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Molecular Cloning, Characterization and Tissue Distribution of MyoGenin Genein Goose (*Anser cygnoides*)

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Abstract: MyoGenin (*MyoG*) is a basic Helix-Loop-Helix (bHLH) transcription factor that belongs to the Muscle-specific transcription Factors (MRFs) family which plays critical roles in regulating the skeletal muscle development and growth. In this study, the complete coding sequence and genomic DNA sequence of goose *MyoG* gene were cloned and characterized. The goose *MyoG* CDS was composed of 684bp that encoded a 227 amino acid protein, including a highly conserved basic helix-loop-helix domain. Multiple sequence alignments and phylogenetic analysis indicated that the deduced goose *MyoG* protein was conserved in vertebrates, especially in the avian species. The goose *MyoG* genomic DNA sequence we obtained was 3444bp and consisted of 3 exons and 2 introns.Sem-quantitative RT-PCR analysis demonstrated that the goose *MyoG* mRNA was specifically expressed in the breast muscle and leg muscle tissues, little or no expression was observed in heart, liver, spleen, lung, kidney, muscular stomach, brain, intestine and sebum. These data will serve as a foundation for further insight into the functions of the *MyoG* gene in Chinese domestic goose.

Key words: MyoGenin, transcription, kidney, characterized, stomach

INTRODUCTION

The MyoGenic Regulatory Factors (MRFs) family is a kind of MyoGenic basic Helix-Loop-Helix (bHLH) transcription factors and consists of four structurally and functionally related genes including *MyoG*, *Myf-5*, *MyoD* and *Myf-6*. All the four genes shared homology with the region coding for bHLH domain and were involved in the muscle development from commitment and proliferation through muscle fiber formation during embryonic development to their postnatal maturation and function (Fujis-Sehara *et al*., 1990; Pas *et al*., 1999). Therefore, the MRFs family genes are considered as candidate genes for meat production traits in farm animals (Verner *et al*., 2007).

MyoGenin, also known as *MyoG* or *Myf4* has a central position within the *MRFs* gene family because it is the only factor expresses in all skeletal muscle cells and has a major influence on the number of muscle fibers during Myogenesis (Pas *et al*., 1999; Wright *et al*., 1989; Megeney *et al*., 1996). In mice, *MyoG* was essential for the development of functional skeletal muscle (Hasty *et al*., 1993). In chickens, *MyoG* gene was the major gene affecting the muscle fiber traits (Wang *et al*., 2007). In pigs, studies showed that the polymorphisms on *MyoG* gene were related to the meat production traits, including the number of muscle fibers, birth weight, growth rate and thickness of backfat (Pas *et al*., 1999; Tepas *et al*., 1996; Lin *et al*., 2001; Kim *et al*., 2009). All the above results suggest that *MyoG* gene is associated with the formation of muscle and plays crucial roles on the meat yield and quality.

As an important agricultural poultry, meat yield and quality traits are main economically traits in geese. However, our understanding on the biological function of goose *MyoG* gene has lagged behind due to the lack of complete coding sequence in GenBank database. Thus, the purpose of the present study was to clone and characterize the goose *MyoG* gene and analyze its tissue expression profile in different tissues of Chinese domestic goose. These data will be useful for the further studies of goose *MyoG* gene function.

MATERIALS AND METHODS

Animals and tissues: Three healthy female Zhedong White geese (12 weeks old) were obtained from the Institute of Zhedong-White Goose (Xiangshan) and all geese were set free in an open ground with a free

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swimming pool and reared under normal management conditions. Muscle tissue from one Zhedong-White goose was used for cloning the goose *MyoG* cDNA sequence. A total of 11 tissues, including heart, liver, spleen, lung, kidney, breast muscle, leg muscle, brain, intestine, muscular stomach and sebum were surgically removed from each goose and used for tissue distribution analysis. All geese were slaughtered by a ventral cut of the neck blood vessels with 10 s after the end of the stun. The tissues were collected immediately, frozen in liquid nitrogen and stored at -80°C until RNA extraction. Blood samples were withdrawn from the three Zhedong-White geese, stored at -20°C until genomic DNA was extracted. All animal procedures were handled in compliance with the law of the people's republic of china on animal protection.

