Evolution of transcriptome profiles during muscle development in Casertana and cosmopolite pig breeds

Mariasilvia D’Andrea, Alberto Pallavicini, Simeone Dal Monego, Renè Dreos, Denis Guiatti, Fabio Pilla

Dipartimento di Scienze Animali, Vegetali e dell’Ambiente, Università del Molise, Campobasso, Italy

Corresponding author: Mariasilvia D’Andrea. Dipartimento di Scienze Animali, Vegetali e dell’Ambiente, Facoltà di Agraria, Università del Molise. Via De Sanctis snc, 86100 Campobasso, Italy - Tel. +39 0874 404818 - Fax: +39 0874 404855 - Email: dandrea@unimol.it

ABSTRACT - In order to determine candidate genes involved in production traits, the mRNA levels of muscle tissue of extremely different pig breeds (genotypes) were studied using microarray tool. Casertana, an autochthonous breed characterized by slow growth and a massive accumulation of backfat, was compared to Large White and to crossbred (Duroc x Landrace x Large White) pigs. The differential expression of muscle genes was evaluated on 3 pigs for each genetic type using a microarray consisting of 10,665 oligos. Animals were of the same age and raised in the same environmental conditions. Muscle tissues were collected at 3, 6, 9, and 11 months and a total of 219 (157 genes in the two main clusters) genes showed differential expression between genetic types. Time series cluster analysis indicated that Casertana breed had a different pattern of gene expressions compared to the Large White and the crossbreed. For Casertana pigs, a first cluster showed 105 genes under expressed at 3 months of age and a second cluster indicated 52 genes over expressed at 3 months of age, in comparison to the other genetic types. As expected, some of the differentially expressed genes were in the category of “contractile fiber” and transcriptional factors involved in muscle development and differentiation.

Key words: Casertana pig, Muscle tissue, Microarray, Time course.

Introduction - Expression profiling using microarrays allows a detailed characterization of gene expression useful to elucidate the metabolic patterns and loci underlining the phenotypic differences in livestock traits (Byrne et al., 2005; Dal Monego et al., 2007). The investigation of the regulation and expression of genes in completely divergent phenotype populations is a useful tool to reveal the effect of genetic variability and to discover candidate genes or eQTL and biochemical pathways involved in the development and regulation of mammalian cells (Lemkin et al., 2000) and could be useful to improve animal breeding schemes and livestock management. The aim of this study was to investigate the differences in gene expression and associated pathways in muscle tissue during post-natal muscle growth of Casertana (CT), Large White (LW), and crossbred (CB; Duroc x Landrace x Large White) pigs. The Casertana pig is a local breed from the South Italy characterized by slow growth and a massive accumulation of backfat (Pietrolà et al., 2006), Large White and the crossbreed were chosen as major commercial breeds with opposite aptitude. Due to the different development of the analyzed breeds, it can be postulated that differences in gene expression in the muscle could be dependent mainly from genetic rather than environmental factors.

Material and methods - Experimental population - Twenty CT, 20 LW, and 20 CB pigs of the same age were reared outdoor in the same environment, were fed twice per day with the same commercial diet, and the productive traits were recorded. Muscle tissues were sampled between the 3rd and 4th...
lumbar vertebrae with biopsies at 3, 6, and 9 months of age; a further sample of muscle tissues was collected at 11 months of age at slaughter. Tissues were immediately frozen in liquid nitrogen and stored at -80°C until analysis. All animals were reared and slaughtered according to European directives and laws. Three pigs within each breed were selected, as closest as possible to the median average daily weight and carcass weight of their group. These pigs were used for gene expression studies. Total RNA form tissue samples was isolated (Trizol Reagent, Life Technologies GIBCOBRL, Carlsbad USA) after grounding with an ultraturrax homogenizer. Spectrophotometer quantification was performed and the RNA quality was checked by 260/280 ratio and gel electrophoresis stained with ethidium bromide.

