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Detection of SNPs in the TBC1D1 gene and their association with carcass traits in chicken

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ABSTRACT

TBC1D1 plays an important role in numerous fundamental physiological processes including muscle metabolism, regulation of whole body energy homeostasis and lipid metabolism. The objective of the present study was to identify single nucleotide polymorphisms (SNPs) in chicken TBC1D1 using 128 Erlang mountainous chickens and to determine if these SNPs are associated with carcass traits. The approach consisted of sequencing TBC1D1 using a panel of DNA from different individuals, revealing twenty-two SNPs. Among these SNPs, two polymorphisms (g.69307744C>T and g.69307608T>G) of block 1, four polymorphisms (g.69322320C>T, g.69322314G>A, g.69317290A>G and g.69317276T>C) of block 2 and four polymorphisms of block 3 (g.69349746G>A, g.69349736C>G, g.69349727C>T and g.69349694C>T) exhibited a high degree of linkage disequilibrium in all test populations. An association analysis was performed between the twenty-two SNPs and seven performance traits. SNPs g.69307744C>T, g.69340192G>A and g.69355665T>C were demonstrated to have a strong effect on liveweight (BW), carcass weight (CW), semi-eviscerated weight (SEW) and eviscerated weight (EW) and g.69340070C>T polymorphism was related to BW, SEW and BMW in chicken populations. However, for the other SNPs, there were no significant correlations between different genotypes and carcass traits. Meanwhile, haplotype CT–TG of block 1 and combined genotype AG–TT–AC–CT of block 3 were significantly associated with BW, CW, SEW and EW. Overall, our results provide evidence that polymorphisms in TBC1D1 are associated with carcass traits and would be a useful candidate gene in selection programs for improving carcass traits.

1. Introduction

With the continued improvement of living standards in China, there is increased consumer demand for consistent quality and constant supply of chicken meat products. Many factors influence chicken meat quality including muscle development and tenderness, especially the subcutaneous, abdominal and intramuscular fat deposition. Recently, through marker-assisted selection (MAS) and/or genome wide association (GWA) methods, researchers have selected candidate genes associated with some economic traits. In chicken, some gene polymorphisms have already been shown to be associated with carcass traits or fat deposition (Xu et al., 2012). TBC1D1 is one of the genes identified as a possible contributor to obesity and other related traits (Fontanesi and Bertolini, 2013).

The TBC1D1 [TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1] is a member of the Rab-GAP (GTPase-activating protein) family that is highly expressed in skeletal muscle and involved in the regulation of glucose transport (Chadt, 2008; Taylor et al., 2008). The human TBC1D1 gene is located on chromosome 4, and consists of 20 core exons, encoding 1168 amino acid residues (Fontanesi and Bertolini, 2013). In humans, SNPs of the TBC1D1 gene are associated with body mass index (BMI) (Arya et al., 2004; Stone et al., 2002, 2006). Using high density SNP chips, Fox et al. (2007) found that rs10517461 (in the TBC1D1 gene) was associated with waist circumference. Meanwhile, the p.Val228Gly variant of TBC1D1 was associated with diabetic nephropathy (Savage et al., 2008). In animals, fat deposition and correlated traits (growth rate, feed conversion rate, and so on) are also important phenotypes that affect production efficiency and profitability.

Hence, Fontanesi identified TBC1D1 as a candidate gene for fat and lean meat deposition in pigs (Fontanesi et al., 2011, 2012). They found that the TBC1D1 SNP was the third most significant associated marker after two other polymorphisms (IGF2 and MC4R) in genes already known to affect fatness in pigs. To date, some indirect evidence of a

Abbreviations: SNP, single nucleotide polymorphisms; BW, liveweight; CW, carcass weight; EW, eviscerated weight; SEW, semi-eviscerated weight; BMW, breast muscle weight; LMW, leg muscle weight; AW, abdominal fat weight; LD, linkage disequilibrium; TFBSs, transcription-factor binding sites; NIT2, the nitrilase family member 2 gene; Lyf-1, the lymphoid transcription factor; HSF, heat shock factor protein; CAP, adenylate cyclase-associated protein.

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possible involvement of the TBC1D1 gene in affecting production traits in chicken has been reported. For example, Nassar et al. (2013) found an important QTL on chromosome 4, affecting visceral, subcutaneous neck and total adipose tissue mass. Other researchers reported QTL for growth rate and body weight in the same region (Ambo et al., 2009; Sewalem et al., 2002; Zhou et al., 2006). Gu et al. (2011) also found a marker positioned only 92 kb downstream of the TBC1D1 gene that was associated with body weight at 12 weeks of age. Interestingly, Rubin et al. (2010) reveal loci under selection during chicken domestication by whole-genome resequencing, in which TBC1D1 was reported as an important gene related to selection sweep. Thus, the TBC1D1 gene might play an important role in carcass fat deposition.

Because there are few studies on the effect of TBC1D1 gene polymorphisms in chickens, the objective of the current study was to identify single nucleotide polymorphisms in the chicken TBC1D1 gene, and to carry out haplotype construction and association analysis in order to contribute to the understanding of the role of TBC1D1 in variation of carcass traits in chickens, which could benefit chicken breeding and genetics.

2. Materials and methods

2.1. Ethics statement

Healthy Erlang mountainous chickens were selected from the experimental farm for poultry breeding of the Sichuan Agricultural University (Ya'an, China). This study was carried out in strict accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals of the State Council of the People's Republic of China. The Committee on Experimental Animal Management of Sichuan Agricultural University approved the study. Chickens involved in this study were humanely sacrificed as necessary to reduce their suffering.