Nucleic acid isolation and c DNA synthesis: Total RNA was extracted from the above 11 tissues with Trizol Reagent (Invitrogen, Carlsbad, CA, USA) using the Tissue Ruptor (TOYOBO, Japan). First-strand cDNA was synthesized using PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Japan). Genomic DNA was isolated from the collected blood samples of the above three geese using the AXYGEN DNA Isolation Kit (AXYGEN, USA). DNA concentration and quality were measured used a ND-1000 spectrophotometer (Nano Drop, USA) and the concentrations were adjusted approximately 300 ng μL and stored at -20°C for use.

Sequence cloning of the goose *MyoG* gene: Based on the partial mRNA sequence of *MyoG* in goose (GI: 83265397) and the complete coding sequence in duck (GI: 296041304) and chicken (GI: 597454037), two pairs of primers (MyoG-F1/MyoG-R1 and MyoG-F2/MyoG-R2, Table 1) were designed to amplify the complete coding sequence of goose *MyoG* gene. The PCR condition initially started with a 94°C 3 min followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature, 30 s at 72°C and an extension step of 5 min at 72°C. PCR products were applied on 2.5 % agarose gel electrophoresis and purified using the PCR purification Kit (TransGen Biotech Co., Ltd., Beijing, China). The purified PCR products were cloned into the PMD19-T vectors (TaKaRa, Japan), the positive clones were sequenced by Sangon Biotech Co., Ltd (Shanghai, China).

Based on the obtained cDNA sequence from the above RT-PCR reactions and the reference genomic DNA sequence of duck *MyoG* gene (NW-004676592), the genomic DNA sequence of goose *MyoG* gene were amplified using the two pairs of primers (MyoG-GF1/MyoGs-GR1 and MyoG-GF2/MyoG-GR2, table) from one DNA sample of Zhedong-White goose. The PCR profile was 5 min at 94°C followed by 36 cycles of 30 s at 94°C, 30 s at annealing temperature, 2-3 min at 72°C and a final extension of 10 min at 72°C. PCR products were gel purified, cloned and sequenced according the above methods.

Sequence analysis: The obtained cDNA and genomic DNA sequences were matched using DNAMAN software (Pointe-Claire, Canada). The amino acid sequence was predicted using ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/orfig.cgi). Sequence similarity was analyzed with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments of the amino acid sequences were performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2) and edited with BOXSHADE (http://bioweb. pasteur. fr/seqanal/interfaces/boxshade.html). Prot Params were used to predict the molecular weight and isoelectric point. Signal peptide sequence was predicted by Signal P 4.1 (http://www.cbs.dtu.dk/services/SignalP/). The transmem brane domain was predicted by TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Phylogenetic tree was constructed using MEGA 6.0 and based on the Neighbor-Joining (NJ) method with a bootstrap of 1000 repetitions (Tamura *et al*., 2013).

Tissue distribution of goose *MyoG* gene: To measure the mRNA expression levels of goose *MyoG* in various tissues, semi-quantitative RT-PCR was performed with primers MyoG-RT-F/MyoG-RT-R (table). Amplification of goose *GAPDH* gene was used as an internal control (GAPDH-F/GAPDH-R, Table 1). The amplified GAPDH fragment spanned intron 4 which was applied to exclude the possibility of DNA contamination during all RT-PCR reactions. The semi-quantitative RT-PCR amplifications were carried out in triplicate in a total volume of 20 μL, PCR conditions were as follows: 94°C, 4 min; 25-36 cycles with 94°, 45 s; 65°, 40 s; 72°C, 45 s and a final extension at 72°C for 10 min. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The PCR fragments were visualized on 2.5% agarose gels stained with ethidium bromide and visualized with ultraviolet light.

