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RESEARCH ARTICLE

Differentiation of expression profiles of two calcineurin subunit genes in chicken skeletal muscles during early postnatal growth depending on anatomical location of muscles and breed

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Abstract

Calcineurin (Cn or CaN) is implicated in the control of skeletal muscle fiber phenotype and hypertrophy. However, little information is available concerning the expression of Cn in chickens. In the present study, the expression of two Cn subunit genes (*CnAα* and *CnB1*) was quantified by qPCR in the lateral gastrocnemius (LG, mainly composing of red fast-twitch myofibers), the soleus (mainly composing of red slow-twitch myofibers) and the extensor digitorum longus (EDL, mainly composing of white fast-twitch myofibers) from Qingyuan partridge chickens (QY, slow-growing chicken breed) and Recessive White chickens (RW, fast-growing chicken breed) on different days (1, 8, 22, 36, 50 and 64 days post-hatching). Although *CnAα* and *CnB1* gene expressions were variable with different trends in different skeletal muscles in the two chicken breeds during postnatal growth, it is highly muscle phenotype and breed specific. In general, the levels of *CnAα* and *CnB1* gene expressions of the soleus were lower than those of EDL and LG in both chicken breeds at the same stages. Compared between the two chicken breeds, the levels of *CnAα* gene expression of the three skeletal muscles in QY chickens were higher than those in RW chickens on days 1 and 22. However, on day 64, the levels of both *CnAα* and *CnB1* gene expressions of the three skeletal muscles were lower in QY chickens than those in RW chickens. Correlation analysis of the levels of *CnAα* and *CnB1* gene expressions of the same skeletal muscle showed that there were positive correlations for all three skeletal muscle tissues in two chicken breeds. These results provide some valuable clues to understand the role of Cn in the development of chicken skeletal muscles, with a function that may be related to meat quality.

Keywords: calcineurin, chicken, skeletal muscle, expression

1. Introduction

Skeletal muscles of vertebrates contain myofibers that differ in contractile function, mitochondrial content and metabolic properties (Choi and Kim 2009). The red slow-twitch myofibers (type I) are oxidative fibers, with high lipid content and many mitochondria. The white fast-twitch myofibers (type IIb) are glycolytic, with high glycogen content and few

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mitochondria. The red fast-twitch myofibers (type IIa) are intermediate oxidative-glycolytic fibers. It has been well documented that myofiber type composition can profoundly influence postnatal muscle growth and meat quality and skeletal muscles containing a higher proportion of the red slow-twitch myofibers is hypothesized to be beneficial for meat quality (Karlsson *et al*. 1999; Pette and Staron 2001; Lefaucheur 2010). However, less is known about the mechanisms that control myofiber type specificity.

Calcineurin (Cn or CaN), also known as protein phosphatase 3 (formerly 2B), is a calcium-calmodulin-dependent serine/threonine protein phosphate that couples intracellular calcium to dephosphorylate selected substrates resulting in diverse biological consequences depending on cell type. In skeletal muscle, Cn participates in a variety of processes including myoblast recruitment, myotube differentiation, fiber type specification, and recovery from muscle injury and dystrophic muscle damage, all processes that are crucial to muscle development, metabolism, and functional adaptations (Friday *et al*. 2000; Naya *et al*. 2000; Horsley *et al*. 2001, 2003; Parsons *et al*. 2003; Sakuma *et al*. 2003; Stupka *et al*. 2006; Mallinson*et al*. 2009; Sakuma and Yamaguchi 2010). Various *in vitro* and *in vivo* studies showed that elevated calcineurin activity influenced the metabolic profiles of fibers by promoting a more oxidative phenotype and hypertrophic growth (Chin *et al*. 1998; Dunn *et al*. 1999, 2000; Bigard *et al*. 2000; Talmadge *et al*. 2004; Oh *et al*. 2005; Jiang *et al*. 2010). With further study of the effects of Cn signaling on muscle differentiation, Cn most likely regulates multiple types of muscle growth that depends both on muscle phenotype and stage of myofiber growth (Mitchell *et al*. 2002; Talmadge *et al*. 2004).

The Cn is composed of a catalytic A subunit with a molecular mass of ~59 kDa (CnA) and a calcium-binding regulatory B subunit with a molecular mass of 19 kDa (CnB). Three isoforms of CnA (α, β and γ) and two isoforms of CnB (1 and 2) were identified in vertebrate species. However, only CnAα, CnAβ, and CnB1 are expressed in skeletal muscle, and the expression of the CnAα isoform predominates over the expression of CnAβ in nearly all muscles analyzed (Parsons *et al*. 2003, 2004). The responses to activation of Cn were muscle specific, and the muscle fiber-specific expression of Cn may explain the importance of Cn in muscle fiber phenotype control. The expression of Cn in different skeletal muscle tissues or myofiber types was analyzed in mammals, including in a rat, a mouse, a pig, an equine and a goat (Spangenburg *et al*. 2001; Parsons *et al*. 2003; Eizema *et al*. 2007; Depreux *et al*. 2010; Wan *et al*. 2014), but to date, little information is available concerning the expression of Cn in the skeletal muscles of chickens.

The Qingyuan partridge chicken (QY), an important indigenous breed distributed in Qingyuan, Guangdong Province, China, is a light-body type breed with good meat quality that is famous for the three “yellow”, two “thin”, and one “partridge” morphological features, i.e., yellow break, shanks and skin, thin head and bones, and partridge feathers (Shu *et al*. 2014). In this study, monitored for the first time, the expression patterns of two Cn subunit genes (*CnAα* and *CnB1*) in the lateral gastrocnemius (LG, mainly composing of red fast-twitch myofibers), the soleus (mainly composing of red slow-twitch myofibers) and the extensor digitorum longus (EDL, mainly composing of white fast-twitch myofibers) were compared between QY (slow-growing chicken breed) and Recessive White chickens (RW, fast-growing chicken breed) during early postnatal development.

2. Results

2.1. Expression of *CnAα* and *CnB1* genes in different types of muscle during early postnatal growth of the two breeds of chicken

The expression of the *CnAα* and *CnB1* genes was analyzed on days 1, 8, 22, 36, 50 and 64 in LG, soleus and EDL from QY and RW chickens. The effects of breed, sex, skeletal muscle phenotype and development stage were tested for statistical significance. There was no significant effect of sex ($P>0.05$) on the expression of the two genes; therefore, we analyzed the expression differences of *CnAα* and *CnB1* genes with pooled data from male and female individuals (Figs. 1–4). The values of gene expressions in skeletal muscles of QY chickens were divided by those in RW chickens and are shown in Table 1.

2.2. Expression of *CnAα* gene in different types of skeletal muscle during early postnatal growth of the two breeds of chicken

The patterns of the *CnAα* gene expression of the LG in the two chicken breeds were different (Fig. 1-A). The expression of the *CnAα* gene of the LG in QY chickens first increased significantly ($P<0.05$) from days 1 to 22, decreased significantly ($P<0.05$) to day 36 and finally increased to day 64. On day 22, the expression level of the gene was significantly higher ($P<0.05$) than that in the other stages, and on day 36, the expression level was the lowest; however, there were no significant ($P>0.05$) differences among days 1, 36, 50 and 64. The expression of the *CnAα* gene of the LG in RW chickens first increased significantly ($P<0.05$) from days 1 to 8, then continuously increased to day 22, decreased to day 50 and finally increased significantly ($P<0.05$) by day 64. The highest expression level of the gene occurred on day 64, and there were no significant differences ($P>0.05$) among days 8, 22, 36 and 50. The levels of *CnAα* gene expression