Saudi Journal of Biological Sciences (2017) 24, 945–949

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Differential expression of six genes and correlation with fatness traits in a unique broiler population

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Received 4 January 2015; revised 16 April 2015; accepted 22 April 2015
Available online 30 April 2015

KEYWORDS

Chicken;
Abdominal fat;
Liver tissue;
Candidate genes;
Differential expression

Abstract Previous results from genome wide association studies (GWASs) in chickens divergently selected for abdominal fat content of Northeast Agricultural University (NEAUHLF) showed that many single nucleotide polymorphism (SNP) variants were associated with abdominal fat content. Of them, six top significant SNPs at the genome level were located within *SRD5A3*, *SGCZ*, *DLC1*, *GBE1*, *GALNT9* and *DNAJB6* genes. Here, expression levels of these six candidate genes were investigated in abdominal fat and liver tissue between fat and lean broilers from the 14th generation population of NEAUHLF. The results showed that expression levels of *SRD5A3*, *SGCZ* and *DNAJB6* in the abdominal fat and *SRD5A3*, *DLC1*, *GALNT9*, *DNAJB6* and *GBE1* in the liver tissue differed significantly between the fat and lean birds, and were correlated with abdominal fat traits. The findings will provide important references for further function investigation of the six candidate genes involved in abdominal fat deposition in chickens.

1. Introduction

Because of the strong association with a number of diseases, including insulin resistance, type 2 diabetes mellitus, atherosclerosis and ischemic heart disease, obesity produces adverse health consequences in humans (Spiegelman and Flier, 2001; Hotamisligil, 2006; Shoelson et al., 2006). A similar problem exists in chickens. Excessive accumulation of fat in chicken abdomens does not only reduce carcass yield and feed efficiency, but is also a less desirable product for consumers. Therefore, mechanisms of obesity occurrence, genes regulating fat deposition and the development of adipose tissue are issues

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.04.014

identified either by traditional research methods or high-throughput techniques (Wang et al., 2006; Gesta et al., 2007).

Notwithstanding increased knowledge of obesity, the genes influencing fatness remain incompletely detected. As one of the major tools, genome wide association studies (GWAS) have resulted in a dramatic increase in the identification of susceptibility variants associated with obesity in humans and domestic animals (Scherag et al., 2010; Day and Loos, 2011; Hu et al., 2013).

In recent years, many variants and genes associated with obesity in chickens have been successfully identified using GWAS. Abasht et al. revealed cryptic alleles as an important factor in heterosis for fatness in a chicken F_2 population (Abasht and Lamont, 2007). Liu and Sun identified some candidate genes associated with abdominal fat traits in an F_2 resource population derived from a cross between a Chinese local breed and a commercial rapid-growing broiler line (Liu et al., 2013; Sun et al., 2013).

Previously, many variants associated with abdominal fat traits have been identified using GWAS in our laboratory (unpublished data). Of them, six top significant single nucleotide polymorphisms at the genome level were located within *SRD5A3* (Steroid 5α-reductase 3), *SGCZ* (Sarcoglycan, zeta), *DLC1* (Deleted in liver cancer 1), *GBE1* (Glucan (1,4-alpha-), branching enzyme 1), *GALNT9* (N-acetylgalactosaminyltransferase 9) and *DNAJB6* (DNAJ homology subfamily B member 6), suggesting that these genes play important roles in fat deposition in chickens. Here, we investigate whether these six genes are differentially expressed in fat and liver tissues between fat and lean broilers and the relationship between their expression levels and abdominal fat content, which would help in our understanding of the roles these genes play in chicken adipose tissues.