RESULTS AND DISCUSSION

Molecular cloning and characterization of goose *MyoG* gene: A 684 bp cDNA sequence was amplified by RT-PCR using two pairs of primers from the muscle tissue of Zhedong-White goose. The cDNA (GenBank: KP893286) comprised an ORF of 681 bp encoding a 227 amino acids

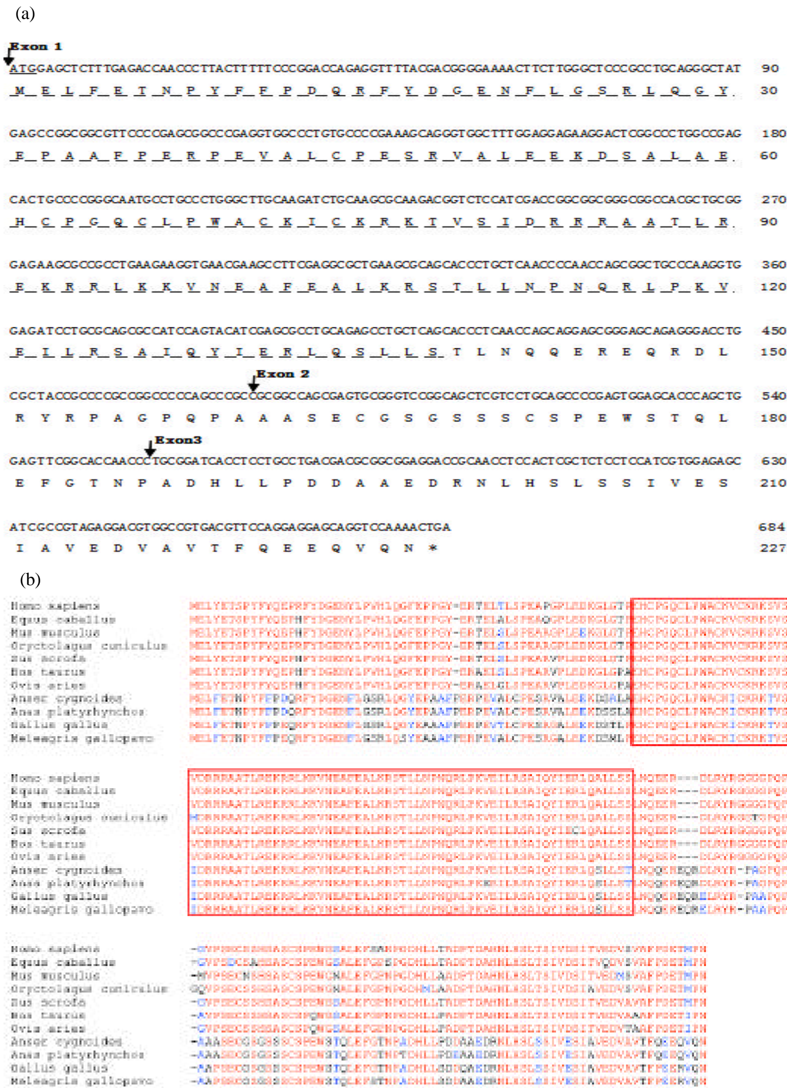

Fig. 1: Nucleotide and deduced amino acid sequences of the goose *MyoG* gene and multiple alignments of *MyoG* amino acid sequences among different species: a) The letters underlined indicate the start codon (ATG) and the stop codon (TAA) is indicated with an asterisk. The bHLH domain is indicated with dotted lines; b) Red amino acids indicate 100% conserved sequences. Less-conserved and non-conserved amino acids are shown in blue and black, respectively. Whereas "-" indicates deletion of an amino acid. The highly conserved bHLH domains are boxed

