Livestock Science 182 (2015) 93–100

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Whole transcriptome analysis of the porcine muscle tissue of breeds differing in muscularity and meat quality traits

K. Ropka-Molik [a,*], K. Żukowski [b], R. Eckert [b], K. Piórkowska [a], M. Oczkowicz [a], A. Gurgul [a], T. Szmatoła [a]

[a] Laboratory of Genomics, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice, Poland
[b] Department of Animal Genetics and Breeding, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice, Poland

ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form
13 October 2015
Accepted 30 October 2015

Keywords:
Pig
Muscle tissue
RNA-seq
Native breed
Candidate genes

ABSTRACT

In pigs, an intense research have been performed in order to select genetic markers, which could be associated with important production traits and may be included in breeding program. The aim of the present study was to compare the whole gene expression profile of *semimembranosus* muscle from three pig breeds differing in muscularity, fatness traits and meat quality. The RNA-seq analysis was performed on 16 animals: Pietrain (5), Polish Landrace – PL (4) and Pulawska pigs (7). The transcriptome sequencing of muscle tissue (*m. semimembranosus*) was carried out on HiScanSQ System (Illumina) with 50 single-end cycles. The validation of obtained results and exact estimation of transcript abundance was performed by real-time PCR method. The present study showed the differential expression of 229 genes between Pulawska and PL pigs, and 87 between Pulawska and Pietrain ($p \leq 0.05$). The Pulawska breed was characterized by significantly higher expression of only 9 and 43 genes compared to Pietrain and PL pigs, respectively. Accordingly to functional annotation of DEGs, several novel genes were proposed which were involved with processes such as purine and pirymidine biosynthesis, cell cycle, protein transport and metabolism. These genes may be potentially responsible for muscle cell growth and development. On the other hand, the up-regulation of genes involved in immune processes, observed in native Pulawska pigs, may be related with a very good resistance to diseases of this breed. The different expression profile of selected genes may illustrate the effect of long-term selection to improve the meat content in carcasses conducted on pigs.

1. Introduction

The whole genome or transcriptome analysis performed with the use of Next Generation Sequencing (NGS) create new possibilities in animal genetics. The transcriptome sequencing (RNA-seq) is a high-throughput method which enables gene expression measurements, including transcripts expressed at low level, detecting alternative splice sites or isoforms, analysis of non-coding RNAs and post-transcriptional mutations (Wang et al., 2009; Wickramasinghe et al., 2014). To date, RNA-seq method has been successfully used to investigate the porcine transcriptome from gonad (Esteve-Codina et al., 2011) and endometrium tissues (Samborski et al., 2013). Authors proposed a new panel of genes involved in spermatogenesis and genes which play an important role in endometrial remodeling during implantation process. Sodhi et al. (2014) profiling the transcriptomes from porcine muscle tissue (*m. longissimus dorsi*) and liver proposed breed specific markers which may be useful to monitor growth performance and meat quality traits. Furthermore, Jung et al. (2012) using RNA-seq method showed that selective nonsynonymous single nucleotide variation (nsSNV; SNP) were associated with porcine meat quality traits.

In the recent few decades breeding and selection programs of pigs have been focused on improving muscularity as well as decreasing the fatness of carcasses. In commercial pig lines animals are characterized by even 60% and above of lean meat content in carcass as well as low fatness (Pulkrabek et al., 2006). Simultaneously with the increase of meat content, the substantial decline of pork quality has been observed. On the other hand, consumers prefer high quality meat i.e. tender, juicy pork with optimal intramuscular fat content (IMF). Therefore, an intense research is conducted in order to select genetic markers which may be included in Marker Assisted Selection (MAS) and allow to improve meat quality without the decrease of meatiness.

The comparison of global gene expression patterns of animal

* Corresponding author. Fax +48 012 285 67 33.
E-mail address: katarzyna.ropka@izoo.krakow.pl (K. Ropka-Molik).

http://dx.doi.org/10.1016/j.livsci.2015.10.024
1871-1413/

groups which varied in a given trait can be used to search for genes associated with exactly these features. Such approach would be helpful in selection of candidate genes related with important production traits in farm animals. The aim of the present study was to compare the whole gene expression profile of *semimembranosus muscle* from three pig breeds differing in muscularity, fatness traits and meat quality. The use of RNA-seq screening method will allow to identify metabolic pathways, biological processes and single genes with a potentially large effect on pig production traits.

2. Material and methods

2.1. Animals and tissues

In total, analysis was performed on 16 animals: Pietrain (5), Polish Landrace – PL (4) and Pulawska pigs (7). All pigs were maintained in the Pig Test Station of the National Research Institute of Animal Production in Chorzelów under the same housing and feeding conditions. 48 h before the slaughter, pigs (sows with an average weight of 100 kg) were fasted. During slaughter the samples of muscle tissues (*m. semimembranosus*) were collected in tubes with RNAlater solution (Ambion Inc., Austin, USA) and stored at −20 °C. The total RNA from muscle tissue was isolated using TRI-Reagent (Sigma-Aldrich, Poznan, Poland) and purified using lithium chloride precipitation (Ambion). The RNA quantity and quality was assessed with the NanoDrop 2000 (Thermo Scientific, Wilmington, USA) and 2% agarose gel electrophoresis.