Microarray and Experimental Design - For the experiment, an array consisting of 10,655 oligo 70mer (Operon Pig Genome Set, Version 1.0, QIAGEN) was used. A pool of identical amount of RNA extracted from samples of 3, 6, 9, and 11 was assembled for each animal, according to the “pooled reference sample” strategy (Townsend, 2003). The differential expression of genes at 3, 6, 9, and 11 months (target sample) was assessed for the individual pig using a competitive hybridisation of the mRNA of each target sample and the respective pooled reference sample (Kerr et al., 2001). Microarray experiment hybridizations were performed in duplicate dye swap, switching the colour between the pooled reference sample and the target sample. Microarray hybridization and data analysis - RNA was initially amplified with an antisense RNA (aRNA; MessagAmp kit Ambion, Foster City, USA) and 3 µg of aRNA was indirectly labelled using Cy3 and Cy5 fluorochrome. Cy3/Cy5-labelled aRNA was purified and hybridized for 16 h at 40°C to the slides in a hybridization chamber (BIO-RAD, Hercules USA). After washing, microarray images were acquired using VersArray Analyzer 5.0 (BIO-RAD, Hercules USA).

The statistical analysis was performed using the open source software project Bioconductor (Gentleman et al., 2004). Low quality spots were filtered using the “filtergene” library as follow: only spots with a ratio between foreground and background intensity greater than 3 in at least one third of all the hybridizations were used for the analysis. This approach filters low quality spots but allows genes that are expressed only in one or two time points. After filtering, the data were normalized using the “scalePrintTipMAD” algorithm implemented in the “marray” library (Yang et al., 2002). Analysis of variance (ANOVA) models were employed to detect differentially expressed genes using the maSigPro library (Conesa et al., 2006). This is a two steps filter. First, differentially expressed genes within each time point are selected using the FDR corrected P-value (Reiner et al., 2002). Second, only genes that change their expression between time points are maintained. Genes were then grouped in cluster considering their expression trend. Data mining of the differentially expressed genes were performed with the freeware Babelomics (http://babelomics.bioinfo.cipf.es/index.html), using the tools “biological function”, “cellular localization” and “transcriptional factors”.

Results and conclusions - The statistical comparison of microarray data of CT, LW, and CB resulted in 219 (157 genes in the two main clusters) genes differentially expressed at different ages. These genes were then grouped in clusters to uncover important trends. In fact, differences during post-natal growth between breeds are highlighted in cluster analysis. The Figure 1 shows the genes differentially expressed during animal growth at each time considering the single genetic type. The dotted line shows the ongoing of the expression in the CT breed. The LW breed and the CB have a similar gene expression trend for all the four considered ages.

The cluster #1 reports genes under-expressed in CT only at 3 months of age and with a similar expression trend in the 3 breeds from the 6th month onward. From these results, it appears that CT pigs have a different gene expression profile than LW and CB. The cluster #2 shows the genes that in CT were over-expressed only at 3 months of age in comparison to LW and CB.

Genes over or under-expressed in CT at 3 months of age in comparison to LW and CB were annotated in the “cellular macromolecule metabolic process (GO:0044260, p<0.01)”, signal transduction (GO:0007165, p<0.01), amine metabolic process (GO:0009308, p<0.05), lipid metabolic process (GO:0006629, p<0.10), and organic acid metabolic process (GO:0006082, p<0.05). In addition, tran-
scriptional factors involved in muscle development and differentiation were found (Evi-1, Myogenin p<0.01; Pit-1 Pax-3 p<0.05).

These results indicate the different involvement of genes during postnatal growth in the genotypes. As expected, some differentially expressed genes were in the category of "contractile fiber" and transcriptional factors involved in muscle development and differentiation.

The unambiguous genetic differences between the CT and the cosmopolitan breed are at 3 months of age and the similar trend observed from 3 months onward, suggests that phenotypic differences in growth and development between these pigs are genetic based. Furthermore, gene expression profiles open new insight for the understanding of cellular processes during muscle post-natal growth and for the identification of candidate genes that could influence muscle phenotype. Further investigations are in progress in order to validate the microarray results through Real-Time PCR.

Figure 1. Gene clusters: Differentially expressed genes during animal growth at 3, 6, 9, and 11 months of age. Each genetic type considered has been represented using a different colour. Unbroken lines represent true expression trends and dotted lines show the ideal expression trends generated by the analysis.

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