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Differential expression of six chicken genes associated with fatness traits in a divergently selected broiler population

Xianwen Wu [a, b, c], Qi Zhang [a, b, c], Songsong Xu [a, b, c], Pengcheng Jin [a, b, c], Peng Luan [a, b, c], Yumao Li [a, b, c], Zhiping Cao [a, b, c], Li Leng [a, b, c], Yuxiang Wang [a, b, c], Shouzhi Wang [a, b, c, *]

[a] Key Laboratory of Chicken Genetics and Breeding, Ministry of Agriculture, Harbin 150030, China
[b] Key Laboratory of Animal Genetics, Breeding and Reproduction, Education Department of Heilongjiang Province, Harbin 150030, China
[c] College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China

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ABSTRACT

A genome-wide association study has shown a number of chicken (*Gallus gallus*) single nucleotide polymorphism (SNP) markers to be significantly associated with abdominal fat content in Northeast Agricultural University (NEAU) broiler lines selected divergently for abdominal fat content (NEAUHLF). The six significant SNPs are located in the kinase insert domain receptor (*KDR*), tumor suppressor candidate 3 (*TUSC3*), phosphoribosyl pyrophosphate amidotransferase (*PPAT*), exocyst complex component 1 (*EXOC1*), v-myb myeloblastosis viral oncogene homolog (avian)-like 2 (*MYBL2*) and *KIAA1211* (undefined) genes. In this study, the expression levels of these genes were investigated in both abdominal fat and liver tissues using 32 14th generation chickens from the NEAUHLF. The levels of expression of *KDR* in abdominal fat and *KDR* and *TUSC3* in liver differed significantly between the two lines. The expression level of *KDR* in the abdominal fat was significantly correlated with the abdominal fat weight (AFW) and abdominal fat percentage (AFP). The expression levels of *KDR*, *TUSC3* and *PPAT* in liver were significantly correlated with AFW and AFP, indicating that the six genes, especially *KDR* and *TUSC3*, could be associated with fat traits in domestic chickens. This study could provide insight into the mechanisms underlying the formation of abdominal fat in chickens.

1. Introduction

Chickens selected for rapid growth have an increased risk of physiological disorders such as obesity [1]. The excessive deposition of abdominal fat can lead to diseases such as ascites, leg malformation and sudden death syndrome in broiler chickens [2,3]. Breeding chickens with less abdominal fat has become a goal of the poultry industry.

Genetic improvement of meat quality and carcass traits through traditional selection strategies is difficult because these traits have low or moderate heritability and, in general, can only be measured post slaughter [4,5]. Abdominal fat is an important factor in meat quality and carcass traits in chickens. Efficient selection for improved meat quality and carcass traits through marker-assisted selection (MAS) or genomic selection using high-throughput genomic techniques is achievable. Genome-wide association studies (GWASs) are commonly used for the identification of genes responsible for complex traits in farm animals, which greatly facilitates MAS or genomic selection. GWASs have been used to identify major genomic loci associated with important economic traits in chickens [6].

In this study we have used a GWAS to identify a number of single nucleotide polymorphisms (SNPs) associated significantly with abdominal fat weight (AFW) and abdominal fat percentage (AFP) in chickens. Six significant SNPs located in the *KDR*, *TUSC3*, *PPAT*, *EXOC1*, *MYBL2* and *KIAA1211* genes were chosen to investigate whether these six genes affect the accumulation of abdominal fat via analysis of differential expression in abdominal fat and liver and by analysis of the correlation between the level of gene expression and AFW and AFP values.

* Corresponding author. College of Animal Science and Technology, Northeast Agricultural University, No. 59 Mucai Street, Xiang Fang District, Harbin, Heilongjiang, 150030, China.
E-mail address: shouzhiwang@126.com (S. Wang).

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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 using the AFP (abdominal fat weight/body weight) and the plasma very low-density lipoprotein (VLDL) concentration as selection criteria. The entire G_0 generation of NEAUHLF came from the same Arbor Acres broiler grandsire line, which was then divided into two lines according to VLDL concentration at seven weeks of age. From G_1 to G_{14}, birds from each line were raised in two hatches. Plasma concentrations of VLDL were measured in all male birds at seven weeks of age and the AFP of the male birds in the first hatch was measured after slaughter at seven weeks of age. Sibling birds from the families with AFPs lower or higher than the average value of the population were selected as candidates for breeding. The plasma concentration VLDL and the body weight (BW) of male birds in the second hatch and egg production of female birds in both hatches were taken into consideration. The selection procedure and rearing conditions have been described [7]. The AFW and AFP differed significantly between the two lines from the fourth generation onwards, with the AFP of the fat line being nearly 4.45-fold greater than that of the lean line at 49 days old. BW at seven weeks of age was not significantly different between the two lines, indicating that selection for AFP was very efficient in the subsequent generations (Fig. 1). All birds were housed under identical environmental conditions with free access to food and water. They were fed a commercial soybean-based diet that met all of the NRC requirements. The birds received a starter diet of 3000 kcal ME/kg and 210 g/kg CP until they reached three weeks of age. They were fed a grower diet of 3100 kcal ME/kg and 190 g/kg CP from three to seven weeks of age [8].

