Transcriptome analysis of porcine skeletal muscle: differentially expressed genes in Italian Large White pigs with divergent values for glycolytic potential

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ABSTRACT: Glycolytic Potential (GP) is an important parameter influencing pig meat quality. In order to identify genes and gene networks influencing glycolytic potential we utilized microarray analysis and studied the expression profile of skeletal muscle tissue from Italian Large White pigs with extreme high/low levels of GP. The knowledge of differentially expressed genes in groups of pigs with substantively different GP values could be a first step to understand the metabolic regulatory pathways influencing GP. The genes more expressed in the samples with low GP are mainly associated with energy metabolism while those more expressed in the samples with high GP are involved in intracellular transport. These results can give useful information to identify candidate genes for glycolytic potential level of pig meat.

Key words: Pig, Gene expression study, Glycolytic potential, Meat quality.

INTRODUCTION – Several biochemical processes occurring during the post-slaughter conversion of muscle to meat are relevant for meat quality. In particular the post-mortem pH fall and ultimate pH value are crucial factors that are mainly determined by the muscle glycogen content at time of slaughter. As proposed by Monin and Sellier (1985) an estimate of glycogen content and therefore the potential of lactic acid formation can be represented by the glycolytic potential (GP) which includes the main intermediates of glycogenolysis and glycolysis of pig muscles (Hamilton et al., 2003). GP is an important parameter for the determination of meat quality that affects meat colour, pHu, water holding capacity, drip loss, processing yield and attitude of the meat to seasoning (Enfalt et al., 1997; Nanni Costa et al., 2000). Excessively high or low levels of GP influence negatively the qualitative characteristics of meat. This parameter is largely influenced by the PRKAG3 gene (Milan et al., 2000). In particular the RN mutation increases the glycogen content of muscle and determines the defect called acid meat. However other genes may affect GP as this parameter shows some degree of heritability in pig populations where the negative allele of RN locus was not found. The expression profile of skeletal muscle genes influencing the level of GP in pigs is not known and this information could be useful to identify candidate genes for this trait and clarify the biological and physiological processes involved in the regulation of GP in skeletal muscle. This study focus on the identification of differentially expressed genes in Italian Large White pigs with divergent levels (high/low) of GP using microarray technology that allows to screen thousands genes in the same analysis.

MATERIAL AND METHODS – Eight subjects with extreme GP values were chosen among 277 sib-tested Italian Large White pigs (Nanni Costa et al., 2006) for the present study. The genotype of the pigs at the PRKAGE R200Q polymorphic site was determined as described in Fontanesi et al., 2003. RNA extraction was performed on muscle samples (m. semimembranosus) obtained from 8 animals with divergent values for GP and two pools of RNA were constructed: one with low GP (“pool –” with 4 samples showing GP<65 µmol lactate equivalent g-1 muscle wet
weight) and one with high GP (“pool +” with 4 samples presenting GP>145 µmol). RNA quality was checked using Bioanalyzer 2100 (Agilent) and 20 µg of RNA of each pool was retrotranscribed. cDNAs were labelled with Cy3 and Cy5 dyes with SuperScript Indirect cDNA Labeling System (Invitrogen) for the hybridization of 6 slides spotted in duplicate with the 11k Pig Oligo set version 1.0 (Operon-Qiagen). Dye swap approach was followed and replications were performed reversing the dye assignment to “pool –” and “pool +” samples to reduce systematic bias. The hybridisation conditions were carried out in a humid chamber at 42° C for 48 hours. After hybridisation the slides were scanned using ScanArray Gx (Perkin Elmer) scanner and the image acquisition was done using ScanArray Express (PerkinElmer) software. The data extracted from image analysis were pre-processed to exclude poor-quality spots and normalized to remove systematic errors before downstream analysis. The fluorescent signals were normalized with LOWESS adjustment using the same software. Data analysis was obtained using a fixed model with GLM procedure of SAS package (SAS, 1989) and a t-test was performed in order to identify genes differentially expressed between “pool +” and “pool –” (P<0.05 and FDR= 10%). Functional annotation and data analysis of differentially expressed genes were obtained using the tools present in DAVID (Database for Annotating, Visualization and Integrated Discovery; Dennis. et al., 2003).

Some differentially expressed genes were chosen on the basis of their functional relevance to validate the results of the microarray experiment and qRT-PCR was performed. Therefore total RNA from the eight analysed samples was treated with DNAse and retrotranscribed using the Improm-II™ Reverse Transcription System and Oligo-dT Primers (Promega). After optimization of qRT-PCR conditions on cDNAs, standard curves with serial cDNA dilutions (from 10^8 to 50 molecules of RNA/µl) were constructed to obtain absolute quantification of the RNA level of the chosen genes in the individual samples.

RESULTS AND CONCLUSIONS – The genotyping of the 8 samples used for the preparation of two RNA pools showed that none of the tested pigs carried the RN- mutation. The investigation on gene expression profile in high and low GP samples was performed using replicated slides and dye-swap procedure to eliminate systematic errors and experimental variables unrelated to the differences in gene expression. Data analysis of the fluorescent signals of the spots was performed using five slides out of six owing to the high level of background in one of the slides. Preliminary results of microarray analysis showed that 88 genes present a significant overexpression in pool with low GP (pool –) and 50 genes were significantly upregulated in the pool with high level of GP (pool +). The genes more expressed in the “pool –” were involved in oxidative phosphorilation and ATP synthesis coupled to electron transport, while the “pool +” presented more genes with roles in signalling and intracellular transport. For “pool +” the level of significance for the differences in gene expression was lowest (P values ranged from 1.08E-02 to 4.99E-02 and FDR varied from 1.02E-04 to 1.25E-03) than that of “pool –” (P values ranged from 1.35E-03 to 5.00E-02 and FDR varied from 9.23E-06 to 1.26E-03). This might indicate a more explicit and defined gene expression profile for “pool –” rather than for “pool +”. The results obtained for “pool –” suggest a metabolic situation answering to a likely low level of ATP in the cells. The energy request could be the condition causing the activation of specific processes (oxidative phosphorilation and electron transport) to counterbalance this lack and to restore ATP levels. The low GP level could be also relat-ed to the degradation of glycogen by the overexpression of the enzyme UDP-pirophosphorylase in order to produce precursor for ATP synthesis. Moreover it is interesting to point out that the top list gene of “pool –” is Stearoyl-CoA desaturase, a gene with a relevant role in the energetic metabolism. The higher level of expression of this gene in pool - has been verified and validated also by qRT-PCR (Figure1). The validation of the results on expression profile obtained in the microarray experiment for other genes is underway using real time PCR. Aiming to identify genes differentially expressed in skeletal muscle samples with high and low GP levels, the present study showed interesting differences in metabolic pathways and could help explaining the complex network of molecular processes involved in regulation of GP level. The use of expression profiling techniques can give useful information about the genetic regulation of metabolic and physiological processes and about candidate genes for association studies with meat quality in pigs.
Figure 1. Stearoyl-CoA desaturase gene expression level in the individual samples used to construct the two pools. The inset image represents the averaged expression.

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