protein with a calculated molecular mass of 25.74 kDa and an isoelectric point of 5.31 (Fig. 1a). A Gen Bank database search using BLAST revealed that the goose *MyoG* gene was consistent with those of other species with 97, 91, 90, 84, 81, 76, 75, 75, 75 and 74% identity to *Anas platyrhynchos* (XM_013094032), *Gallus gallus* (FJ882411), *Meleagris gallopavo* (NM_001303170), *Mus musculus* (NM_031189), *Oryctolagus cuniculus* (FJ605116), *Equus caballus* (AB608013), *Homo sapiens* (NM_002479), *Sus scrofa* (NM_001012406), *Bos taurus* (NM_001111325) and *Ovis aries* (GU550517). Meanwhile, BLASTP analysis displayed that the predicted protein shares 98, 94, 94, 70, 71, 68, 69, 70, 71 and 70% amino acid sequence similarity with the *MyoG* protein sequences of the above species. The results of multiple alignments analysis showed that the deduced goose *MyoG* amino acid sequence was more similar to those of avian species, particularly to ducks (Fig. 1b). To elucidate the genomic organization, a total of 3444 bp in length genomic sequence (GenBank: KP893286) of goose *MyoG* gene was

Table 1: Primers used in this study

Primers purpose	Primer name	Primer sequence (5'→3')	Product size (bp)	Tm(°C)
cDNA	MyoG-F1	TTTTTCCCGGACCAGAGGTT	429	59.8
cloning	MyoG-R1	CTGCTGGTTGAGGGTGCTGA		
	MyoG-F2	GCAGCGCCATCCAGTACATCGAG	314	62.7
	MyoG-R2	TCAGTTTTGGACCTGCTCCTCCT		
Genomic DNA	MyoG-GF1	CAGCGCCATCCAGTACATCGAG	862	58.5
cloning	MyoG-GR1	ATGTCCATCTGTATCCGTCTGT		
	MyoG-GF2	GGTACAGAGGGAGGTGGCGTG	2338	63.8
	MyoG-GR2	TCCTCTACGGCGATGCTCT		
Expression	MyoG-RT-F	GAGAAGCGCCGCCTGAAGAA	351	62.4
Profile	MyoG-RT-R	GATGGAGGAGAGCGAGTGGA		
Internal	GAPDH-F	GGCTGAGAATGGGAAACTT	188	58.0
Control	GAPDH-R	CAACATATTCAGCACCAGCA		

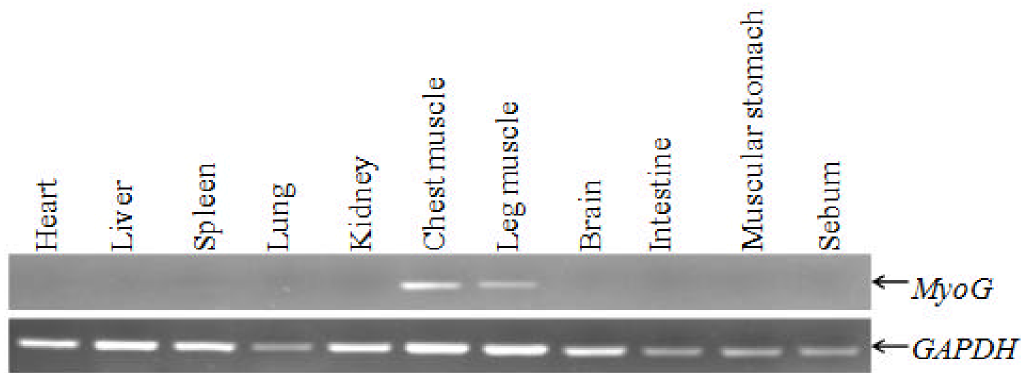

Fig. 2: Tissue distribution of the goose *MyoG* gene in different tissues

cloned using the primers MyoG-GF1/MyoG-GR1 and MyoG-GF2/MyoG-GR2 (Table 1). BLAST similarity analysis showed that the goose *MyoG* genomic sequence (GenBank: KT290042) shared high similarity with duck and chicken *MyoG* genomic DNA sequences. The goose *MyoG* genomic DNA sequence consisted of three exons of 480, 82 and 122 bp as well as two introns of 2022 and 737 bp, respectively. All exon-intron splice junction sequences conformed to the "GT-AG" rule.