Sixteen fat (ten male and six female) and 16 lean (ten male and six female) age-matched birds of 14th generation (G_{14}) were used in this study. The birds were slaughtered when they were seven weeks old. There were significant differences in both the AFW and AFP between the two lines. The average (±standard error) AFW and AFP values were 12.53 (±1.17) g and 0.59 (±0.05)%, respectively, for the lean line, and 54.09 (±1.93) g and 3.29 (±0.13)%, respectively, for the fat line. The average (±standard deviation) plasma concentration VLDL value of the birds of the G_{14} was 0.18 (±0.06) $mmol \cdot L^{-1}$ for the lean line, and 0.32 (±0.14) $mmol \cdot L^{-1}$ for the fat line, respectively.

Abdominal fat and liver samples were collected and immediately frozen in liquid nitrogen and stored at −80 °C [9].

2.2. RNA extraction and cDNA preparation

Total RNA was extracted from the abdominal fat and liver samples using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions then dissolved in DEPC-treated water. The concentration, purity and integrity were assessed using an Eppendorf Biophotometer (Eppendorf, Hamburg, Germany) to measure the 260 nm/280 nm absorbance ratio (range 1.8–2.0 indicates pure RNA) and electrophoresis in 1% (w/v) agarose gel was used to verify the integrity.

Total RNA was reverse transcribed to cDNA in a reaction volume of 20 μL containing 1 μg of total RNA, 0.5 μL of 50 pmol/L oligo$(dT)_{18}$ primers and 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 then incubated in ice-water for 5 min. Subsequently, 4 μL of 5× reverse transcription buffer, 2.5 μL of 25 mM $MgCl_2$, 1 μL of dNTP mixture, 0.5 μL of RNase inhibitor (Promega Biotech Co. Ltd, Beijing, China), 1 μL of Improm-II Reverse Transcriptase (Promega, Madison, WI, USA) and nuclease-free distilled water were added to make a final volume of 20 μL. The mixture was incubated at 25 °C for 5 min, 42 °C for 60 min and inactivated by heating at 70 °C for 15 min. The cDNA was subsequently used in real-time quantitative PCR (qPCR) [9].

2.3. Quantitative analysis of mRNA expression of six chicken genes

The expression levels of six genes were measured using real-time PCR. Primers for the amplification of the six target genes and two internal controls were designed spanning one intron to avoid genomic DNA contamination. Premier 5.0 software was used to design the oligonucleotide primers set for the eight genes (Table 1).

SYBR Green real-time PCR amplifications were performed using an Applied Biosystems® 7500 Real Time PCR System (Life Technologies, Gaithersburg, MD, USA). The internal controls *GAPDH* and β-*actin* served as endogenous references. qPCR amplifications were performed in a reaction volume of 10 μL consisting of 5 μL of SYBR® Permix ExTaq™ (Perfect Real Time, Dalian, China), 0.2 μL of ROX™ Reference Dye II (50×), 0.4 μL of 10 μM primer for target or internal control and supplemented with 3.4 μL of water and 1 μL of cDNA.

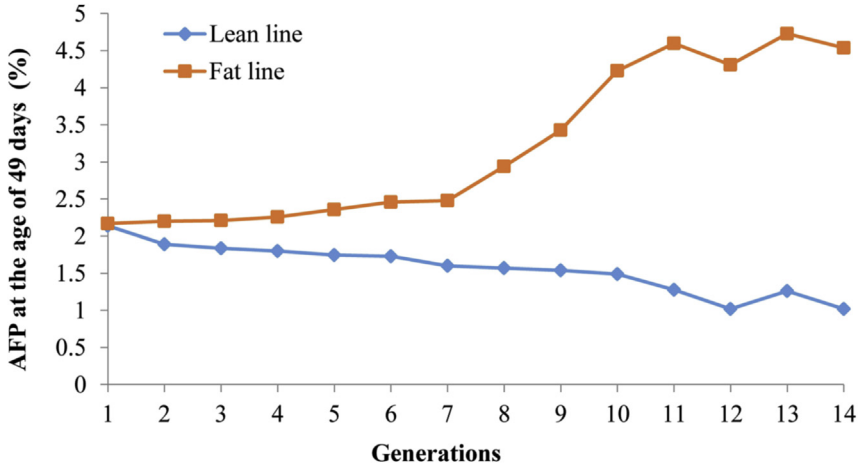

Fig. 1. Phenotypic changes after 14 generations of divergent selection for high and low abdominal fat content.