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HMG1A and *PPARG* are differently expressed in the liver of fat and lean broilers

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Abstract The expression of nine functional candidates for QT abdominal fat weight and relative abdominal fat content was investigated by real-time polymerase chain reaction (PCR) in the liver, adipose tissue, colon, muscle, pituitary gland and brain of broilers. The high mobility group AT-hook 1 (*HMG1A*) gene was up-regulated in liver with a ratio of means of 2.90 ($P \leq 0.01$) in the «fatty» group (relative abdominal fat content 3.5±0.18%, abdominal fat weight 35.4±6.09 g) relative to the «lean» group (relative abdominal fat content 1.9±0.56%, abdominal fat weight 19.2±5.06 g). Expression of this gene was highly correlated with the relative abdominal fat content (0.70, $P \leq 0.01$) and abdominal fat weight (0.70, $P \leq 0.01$). The peroxisome proliferator-activated receptor gamma (*PPARG*) gene was also up-regulated in the liver with a ratio of means of 3.34 ($P \leq 0.01$) in the «fatty» group relative to the «lean» group. Correlation of its expression was significant with both the relative abdominal fat content (0.55, $P \leq 0.05$) and the abdominal fat weight (0.57, $P \leq 0.01$). These data suggest that the *HMG1A* and *PPARG* genes were candidate genes for abdominal fat deposition in chickens. Searching of rSNPs in regulatory regions of the *HMG1A* and *PPARG* genes could provide a tool for gene-assisted selection.

Keywords *Gallus gallus* · Quantitative trait · Abdominal fat · Gene expression profiling

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Biological mechanism of the deposition of abdominal fat seems to be important for both the understanding of obesity in humans and increasing productivity in farm animals. Reduction of abdominal fat deposition could allow increasing feed efficiency and carcass value significantly. Based on previously performed research in the Department of Molecular Cytogenetics at the Institute of Genetics and Animal Breeding and the Laboratory of Molecular Genome Organization, Institute of Farm Animal Genetics and Breeding, we selected nine most probable functional candidate genes (FABP1, FABP2, FABP3, HMGA1, MC4R, POMC, PPARG, PPARGC1A and PTPN1) for expression profiling in the adipose tissue, brain, colon, liver, muscle and pituitary gland of broilers.

Fatty acid-binding proteins (FABPs) belong to the family of small cytoplasmic proteins. FABP family members are thought to play roles in fatty acid uptake, transport and metabolism. The *FABP* genes expression level was significantly increased in obese rats compared with controls (López et al. 2003). The *HMGA1* gene encodes high mobility group AT-hook 1 protein. This non-histone protein was involved in a number of cellular processes, such as the integration of retroviruses into chromosomes, metastatic progression of malignant cells and the regulation of

Table 1 Abdominal fat candidate genes and primers

Locus	Accession number	Tissue	Primers (annealing temperature 60°C)	Product size (bp)
FABP1	NM_204192	Liver	FW: GGGGAAGAGTGTGAGATGGA RV: GTTGAGTTCGGTCACGGATT	120
FABP2	NM_001007923	Colon	FW: TGGCATTTAACGGTACTTGGA RV: TCAGATTATCGTGGGCTCCT	111
FABP3	NM_001030889	Muscle	FW: TGAGGAGTTCGATGAGACCA RV: GTCTCCTTCCCATCCCACTT	105
HMGA1	NM_204369	Liver	FW: CAGGAAGAAACCGGAGGAT RV: CTGAGGATTTCCTGCCTTTG	107
MC4R	NM_001031514	Brain	FW: CTCCAGCCTCTCCATTTCTG RV: AAGCTGATGATGCCCAGAGT	146
POMC	NM_001031098	Pituitary gland	FW: ATGCTGGGAGAACAGCAAGT RV: GAACTTGTTCCAGCGGAAAT	181
PPARG	NM_001001460	Liver, adipose tissue	FW: GGGGAAGAGTGTGAGATGGA RV: GTTGAGTTCGGTCACGGATT	120
PPARGC1A	NM_001006457	Liver	FW: TGGCATTTAACGGTACTTGGA RV: TCAGATTATCGTGGGCTCCT	111
PTPN1	NM_204875	Brain, colon	FW: TGAGGAGTTCGATGAGACCA RV: GTCTCCTTCCCATCCCACTT	105
GAPDH	NM_204305	Adipose tissue, brain, colon, liver, muscle, pituitary gland	FW: CCTCTCTGGCAAAGTCCAAG RV: CATCTGCCCATTTGATGTTG	180

inducible gene transcription. The *MC4R* gene encodes melanocortin 4 receptor. *HMGA1* and *MC4R* were significantly associated with a fat deposition measurement in pigs (Kim et al. 2004). The mutations of the *MC4R* gene cause several obesity forms in humans (Tan et al. 2009; Calton et al. 2009; Wangensteen et al. 2009) and could be associated with feed intake, fatness and growth in pigs (Meidtner et al. 2006; Bruun et al. 2006). Pro-opiomelanocortin (POMC) plays a key role in the regulation of body weight. The *POMC* gene mutations were reported to be associated with human obesity (Dubern et al. 2008). The *PPARG* gene encodes the peroxisome proliferator-activated receptor gamma. It was implicated in the pathology of obesity, diabetes, atherosclerosis and cancer (Qi et al. 2000). *PPARGC1A* encodes peroxisome proliferator-activated receptor gamma coactivator 1 alpha. This gene plays a significant role in the development of obesity in humans (Okauchi et al. 2008; Lu et al. 2007; Franks et al. 2007) and affects back fat in pigs (Stachowiak et al. 2007). The *PTPN1* gene encodes tyrosine phosphatase, non-receptor

