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Expression of variant transcripts of the potassium channel tetramerization domain-containing 15 *(KCTD15)* gene and their association with fatness traits in chickens

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ABSTRACT

The aim of this study was to characterize the structure, expression, and biological functions of potassium channel tetramerization domain containing 15 (*KCTD15*) in chickens. We compared the *KCTD15* expression level in samples of hypothalamic, adipose, and liver tissue of Xinghua chickens that were maintained on different dietary status. An association analysis of *KCTD15* gene variant transcripts with fatness traits in a F2 resource population of chickens was performed. Three *KCTD15* transcripts were identified in which the complete transcript was predominantly expressed in adipose tissue and the hypothalamus. The chicken *KCTD15* gene was regulated by both feeding and fasting and consumption of a high-fat diet. The expression level of *KCTD15* gene was markedly decreased in hypothalamus and liver of fasted and refed chickens ($P < 0.05$) and significantly downregulated in adipose tissue by the high-fat diet ($P < 0.05$). Three single-nucleotide polymorphisms of the *KCTD15* gene were significantly associated with a number of fatness traits in chicken ($P < 0.05$). These results suggest that *KCTD15* have a potential role regulation of obesity and fat metabolism in chickens.

1. Introduction

Obesity is a major public health problem because of an increased risk for development of several diseases and imposition of severe economic burdens on health care systems [1,2]. The prevalence of obesity has dramatically increased worldwide during the past years. Recently, many genome-wide association studies showed that potassium channel tetramerization domain containing 15 (*KCTD15*) gene was significantly associated with both body mass index and weight in adult of various populations, including European [3,4,5,6], Chinese [7,8], Americans [9], and Japanese [10]. This result was also confirmed in children and adolescents [11,12,13,14].

A family of T1 domain containing proteins (the KCTD family) was described in recent years [15,16]. The KCTD family shares a common N-terminal domain. It is a close relative of the BTB (Broad-Complex, Tramtrack and Bric-a-brac)/POZ (poxvirus and zinc finger) domain, which is a major protein-protein interaction motif found in viruses and throughout eukaryotes [17]. The KCTD proteins play an important role in cell differentiation and vertebrate development [18]. As a member of the KCTD family, *KCTD15* encodes a protein with BTB domain and inhibits neural crest induction [19,20], and it could have transcription factor activity because of its sequence homology to *KCTD1* [21]. Another voltage-gated K channel, Kv1.3, has been reported to regulate body weight, glucose uptake, insulin sensitivity, and energy homeostasis [22,23]. It is possible that *KCTD15* could have similar functions.

Obesity is a result of an imbalance between food intake and energy expenditure. The *KCTD15* gene was highly

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expressed in the hypothalamus, which is a crucial center for energy balance and regulation of food intake [4,24,25]. The highest expression of *KCTD15* was also found for rats and mice in the hypothalamus [26,27]. The single-nucleotide polymorphisms (SNPs) of *KCTD15* were associated with dietary intake, including carbohydrate and fat intake. A recent study in mice showed that the messenger RNA (mRNA) level of *KCTD15* was dependent on the nutritional status [27], its expression was upregulated in hypothalamus and adipose tissue of fed mice and downregulated by high-fat feeding in adipose tissue and the hypothalamus. Decreased *KCTD15* expression was also found in hypothalamus and adipose tissues of rats that fed on a high-fat diet [28]. This suggests a central regulation role of *KCTD15* gene on energy balance.

Fatness traits are important economic traits in chickens, the fat mass play an important role in the meat quality and flavor of chicken. The *KCTD15* gene was found to be associated with multiple meat quality traits in swine [29]. There were very less information about *KCTD15* gene in chicken and whether it might be linked to fatness traits remains unknown. In this study, we identified the chicken *KCTD15* gene and monitored its mRNA level in various tissues under different conditions of nutrition and performed association analysis of *KCTD15* SNPs with fatness traits in chicken, so as to first characterize the *KCTD15* gene in poultry.

2. Materials and methods

2.1. Ethics statement

All animal experiments were handled in compliance with and approved by the Animal Care Committee of South China Agricultural University (Guangzhou, People's Republic of China) (approval number: SCAU#0011). All efforts were made to minimize suffering to animal.

