Gene expression profile in two pig breeds using the Affymetrix GeneChip® Porcine Genome Array

L. Pannier[1], D. Corcoran[1], R.M. Hamill[1], T. Sweeney[2] & A. Mullen[1]
[1]Department of Meat Technology, Ashtown Food Research Centre Teagasc, Ashtown, Dublin 15, Ireland.
[2]Department of Animal husbandry and Production, University College Dublin, Belfield, Dublin 4, Ireland.
Email: liselotte.pannier@teagasc.ie

Abstract

The objective of this study was to identify differentially expressed genes associated with pork meat quality in Duroc and Pietrain breeds. Five purebred gilts of each breed were fed a standard diet for 6 weeks and subsequent post slaughter samples were taken for meat quality analysis and RNA studies. High quality RNA was processed and hybridised to the Affymetrix GeneChip® Porcine Genome Array. Results indicate that 108 genes were identified as significantly differentially expressed between the two breeds. Annotation of the differentially expressed sequences identified some interesting putative genes with diverse biological functions (protein modification, proteolysis, energy metabolism, nucleotide metabolism). These genes are targets for further research to identify markers for meat quality. Subsequently, gene expression levels were validated using real-time reverse transcription PCR.

Introduction

At present relatively little is known about the relationship between the genome and meat quality traits such as tenderness and nutritive value of pork muscle. Identifying important genes relating to pork quality will impact our understanding of the biology of meat quality and muscle biology. This will ultimately lead to improving our ability to predict and optimise pork quality parameters. Improvements in genetic evaluation procedures, breeding systems and the identification of genes that contribute to variation of meat quality, have had a significant impact on meat production systems. Divergent breeding goals over time have led to variation in certain meat quality traits among specialised porcine breeds. Therefore, unraveling complexities in gene expression patterns in animals varying for meat quality traits and between-breed variation, using microarray technology, can expand our understanding of the genomic, and hence biological basis for this divergence. Duroc and Pietrain breeds were used in this study and previous studies have indicated their divergence for a number of meat quality traits (Edwards et al., 2003; 2006).

Materials & Methods

Five gilts of each breed were fed a standard diet for 6 weeks and were slaughtered in a pilot abattoir. Porcine *Musculus longissimus thoracis et lumborum* (*LTL*) samples (n=10) were collected post-slaughter and meat quality measurements were carried out (composition, fatty acid analysis, instrumental tenderness). The muscle samples for RNA analysis were excised within 5 minutes post-mortem, samples were finely minced using a sterile scalpel and placed in RNAlater (Ambion, Inc., Alameda CA), then stored at -20°C until further extraction. Samples were homogenised in the Fastprep® Instrument (FP120, Bio101 Thermo Savant) and total RNA was extracted from 100 mg tissue using the RNeasy® Mini Kit (Qiagen, UK) according to manufacturer's instructions. RNA quality and quantity was assessed using the Agilent 2100 Bioanalyser (Agilent Technologies, Germany) and the ND-1000 Nanodrop spectrophotometer (ND-1000 V3.1.2) respectively.

Double-stranded cDNA was amplified and labelled using the GeneChip® Expression 3'-Amplification IVT Labelling Kit (Affymetrix). Samples were further hybridised to the Affymetrix GeneChip® Porcine Genome Array. The array consisted of 23,937 probe sets to interrogate 23,256 transcripts in pig, which represents 20,201 genes. Ten chips in total were used in this experiment.

Real-time RT-PCR was carried out to validate the gene expression differences between the 2 breeds. Ten of the 108 differentially expressed genes were selected, according to low, medium and high fold change. Total RNA was converted into first-strand cDNA using SuperScript™ III Reverse Transcriptase (Invitrogen, CA, USA) and Oligo$(dT)_{23}$ primers (70 µM, Sigma-Aldrich, USA) according to the manufacturer's instructions. Expression level analysis was performed on an ABI PRISM® 7500 Real Time System using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). Samples were analysed in duplicate and a 5-fold dilution of the gene under investigation was included on every 96 well plate to test PCR efficiency. Real-time RT-PCR conditions were as follows: 50 ºC for 2 min and 95 ºC for 10 min, followed by

40 cycles of 95 ºC for 15 s and 60 ºC for 1 min, concluding with a dissociation step. All primers for the target genes were designed from the oligonucleotide sequences using Primer3 software and primer quality was checked using NetPrimer software. The house-keeping genes (HKG) tested were β-actin, B2M, TBP, YWHAZ, HMBS, RPLI3A (Erkens et al., 2006).