2.2. Transcriptome sequencing

For each sample, a cDNA library was synthesized using 300 ng of total RNA with the use of TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA) in accordance with the protocol and with the use of differently indexed adapters (Table 1). The concentration of obtained cDNA libraries was estimated by Qubit® 2.0 Fluorometer (Life Technology, Invitrogen). Then, normalized sample libraries (diluted to the 10 nM with Tris–Cl) were pooled and sequenced on a HiScanSQ System (Illumina). For each library two technical replicates were performed. The clustering of the flowcell was performed using TruSeq SR Cluster Kit v3-cBot-HS (Illumina) and sequencing was performed using TruSeq SBS Kit v3-HS chemistry (50 single-end cycles)(Illumina).

2.3. Data analysis

CASAVA software (Illumina) was used to obtain raw fastq sequences and demultiplexing of the samples. Then, Flexbar software (Dodt et al., 2012) was used to remove sequences of Illumina adapters and poliA stretches. Moreover, the reads shorter than 32 bp and those with the quality lower than 10 were removed from the dataset. TheBowtie2 software (Langmead and Salzberg, 2012), implemented in TopHat splice junction mapper (Trapnell et al., 2009), was used to align filtered reads to the *Sus scrofa* genome assembly (Sscrofa10.2.70)(*–b2-very-sensitive option*). The quality statistics were generated in SAMStat and RNA-SeQC (Lassmann et al., 2011; DeLuca et al., 2012). The filter for detection of gene expression was set to 5 reads per gene.

Differentially expressed genes (DEGs) were evaluated with the use of three software: Cufflinks (Trapnell et al., 2010), DESeq (Anders and Huber, 2010) and edgeR (Robinson et al., 2010). The general pipeline of performed analysis was described previously by Ropka-Molik et al. (2014). The functional annotation of differentially expressed genes was performed by using Panther software (Statistical Overrepresentation test) (Huaiyu and Paul, 2009).

2.4. qPCR validation of RNA-seq data

The validation of RNA-seq data was carried out on the same group of animals as transcriptome analysis. The 1 μg of total RNA was reverse transcribed to cDNA by using Transcriptme RNA Kit (Blirt, Poland) according to the protocol. The expression level was evaluated for 9 genes with the use of *OAZ1* gene as an endogenous control. Specific primers were designed using Primer3web version 4.0.0 software (Table S1).The transcript abundance was evaluated on 7500 Real-Time PCR System using AmpliQ 5x HOT EvaGreen® qPCR Mix Plus (ROX) (Novazym, Poland). Reactions for each sample (in a total volume of 20 μl) were performed in three repeats and in accordance to the attached protocol: initial denaturation – 95 °C (15 min) and quantification – denaturation 95 °C (15 s); annealing 60 °C (32 s); elongation 72 °C (20 s) through 40 cycles. The efficiency of real-time PCR reactions was defined by using the standard curve method as E=efficiency ($10^{[-1/\text{slope}]}$). Relative quantity of transcripts abundance was calculated according to Pfaffl (2001).

3. Results

3.1. Quality control of RNA-seq data

The cluster density obtained per lane was between 334(± 106) and 798(± 55) K/mm^2. The total number of the obtained reads that passed the filtering procedure ranged from 44.7 to 95.3 million per lane and on average 23.6 million of reads were obtained per sample (Table 1). After removal of adapter sequences, trimming and filtering, the average of 87.69% of reads were mapped to the porcine reference genome (Sscrofa10.2.70) of which approximately 76.8% were mapped to annotated exons, 5.4% to introns and 17.8% to intergenic regions. In Pietrain and Polish Landrace breeds, the distribution of the annotated reads was similar across individual samples, while Pulawska samples showed a higher number of reads mapped to the intergenic regions (Fig. 1).

Table 1
The overall quality statistics and reads annotation of obtained RNA-seq data for each cDNA library.

	PL1	PL2	PL3	PL4	Pi1	Pi2	Pi3	Pi4	Pi5	Pu1	Pu2	Pu3	Pu4	Pu5	Pu6	Pu7
Applied indexes	*2*	*4*	*7*	*16*	*6*	*12*	*5*	*22*	*19*	*1*	*8*	*10*	*11*	*3*	*9*	*23*
Raw reads obtained per sample (mln)	18.9	21.4	21.4	40.3	46.5	48.2	36.3	40.1	43.5	9.38	10.1	9.06	11.3	9.65	8.88	9.32
Reads obtained after																
Removing adapters and poliA tail (mln)	18.6	21.0	21.0	39.0	45.5	47.2	35.1	38.7	42.2	10.6	10.0	9.08	11.2	9.57	8.82	9.24
Tophat filtered (mln)	18.5	20.9	21.0	38.9	45.5	47.2	35.1	38.7	42.2	9.31	10.0	8.98	11.2	9.57	8.82	9.24
Total purity filtered reads sequenced																
Tophat/accepted_hits.rg.dedup (mln)	17.0	18.4	18.6	31.4	40.8	42.1	29.0	32.9	36.0	8.38	8.95	8.03	9.86	8.45	7.87	8.33
% of reads alignment to reference Genome	**91.6**	**87.6**	**88.5**	**80.8**	**89.7**	**89.3**	**82.5**	**84.8**	**85.4**	**90.0**	**89.9**	**88.3**	**87.8**	**88.2**	**88.5**	**90.1**

PL – Polish Landrace; Pi – Pietrain; Pu – Pulawska pigs.