2.2. Animals and DNA samples

A total of 35 Xinghua (XH) chickens (10 males and 10 females at 14 wk of age; 15 females at 20 wk of age, respectively) were raised in individual cages and kept in identical light/dark cycles. The 14-wk-old chickens were divided into 2 groups (n = 10, 5 males and 5 females each) and fed a high-fat diet (lard oil 20%, cholesterol 2%, cholate 0.5%, yolk power 5%, and basal diet 72.5%) or a basal diet, respectively. The high-fat diet was bought from Botai biology Co Ltd (Beijing, China), and nutrient information was listed in Supplementary Table 1. Chickens had ad libitum access to water and their respective diets for 2 wk. The Xinghua chickens at 20 wk were divided into chow fed, fasted, and refed groups, each group comprising 5 female chickens. The fasted group was fasted for 3 d during which they had ad libitum access to water. The refed group was fed the basal diet 1 d after the chickens were fasted for 3 d. The DNA samples from an F_2 resource population crossed from XH and white recessive rock (XH and WRR), previously described by Lei et al [30], were used for association analysis of *KCTD15* variations with fatness traits.

2.3. RNA isolation and complementary DNA synthesis

Chickens were euthanized, and 16 tissues (cerebrum, cerebellum, hypothalamus, pituitary, abdominal fat, subcutaneous fat, breast muscle, heart, liver, spleen, lungs, kidney, muscular stomach, glandular stomach, duodenum, and ovary and/or testis) were rapidly dissected and immediately placed in liquid nitrogen then stored at −80°C. Total RNA was extracted from each tissue using Trizol reagent (Invitrogen, Foster City, CA), following the manufacturer's protocol. The quality and quantity of all obtained RNA samples were determined by 1.5% agarose gel electrophoresis and evaluated for optical density 260/280 ratio. A RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Femantas, CA) was used to synthesize complementary DNA (cDNA) from 2 μg total RNA.

2.4. Primers

Primers were designed by Premier Primer 5.0 software (Premier Biosoft International, Palo Alto, CA) and synthesized by Biosune Co Ltd (Shanghai, China). Primers of KF1-KR1 and KF2-KR2 were used to clone partial cDNA of c*KCTD15*. Other 3 primers (K5′-R1, K5′-R2, and K5′-R3) were used to clone the full-length *KCTD15* cDNA of chicken. QKF1-QKR1 was used for real-time polymerase chain reaction (PCR) analysis of c*KCTD15*. SKF1-SKR1, SKF2-SKR2, and SKF3-SKR3 were used to identify and genotype SNPs of c*KCTD15* (Supplementary Table 1).

2.5. 5′RACE and 3′RACE PCR

The hypothalamus and adipose tissue total RNA were used as template for RACE PCR, which was performed with the SMARTer RACE cDNA Amplification Kit (Clontech, Osaka, Japan) following the manufacturer's instructions. Products of RACE PCR were cloned into pMD-18T vector (Takara, Osaka, Japan) and sequenced by Invitrogen Co Ltd (Guangzhou, China).

2.6. KCTD15 database and phylogenetic analysis

The full-length KCTD15 cDNA sequences of the other 16 species were obtained from Genebank (human NM_001129994.1, mouse NM_146188.1, rat NM_001109141.1, cat XM_003997946.2, dog XM_541709.3, cattle NM_001075568.1, goat XM_005692247.1, horse XM_001489117.2, frog NM_203908.1, mallard XM_005019976.1, zebra finch XM_002188719.2, ground finch XM_005428465.1, ground tit XM_005530122.1, budgerigar XM_005152184.1, pigeon XM_005514436.1, and saker XM_005444627.1). The obtained cDNA sequences were analyzed by Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). On the basis of the 17 *KCTD15* sequences, a phylogenetic tree was constructed by using neighbor-joining method of the MEGA 4.1 (http://www.megasoftware.net/mega41.html) program.

2.7. Real-time PCR analysis

The relative quantity of mRNA was detected using SsoFast EvaGreen Supermix and CFX9600 (BIO-RAD, Hercules), for which the chicken β-actin was used as an internal