Data analysis

The microarray data was analysed using the commercial GeneSpring GX analysis (Agilent Technologies, Inc., http://www.chem.agilent.com). Per chip and per gene normalisation was followed by filtering on flags. Hierarchical clustering and principal component analysis were included as quality control steps. The standard 2-fold cut-off and subsequent t-test for microarray data analysis were applied to identify genes that were significantly different in expression between the 2 breeds ($P < 0.05$).

Since the porcine genome is not yet published, the sequence annotations from the differentially expressed oligonucleotide probes were compiled by using a number of available database resources. Putative pathways and regulatory mechanisms were inferred on the basis of literature searches and Gene Ontology annotations and the vast majority of the differentially expressed genes could be associated with a biological function and were assigned to functional classes.

Real-time RT-PCR data was analysed using the software package geNorm (http://medgen.ugent.be/~jvdesomp /genorm/; VBA applet for Microsoft Excel). The 6 HKG were analysed in geNorm to determine the expression stability of the non-normalised HKG genes, this was done by assigning each HKG gene a gene-stability measure (*M*) (Vandesompele et al., 2002). Stepwise exclusion of the gene with the highest *M* value allows ranking of the tested genes according to their expression stability until only the two most stable HKG remained. Further expression levels were calculated according to the *M* value.

Results & Discussion

Samples with an RNA Integrity Number (RIN) of 7 or greater and with an absorbance wavelength ratio (A_{260}/A_{280}) ranging from 1.8 to 2.1 were accepted for this study.

The gene expression intensities of a single Duroc pig did not approximate a normal distribution and it was an outlier in hierarchical clustering (Figure 2) and principal component analysis hence it was removed from further analysis. Statistical analysis of the Affymetrix GeneChip® data indicated that 108 genes were identified as significantly differentially expressed between the two breeds. Fifty percent of the differentially expressed gene could be assigned to putative biological function and are depicted in Figure 1.

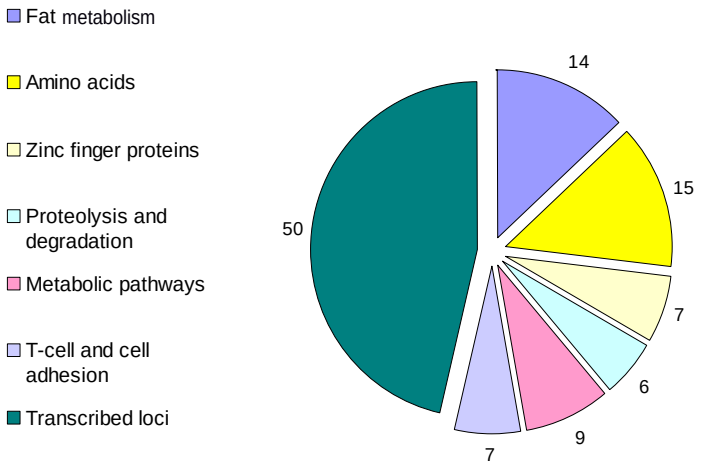

Figure 1: Putative functional categories.

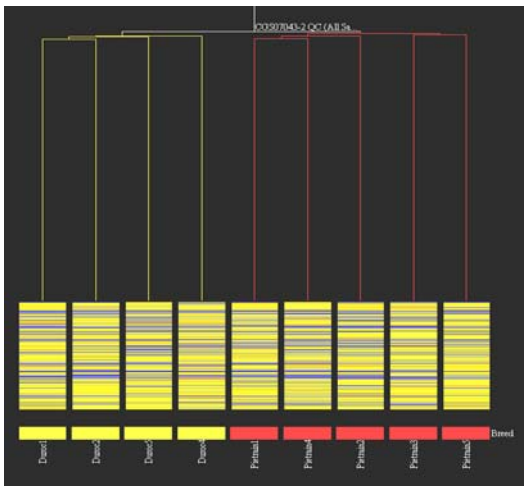

Figure 2: Cluster analysis.

