high variation in IMF content. All pigs were born the same day and raised under identical conditions until 185 days of age. They were controlled regularly during the fattening period for weight and ultrasonic backfat and loin depth. A biopsy of the longissimus dorsi muscle (LM) was taken at 115 and 150 days, which were immediately frozen in liquid nitrogen. The pigs were slaughtered in a slaughterhouse equipped with the Autofom automatic carcass grading. A sample of LM was taken from the carcass, vacuum packaged and stored in deep freeze. LM samples taken at 115 and 185 days were used to determine IMF content and composition while the sample taken at 150 days was used for the gene expression profiling analysis. IMF content was determined by quantitative determination of the fatty acids by gas-liquid chromatography. A comparison of some relevant characteristics of the DU and the DUxPI littermates used in the experiment is given in Table 1. Experimental procedures were approved by the Ethics Committee for Animal Experimentation of the University of Lleida.

Table 1. Means ± SEM of some characteristics of the three pairs of Duroc (DU) and Duroc x Pietrain (DUxPI) littermate barrows used in the experiment

Genetic type	Trait[a]							
	W150 (kg)	DG (g/d)	CW (kg)	LEAN (%)	IMF (g/g DM)	SAFA (%)	MUFA (%)	PUFA (%)
DU	75.2±2.5	796±53	78.2±1.4	44.5±1.0	12.1±0.9	46.4±0.3	38.2±0.1	15.5±0.4
DU x PI	93.0±4.5	962±106	97.7±5.9	53.8±1.2	8.2±0.8	40.9±1.7	40.8±3.9	18.3±2.2

[a] W150: Live weight at 150 days; DG: Daily gain from 115 to 185 days; CW: Carcass weight ; LEAN: Carcass lean content; IMF: Intramuscular fat content in *longissimus* muscle; SAFA (PUFA, MUFA): Saturated (Monounsaturated, Polyunsaturated) fatty acid percentage in IMF.

Expression profiling. Expression profiling was conducted with the Affymetrix Porcine Genome array. Total RNA from muscle samples was extracted using Trizol Reagent (Life Technologies) and purified by RNeasy (Qiagen). RNA quality was assessed after agarose gel electrophoresis and spectrophotometric methods. Biotynilated cRNA was then synthesized using the associated protocol of Affymetrix. The fragmented cRNA was then hybridized to arrays, which were processed using the Gene Chip Operating Software (GCOS 1.4). The six hybridized arrays fulfilled the standard quality control metrics established in GCOS.

Data analysis. For the bioinformatic analysis dChip (www.dchip.org) and Affy/AffyPLM (Bioconductor) software were used to look for samples acting as outliers and Genespring v 7.3 (Agilent) to perform gene expression analysis. RMA processing was used for normalizing the data as well as a global median normalization. Finally, each probe set in DUxPI was compared with the paired DU. One such pairwise comparison was performed for each of the three litters. Because each paired sample was from the same litter, average changes were calculated by averaging changes obtained from the three litters and paired t-test statistics were calculated.

Results and Discussion

A total of 374 probe sets showed a significant ($p<0.05$) fold change (189 were more highly expressed in DU and 185 in DUxPI). Significant fold changes were low, ranging from 0.28 to 5.9 However, a supervised clustering based on their expression profiling was able to discriminate pigs into the two genetic types. A selected list of genes differentially transcribed related to fat or muscle metabolism is presented in Table2. Genes included in the list showed consistent results in all the three pairwise comparisons.

Table 2. A selected list of genes differentially transcribed in littermate Duroc-Pietrain and Duroc pigs.

Gene Name	GeneBank Accession No	Fold increase[a] (Mean ± SE)
Glycogen synthase 1(muscle) (GYS1)	BG384816	1.15±0.01
Beta-tropomyosin (TPM2)	CF180239	0.87±0.02
GTP binding protein overexpressed in skeletal muscle (GEM)	Z80109.1	0.61±0.06
Lipoprotein lipase (LPL)	X62984.1	0.66±0.05
Diacylgycerol acyltransferase (DGAT)	NM_214051.1	0.86±0.03
Fatty acid synthase (FASN)	CF 179563	0.63±0.08
Adiponectin receptor 2 (ADIPOR2)	CN156813	0.72±0.08
Acetyl-CoA synthetase long chain family member 4 (ACSL4)	BI403313	1.18± 0.07
Hormone-sensitive lipase (LIPE)	AY686758.1	0.60±0.11
Adipocyte fatty acid binding protein (FABP4)	AU059657	0.72±0.09
Plasma phospholipid trasfer protein (PLTP)	BX667243	1.44±0.20

[a]Fold changes in the longissimus muscle of Duroc-Pietrain relative to Duroc. Genes listed were a selected sample of genes showing differentially ($P<0.05$) mRNA abundance.

Most of the genes in Table 2 have been proposed as candidate genes for meat quality traits. Association studies of these gene polymorphisms with meat quality traits did not lead to clear-cut results. Gene expression changes are greatly influenced by the adipocyte number and therefore the observed differences may be a consequence of adipogenesis rather than a cause (Damon et al., 2006). Our small sample size precluded statistical corrections for multiple testing and false discovery rates. Accordingly, results need to be validated with more samples and discussed changes be confirmed by either quantitative mRNA or protein studies.

Conclusions

A different expression pattern was found between DU and DUxPI dam half sibs in genes affecting lipid metabolism. The results may be valuable for finding biological markers for intramuscular fat content.

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Acknowledgements. Work funded by CICYT, Spain (grants No. AGFL2003-05361 and AGL2006-01243).