2.2. Animal material and DNA extraction

The chicken lines/breeds used for the experiment were Erlang Mountainous chickens (including SD02 and SD03 strains), an indigenous chicken breed distributed in Tianquan of the Sichuan Province, with superior performance traits and juicy meat characteristics. These strains were developed on an experimental farm for poultry breeding at the Sichuan Agricultural University (Ya'an, China) (Lan et al., 2010; Yin et al., 2013). All chickens involved in this study were housed in a modern, nationally certified animal facility under the supervision of board-certified veterinarians. The first group was comprised of 64 SD02 chickens (32 females and 32 males) with yellow partridge plumage, blue shanks and white skin. These chickens have a favorable meat quality and body weights. The second group of chickens was comprised of 64 SD03 chickens (32 females and 32 males) with yellow partridge plumage and normal body weight. During the growth period, all birds had free access to food and water ad libitum under the same temperature and lighting conditions, meanwhile, their nutrition levels were completely consistent. Venous blood samples taken from under the wing of the adult chickens were prepared for DNA extraction. Then, all chickens were sacrificed by qualified technicians in a clean slaughterhouse by having their carotid severed with clean neck cutters under anesthesia. Total DNA was isolated by the standard phenol/chloroform method. The purity and concentration of them were measured by a Nucleic Acid Protein Analyzer. Based on the machine reading of the concentrated stocks, TE buffer was added to DNA samples extracted from the blood to produce a target concentration of 100 ng/μL. The DNA samples were stored at −20 °C until use.

2.3. Performance measurement

Performance traits were measured for all chickens at the laboratory of livestock and poultry in Sichuan Agricultural University. At 90 d of age, liveweight (BW) was obtained on chickens after a 12 hour food withdrawal. After slaughter on the same day, carcass weight (CW), eviscerated weight (EW), semi-eviscerated weight (SEW), breast muscle weight (BMW), leg muscle weight (LMW) and abdominal fat weight (AW) were obtained. All of these performance traits were determined as described in "The Poultry Production Performance Terms and Measurement Statistics Method" (NY/T823-2004).

2.4. TBC1D1 genetic variant identification

The 22 pairs of primer (Table 1) were designed based on the chicken TBC1D1 gene sequence (EMBL ID: ENSGALG00000013521). Primers were synthesized by Shanghai Yingjun Biotechnology Co. Ltd. (Shanghai, China). Sequences were obtained from Erlang mountainous chicken DNA pool (30 random chicken DNA samples in the each DNA pool). PCR reactions were performed using the Gene Amp PCR System 9700 (Bio-Rad, USA) thermal cycler in a final volume of 25 μL reaction containing 2 μL of pooled DNA, 1.25 μL (10 pmol/μL) of each primer and 12.5 μL 2× Master mix (including Mg^{2+}, dNTPs, *Taq* DNA polymerase; Beijing TIAN WEI Biology Technique Corporation, Beijing, China). The amplification conditions included an initial step of denaturation for 5 min at 95 °C; 35 cycles of 35 s at 95 °C, 35 s at 55 °C (or other apt annealing temperature as shown in Table 1), and 40 s at 72 °C; and a final extension step for 7 min at 72 °C. PCR products were purified with a gel extraction kit (Takara, Dalian, China) and sequenced on an ABI 377 DNA sequencer (Shanghai Sangon Biological Engineering Technology, Shanghai, China). Sequences were analyzed with the DNASTAR software and the CodonCode Aligner software (http://www.codoncode.com/aligner).

Based on the sequencing of the two DNA pools, polymorphisms were identified with nine of the primer pairs. Genotyping was performed using DNA samples extracted from blood samples collected from the 128 chickens. To analyze the mutations, PCR was performed as described above (Table 1). Amplified products were electrophoresed and purified with a gel extraction kit (Takara, Dalian, China) and sequenced by Shanghai Sangon Biology Technique Corporation.

2.5. Data analysis

Allele and genotype frequencies were determined by direct counting. Hardy–Weinberg equilibrium was established with χ^2 testing. The linkage disequilibrium (LD) structure as measured by D′ and r^2 was performed with the Haploview software (Version 3.32) (Barrett et al., 2005).

In order to identify putative gains or losses of transcription-factor binding sites (TFBSs) generated by the alternative SNP alleles, sequences (GenBank accession number NM_001267573.1) surrounding the SNP positions were subjected to in silico analysis using the TFSEARCH tool (http://www.cbrc.jp/research/db/TFSEARCH.html). The vertebrate transcription factor matrices and threshold score of 85.0 were used to reduce the incidence of false positives. The TFSEARCH outputs originated by alternative SNP alleles were visually compared to identify putative gain or loss (abrogation) of TFBSs.

The general linear model (GLM) procedure of SAS 6.12 (Statistical Analysis Systems Institute Inc., Cary, NC) was used to test associations between the genotyped markers and carcass traits. The model is as follows:

$$Y_{ijk} = \mu + S_i + G_j + B_k + G_j \times S_i \times B_k + e_{ijk}$$

where Y is the trait measured on chickens, μ is the population mean, S_i is the fixed effect of sex, G_j is the fixed effect of genotype, B_k is the fixed effect of breed, $G_j \times S_i \times B_k$ is the interaction among genotype, sex and breed, and e is the random error. The values were presented as least square means ± se. Statistical significance was evaluated using Duncan's test. Differences were considered significant at $P < 0.05$.