The differential gene expression identified using the Porcine Affymetrix GeneChip® was validated by performing real-time RT-PCR on 10 genes selected from the 108 differentially expressed genes. Preliminary data indicated that B-actin and TBP were identified as the two most stable HKGs ($M = 0.613$) tested over the whole sample set using geNorm analysis. The Real-time RT-PCR analysis of the selected genes confirmed the results of the microarray experiment for the genes analysed to date and confirmed their differential expression. An example of two genes is depicted in Figure 3.

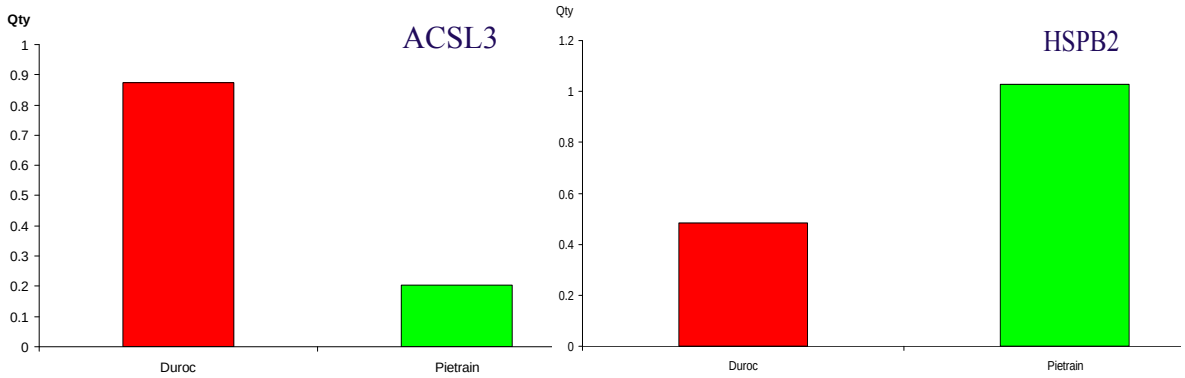

Figure 2: Expression level between the 2 breeds for the HSPB2 and ACSL3 gene.

Acyl-CoA synthetase long-chain family member 3 (ACSL3), involved in lipid biosynthesis and fatty acid degradation, was up-regulated in the Duroc breed in both the microarray and Real-time RT-PCR analysis with 3.6 and 4.3 fold respectively. The Heat-shock protein beta-2;(HSPB2) is involved in proteasomal degradation and apoptotic signalling pathway. This gene was 2.6 and 0.47 fold down-regulated in the Duroc breed according to the microarray and the Real-time analysis, respectively.

Conclusion

In conclusion, 108 differentially expressed genes have been identified between the two pig breeds divergent for meat quality. These genes might be targets for further research on genes influencing pork meat quality. Furthermore, this microarray data will be reanalysed according to meat quality traits on completion of measurements and real-time data will be completed. Extensive data-mining will be beneficial to refine the relationship between these genes and meat quality.

Acknowledgement

This project is funded by the Teagasc Retooling/Biotechnology Programme (Beef Quality Initiative) & by the Food Institutional Research Measure.

References

Erkens, T., Van Poucke, M., Vandesompele, J., Goossens, K., Van Zeveren, A. & Peelman, L.J. (2006). Development of a new set of reference genes for normalization of real-time RT-PCR data of porcine backfat and *longissimus dorsi* muscle, and evaluation with *PPARGC1A, BMC Biotechnology*, 6: 41.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., DePaepe, A. & Speleman, F. (2002). Accurate normalization of realtime quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, 3: 0034.1-0034.11.

Edwards, D.B., Bates, R.O. & Osburn, W.N. (2003). Evaluation of Duroc- vs. Pietrain-sired pigs for carcass and meat quality measures, *Journal of Animal Science*, 81: 1895-1899.

Edwards, D.B., Tempelman, R.J, & Bates, R.O. (2006). Evaluation of Duroc- vs. Pietrain-sired pigs for growth and composition, *Journal of Animal Science,* 84(2): 266-75.