Table 2 Abdominal fat candidate genes expression profiling in broiler chickens

Locus/tissue	$2^{\Delta\Delta Ct}$		Ratio of means, fatty/lean	Correlation	
	Fatty	Lean		With abdominal fat weight	With relative abdominal fat content
FABP1/liver	1.21±0.22	1.22±0.20	0.99	−0.04	−0.01
FABP2/colon	22.75±4.79	21.25±6.32	1.07	0.41	0.32
FABP3/muscle	0.91±0.19	0.54±0,11	1.69	0.35	0.38
HMGA1/liver	4.09±0.75	1.41±0.31	2.90**	0.70**	0.70**
MC4R/brain	0.64±0.144	1.01±0.25	0.64	−0.33	−0.23
POMC/pituitary gland	478.40±376.06	377.77±208.43	1.27	0.08	0.00
PPARG/liver	2.84±0.56	0.85±0.28	3.34**	0.57**	0.55*
PPARG/adipose tissue	0.87±0.37	0.95±0.46	0.92	0.11	0.15
PPARGC1A/liver	2.45±0.60	3.91±1.12	0.63	−0.22	−0.22
PTPN1/colon	0.81±0.07	1.44±0.29	0.56	−0.37	−0.36
PTPN1/brain	0.97±0.105	1.00±0.086	0.97	−0.08	−0.10

*$P \leq 0.05$, ** $P \leq 0.01$

type 1, which was also reported to dephosphorylate epidermal growth factor receptor kinase. SNPs in this gene are associated with human obesity (Kipfer-Coudreau et al. 2004; Ukkola et al. 2005).

Thirty-five-day-old broilers from cross Iza 15, kindly provided by Dr. O.I. Stanishevskaya (Department of Poultry Science, Institute of Animal Genetics and Breeding, Russian Academy of Agricultural Science), were investigated. Ten birds each from the «fatty» group (relative abdominal fat content 3.5±0.18%, abdominal fat weight 35.4±6.09 g) and the «lean» group (relative abdominal fat content 1.9±0.56%, abdominal fat weight 19.2±5.06 g) were used for expression quantification.

The mRNA samples were isolated from frozen tissues using the Aurum total RNA Fatty and Fibrous Kit (Bio-Rad, USA).

Gene-specific primers were designed using database information (http://www.ncbi.nlm.nih.gov and http://www.ensembl.org) and the computer software PRIMER _3 (http://frodo.wi.mit.edu/primer3) (Table 1). *GAPDH* was used as a reporter gene in our experiment. The polymerase chain reaction (PCR) reaction mix was prepared following standard protocols using the iScript One-Step RT-PCR Kit (Bio-Rad, USA). The $2^{\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) for the calculation of the relative ratio was used. Differences between the mean of candidate-expressed sequence tags quantities in tissues of «fatty» and «lean» groups were tested for by a two-tailed *t*-test. Pearson's technique was used for correlation calculation.

No significant difference was detected in expression of the genes *FABP1* and *PPARGC1A* in liver, *FABP2* and *PTPN1* in colon, *MC4R* and *PTPN1* in brain, *FABP3* in skeletal muscle, *PPARG* in adipose tissue and *POMC* in pituitary gland in broilers with different relative abdominal fat content and abdominal fat weight. Correlation of their expression with the investigated traits was also not significant (Table 2). No significant difference in expression of the *FABP* family of genes was also shown by Wang et al. 2009.

The *HMGA1* gene was up-regulated in liver with a ratio of means of 2.90 ($P \leq 0.01$) in the «fatty» group relative to the «lean» group (Table 2). Expression of this gene was highly correlated with the relative abdominal fat content (0.70, $P \leq 0.01$) and the abdominal fat weight (0.70, $P \leq 0.01$) (Table 2). Significant influence of *HMGA1* and *MC4R* in growth and fat deposition in duroc pigs has been shown (Kim et al. 2006); however, Stachowiak et al. 2006 did not find an *MC4R* effect on the production traits of polish large white and polish landrace pig breeds.

The *PPARG* gene was also up-regulated in liver with a ratio of means of 3.34 ($P \leq 0.01$) in the «fatty» group relative to the «lean» group. Correlation of its expression was significant with both the relative abdominal fat content (0.55, $P \leq 0.05$) and the abdominal fat weight (0.57, $P \leq 0.01$) (Table 2), but this gene did not show a significant difference in expression in adipose tissue. The *PPARG* gene encodes the peroxisome proliferator-activated receptor gamma, which participates in adipocytes differentiation. Hyperexpression of this gene correlates with obesity in humans (Hindle et al. 2009). Three SNPs in the *ESR1* and *PPARG* genes were shown to be genetically linked with obesity in Han Chinese (Chen et al. 2009). Association of the *PPARG* expression with fat deposition in broilers was also reported by Sato et al. (2004). This gene was found among the differentially expressed proteins in adipose tissue of divergently selected broilers (Wang et al. 2009).

These data suggest that the *HMG1A* and *PPARG* genes were candidate genes for abdominal fat deposition in chickens. It is interesting that both candidate genes (*HMGA1* and *PPARG*) are responsible for adipocytes proliferation and, until 4–5 weeks of age, increase in the number of fat cells (hyperplasia) dominates in abdominal fat deposition in chickens (Leenstra 1986; Mourot and Hermier 2001).

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