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Identification of polymorphisms and expression of selected porcine fetal skeletal muscle genes

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Objectives

The aim of this study was to verify and quantify differential expression of selected genes in the porcine fetal skeletal muscles using the real-time qPCR. This study was based on the clones from the subtractive hybridization (tester – cDNA from fetal muscle; driver – cDNA from adult muscle).

Introduction

Gene expression is usually related to internal control, also known as reference gene or housekeeping gene. One of the critical steps in comparing transcription profiles is selection of accurate reference genes. We evaluated 6 candidate reference genes for this study and chose two the most stable reference genes used for relative quantification. The clones from subtractive hybridization were sequenced and obtained sequences were tested for homology with human sequences using BLAST (NCBI) and genes were identified (Table 1). Relative quantification of gene expression was calculated by qBase (see [http://medgen.ugent.be/qbase]).

Table 1: Name and function of identified genes

Symbol	Gene name	Function
ACTC	Actin, alpha, cardiac muscle	Cytoskeletal structural protein
CDK4	Cyclin-dependent kinase 4	Regulation protein involved in the cell cycle
CNN3	Calponin-3	Cytoskeletal structural protein
GNAS	Guanine nucleotide-binding proteins	Transport protein
MYH3	Myosin, heavy chain 3, skeletal muscle, embryonic	Muscle-specific contractile protein
POSTN	Periostin	Extracellular matrix protein
YWHAQ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	Regulation protein

Material and Methods

The skeletal muscle-specific RNA was isolated from the adult Large White pig (m. biceps femoris) – Calibrator and 5 samples of the 50-day fetus (hind limbs) – Sample 1–5. Homogenizations of samples were processed in FastPrep FP 120 (ThermoSavant) and total RNA was isolated using FastRNA Pro Green Kit (Q-BIOgene). cDNA was synthesized from 1 µg of the total RNA solution with Omniscript Reverse Transcriptase (QIAGEN) and oligo(dT)20 primers (Invitrogen). Real-time PCR was performed in the MJ Reasearch PTC-200 a Chromo 4 Detector (MJ Reasearch). The PCR reactions were analysed using Power SYBR® Green PCR Master Mix (Applied Biosystems). The samples were cycled under the following conditions: 50°C for 2 min. (uracil N-glycosylase, digest); 95°C for 10 min. (initial denaturation) and 40 cycles of 95°C for 15 s. (denaturation) and 60°C for 1 min. (primer annealing and primer extension). Amplification was performed in duplicate and a blank was incorporated in each assay. The PCR primers were designed (Table 2) using the Primer Express software v2 (Applied Biosystems) with the exception of GAPDH and PPIA (see Vallee et al., 2003; Biol Reprod. Nov;69(5)). For normalization of gene expression levels in our experiment we compared 6 candidate reference genes: ACTB, ESD, GAPDH, H3F, HPRT1 and PPIA using the geNorm application (see [http://medgen.ugent.be/~jvdesomp/genorm/]). The data from real-time PCR analysis were analyzed by the qBase.

Table 2: Primer sequences

Gene	PCR primers	Product size (bp)
ACTC	CCAGCACCATGAAGATCAAGA AAAGAAGGGTGGGTTGGAAG	232
CDK4	TTGGCTGTATCTTCGCAGAGA CAGCCCAATCAGGTCAAAGAT	98
CNN3	AGATGGGCACCAACAAAGG CGAGTTGTCCACGGGTTGT	104
GNAS	ATTCGGGAGGACAACCAGAC AGGACTTTCTCAGCCAGCAGA	137
MYH3	CATGGAACATAGCCTGGTCCT CAGCACGCGGTATCTTTGTT	127
POSTN	ATTCCTGATTCTGCCAAACAAG AGAAAATGCGTTATTCACAGGC	147
YWHAQ	ATCCAGAACTTGCCTGCACA AAGCAACTGCATGATGAGGGT	113
*ACTB**	CATCAGGAAGGACCTCTACGC GCGATGATCTTGATCTTCATGG	129
*ESD**	GGCTCTCTGGTTTAACTTGCAC GCCAAAATCCCAGCTCTCAT	152
*GAPDH**	CAGCAATGCCTCCTGTACCA GATGCCGAAGTTGTCATGGA	70
*H3F**	AAGAAACCTCATCGTTACAGGC TTTGAAGTCCTGAGCAATTTCC	132
*HPRT1**	AAGGACCCCTCGAAGTGTTG CACAAACATGATTCAAGTCCCTG	122
*PPIA**	GCACTGGTGGCAAGTCCAT AGGACCCGTATGCTTCAGGA	71

* reference gene

Results and Conclusion

Reference gene assay for normalization of real-time qPCR data, obtained from normal and fetus muscle were designed. By geNorm aplication we detected that genes PPIA and H3F are the most stable genes from our 6 candidate reference genes (Figure 1). By real-time qPCR was found that all investigate genes (Table 1) were expressed in both the adult and the fetal samples. The expression levels of the investigated genes were different from 38 to 0.11 expression level (Table 3). The study provides new knowledge on genes playing role in muscle development and growth in pigs, with the prospect of their application in marker assisted selection.

Figure 1: Average expression stability values of remaining control genes calculated by geNorm.

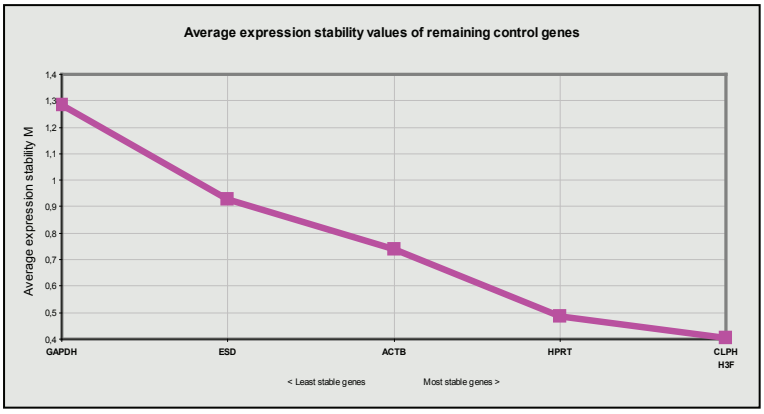

Table 3: Relative expression level of selected genes of fetal muscle (Sample 1–5) compared to adult skeletal muscle (Calibrator).

Sample/Gene	Calibrator	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
ACTC	1.00 ± 0.35	2.36 ± 0.38	3.25 ± 0.70	16.20 ± 2.27	38.00± 5.39	5.41 ± 1.27
CDK4	1.00 ± 0.30	0.39 ± 0.11	2.06 ± 0.42	3.66 ± 0.49	1.62 ± 0.28	7.14 ± 2.45
CNN	1.00 ± 0.48	0.20 ± 0.05	0.16 ± 0.01	0.20 ± 0.02	0.11 ± 0.02	0.66 ± 0.11
GNAS	1.00 ± 0.32	0.42 ± 0.07	0.86 ± 0.05	1.06 ± 0.18	0.67 ± 0.07	3.23 ± 0.88
MYH	1.00 ± 0.34	5.05 ± 0.77	6.94 ± 0.99	7.48 ± 1.12	3.46 ± 0.28	8.29 ± 1.29
POSTN	1.00 ± 0.41	9.17 ± 1.41	13.96 ± 1.46	12.31± 2.27	5.47 ± 0.78	16.67± 4.38
YWHAQ	1.00 ± 0.30	0.84 ± 0.16	0.42 ± 0.07	0.55 ± 0.07	0.36 ± 0.08	0.87 ± 0.13

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