Protein structure prediction and phylogenetic-tree construction of *MyoG*: The goose *MyoG* protein was predicted to a non-secreted protein, due to it did not contain a signal peptide sequence which was consistent with that of sheep (Zhang *et al*., 2014). Meanwhile, it was a non-transmembrane protein, because no transmembrane helices were predicted. Moreover, the analysis from the NCBI conserved domains database showed that the goose *MyoG* protein contained a typical bHLH domain, which was a common feature of the bHLH transcription factors (Fig. 1a). The phylogenetic tree displayed that the *MyoG* proteins from eleven different species were divided into two major groups. The *Anser cygnoides*, *Anas platyrhynchos* (XP_012949486), *Gallus gallus* (NP_989515) and *Meleagris gallopavo* (NP_001290099) were grouped into a cluster, the mammalian species, including *Mus musculus* (NP_112466), *Homo sapiens* (NP_002470), *Oryctolagus cuniculus* (NP_001171220), *Equus caballus* (BAP59006), *Sus scrofa* (NP_001012406), *Bos taurus* (NP_001104795) and *Ovis aries* (NP_001167580) belonged to the other group. The phylogenetic analysis demonstrated that the goose *MyoG* exhibited a closer genetic relationship with those of avian species, particularly with the *MyoG* protein of duck.

Tissue distribution of goose *MyoG* gene: To characterize the tissue expression pattern of goose *MyoG* gene, semi-quantitative RT-PCR was performed with total RNA from eleven different goose tissues. The results showed that the goose *MyoG* mRNA was specificallyexpressed in the breast muscle and leg muscle tissues. Little or no expression was detected in heart, liver, spleen, lung, kidney, brain, intestine, muscular stomach and sebum tissues (Fig. 2).

Like other genes in the *MRFs* gene family, *MyoG* gene belongs to an important member of the MyoGenic bHLH transcription factors with the typical bHLH domain. In this research, molecular cloning, structural and phylogenetic analysis of goose *MyoG* gene were firstly performed. The cloning of goose *MyoG* provided a

material basis for further functional studies of goose *MyoG* molecules. Both of the multiple alignments and the phylogenetic analysis displayed the deduced protein of goose *MyoG* shared high amino acid identities with its counterparts in other species, especially with avian species. This is consistent with our previous studies on other goose genes (Wang *et al*., 2014; Liu *et al*., 2014). The close genetic relationship between goose and these avian species indicates that they may have similar biological functions. Further study should examine whether amino acid differences between the *MyoG* proteins of birds versus mammals contribute to functional differences.

Similar to other genes in MRFs family, the goose *MyoG* genomic DNA is composed of three exons and two introns which is consistent with the genome organization of *MyoG* gene in human, mouse, pig and fish (Soumillion *et al*., 1997, 1998; Tan *et al*., 2002). It is worth mentioning that the three exons encoded three different domains, respectively. The first exon encodes the bHLH domain, second exon encodes the transcriptional activation domain and the last exon encodes the conserved C-terminal domain (Soumillion *et al*., 1997; Schwarz *et al*., 1992). It is surprised that the genome structure of goose *MyoG* gene is different from that of duck, human, mouse and pig. The goose *MyoG* introns (2022 and 737 bp) are longer than the corresponding introns of ducks (1681 and 668 bp), humans (131 and 125 bp), mice (513 and 526 bp) and pigs (785 and 639 bp). In ducks, the expression of *MyoG* was detected both in the breast muscle and leg muscle at different stages of the fetal duck development (Liu *et al*., 2010). Our finding demonstrated that the goose *MyoG* only expressedin the breast muscle and leg muscle tissues. These data indicate that *MyoG* gene may play important roles in regulating muscle development and growth in waterfowl.

CONCLUSION

In summary, the goose *MyoG* gene was isolated and characterized for the first time in this study. The goose *MyoG* transcript was specificallyexpressed in the muscle tissues. All the results derived from this study established the primary foundation for further insight into the function of the *MyoG* gene in goose.

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