2. Materials and methods

2.1. Experimental animals

The broilers used in this study were derived from the Northeast Agricultural University (NEAU) broiler lines divergently selected for abdominal fat content (NEAUHLF). The NEAUHLF line has been selected since 1996 and the selection procedure and raising conditions have been described in detail previously (Wang et al., 2007; Guo et al., 2011). For each line, a total of 10 male and 6 female birds from the 14th generation population were used. Birds were slaughtered at 7 weeks of age, the average abdominal fat weight (AFW) with standard error and average abdominal fat percent (AFP) with standard error of the lean line were 12.53 ± 1.17 g and 0.59% ± 0.05%, respectively, however, for the fat line, they were 54.09 ± 1.93 g and 3.29% ± 0.13%. There were significant differences in both AFW and AFP between the two lines. Samples were collected from abdominal fat and liver tissues, then weighed and immediately frozen in liquid nitrogen, and stored at −80 °C until analysis.

2.2. RNA extraction and cDNA synthesis

Total abdominal and liver RNA was isolated using TRIZOL® Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted RNA was dissolved in DEPC-treated water and the purity and integrity were estimated by an ultraviolet/visible spectrophotometer (Pharmacia, USA) at a 260/280 nm absorbance ratio (range 1.8–2.0 indicates a pure RNA sample) and agarose gel electrophoresis. Total RNA was reverse transcribed to cDNA in a reaction volume of 20 μL containing 1 μg total RNA, 0.5 μL of 50 pmol/L Oligo d(T)18 Primers and finally supplemented with nuclease-free water to a volume of 5 μL for the first step. This mixture was heated at 70 °C for 5 min and incubated on ice-water for 5 min. Then 5× Reverse transcription Buffer 4 μL, $MgCl_2$ (25 mM) 2.5 μL, dNTP Mixture 1 μL, RNase Inhibitor (Promega Biotech Co. Ltd) 0.5 μL, Improm-II Reverse Transcriptase (Promega, Madison, WI, USA) 1 μL and nuclease-free dH_2O were added to a final volume of 20 μL. The RT mixture was incubated at 25 °C for 5 min, then 42 °C for 60 min and finally inactivated by heating at 70 °C for 15 min. The cDNA was directly for use in quantitative real-time PCR.

2.3. Quantitative analysis of mRNA expression

Special primers for amplifications of these genes were designed spanning at least one intron to avoid genomic DNA contamination using Primer Premier 5.0 software according to Ensembl. All primers were synthesized by Invitrogen Biotechnology (Shanghai) Co., Ltd. (Table 1).

SYBR Green real-time PCR amplifications were conducted using an AB Applied Biosystems 7500 Real Time PCR System (Life Technologies, USA). The stably expressed gene, GAPDH, served as the endogenous reference for determination of targeted mRNA profiles (Bustin, 2002). Quantitative PCR amplifications were performed in a final volume of 10 μL reaction mixture under the optimum reaction conditions including 5 μL SYBR® Permix Ex Taq™ II (TaKaRa, Japan), 0.2 μL ROX Reference Dye II (TaKaRa, Japan), 0.2 μL

Table 1 Primer sequences used in this study.

Gene symbol	Forward primer (5′–3 ′)	Reverse primer (5′–3′)	Production size (bp)	Anneal temp (°C)	GenBank No.
SRD5A3	TGGACTTGGCTATTACGTTGCTG	CATCGCAACGCCTATGATGTG	122	60	ID: 422750
SGCZ	GCTCTGCGTCTGTCCCAATG	AGCTCCACAAGCAGATGTTGCTA	92	60	ID: 422739
DLC1	ATGAGAGTTCAACAGACAG	TAAAAGCATAATGGCAG	194	60	ID: 422740
GBE1	ATTTGTGGATGGTGGACT	CATACCCTTTACCCTCAA	132	60	ID: 427964
GALNT9	AGATTGGCTTGCTTGAC	TGTAGGGTTTCTTTGTGC	153	60	ID: 416796
DNAJB6	AGCCTTTGCTGAGGAGT	CTTGCTGCCTTCTTTGTAT	211	60	ID: 420448
GAPDH	AGAACATCATCCCAGCGT	AGCCTTCACTACCCTCTTG	184	60	ID: 374193