Theriogenology 79 (2013) 1218–1223

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

A novel SNP marker of ovalbumin gene in association with duck hatchability

Hsiu-Lin Huang [a,*], Liang-Tsung Huang [a], Yu-Shin Cheng [b]

[a] Department of Biotechnology, MingDao University, Pettow, ChangHua, Taiwan
[b] Livestock Research Institute, Council of Agriculture, Hsin-Hua, Tainan, Taiwan

ARTICLE INFO

Article history:
Received 30 October 2012
Received in revised form 16 February 2013
Accepted 16 February 2013

Keywords:
Hatchability
Ovalbumin gene
SNP genotyping
Marker-assisted selection
Duck

ABSTRACT

Our previous transcriptome analysis using a cDNA microarray identified differentially-expressed transcripts in Tsaiya ducks (*Anas platyrhynchos*); we concluded that the ovalbumin gene might be involved in duck hatchability. In the present study, associations of single nucleotide polymorphism (SNP) genotypes of the duck ovalbumin gene with hatchability were investigated. To confirm the cDNA microarray analysis, real-time polymerase chain reaction (PCR) and Western blot analysis were used to validate ovalbumin gene expression. The messenger RNA and protein expression of the ovalbumin gene were higher ($P < 0.05$) in the low-hatchability group (1.00 ± 0.19; 30.36 ± 3.51 arbitrary units) than in high-hatchability counterparts (0.56 ± 0.07; 8.53 ± 2.97 arbitrary units), consistent with the previous cDNA microarray analysis. The PCR products (506 base pairs) of ovalbumin gene amplified by the primer pair of TovaF and TovaR from the genomic DNA templates of 10 ducks were sequenced and a g.385 C>T SNP site in the 506-base pair sequence of the ovalbumin gene identified. Genotyping of SNP of 187 ducks was then carried out by PCR restriction fragment length polymorphism and minisequencing methods. Based on SNP genotypes of the duck ovalbumin gene, there were three types: CC, TT, and CT. Birds with the CC and TT genotypes had higher hatchability (79.59 ± 3.40, 76.35 ± 1.77) ($P < 0.05$) than those with a CT genotype (65.77 ± 2.07). In conclusion, the ovalbumin gene was an important candidate gene that can be used for marker-assisted selection to increase hatchability in Tsaiya ducks.

1. Introduction

Parental condition at progeny production can affect reproductive success and progeny fitness [1–7]. In mammals, maternal factors can be transferred via the placenta, the colostrum, and milk produced during lactation [8,9]. In birds, reptiles, and fish, maternal factors are transferred via the egg to the offspring [3,4,10–12]. Consequently, the duration of the uptake of maternal factors is limited to the interval before and shortly after birth.

In birds, a high ratio of hatchability and fertility, a long duration of fertility, heavy egg weight, and numerous eggs can augment overall reproductive efficiency. However, there is limited knowledge regarding gene expression in oviducts relative to embryo development and hatchability [13,14]. Ovalbumin is the major protein that constitutes more than 50% of the total proteins in avian albumin [15]. During embryo development, albumin secreted by the magnum is gradually transferred into the amniotic fluid [16,17], and finally accumulates in the embryo [17–19]. The mortality of ovalbumin gene mutant embryos is increased threefold compared with normal embryos [20]; therefore, the ovalbumin gene is critical to poultry hatchability.

Our previous cDNA microarray analysis revealed that the ovalbumin gene might be associated with Tsaiya duck

* Corresponding author. Tel.: +886 4 8876660 ×8316; fax: +886 4 8871774.
E-mail address: hlhuang@mdu.edu.tw (H.-L. Huang).

0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.02.021

hatchability [21]. In the present study, to confirm the results of cDNA microarray analysis, the differentially-expressed transcript of the ovalbumin gene was validated by real-time polymerase chain reaction (PCR) and Western blot analysis. This study used female Tsaiya ducks (*Anas platyrhynchos*; the major egg-laying duck in Taiwan), which is usually crossed with the male Muscovy duck to produce the Mule duck for meat production [22,23]. Identification of the single nucleotide polymorphism (SNP) site and investigation of associations of SNP genotypes of the duck ovalbumin gene with hatchability were also done.

2. Materials and methods

2.1. Experimental birds and animal care

A total of 187 laying Tsaiya ducks raised in the Livestock Research Institute, Council of Agriculture, Taiwan, were used. The care and use of these research animals were within standard ethical guidelines. Hatchability, fertility, duration of fertility, egg weight, and total number of eggs at 40 weeks of age, were determined and used for marker–trait association analysis.

2.2. Assessing transcripts and proteins

To confirm the cDNA microarray findings, in this study, real-time PCR and Western blot analysis were performed for validation of ovalbumin gene expression. Total RNA isolated from the magnum epithelium of laying Tsaiya ducks of high hatchability ($88.20 \pm 1.21\%$; $N = 5$) and low hatchability ($55.60 \pm 1.04\%$; $N = 5$) using the RareRNA reagent (GenePure, Kaysville, UT, USA) was used for real-time PCR. Cell extracts for Western blot analysis were prepared from the magnum epitheliums of each hatchability extreme, as described in this paragraph, using a Mini-Beadbeater (BioSpec Products Inc., Bartlesville, OK, USA), in accordance with the manufacturer's instructions.

Using a High Capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions, complementary DNA was reverse-transcribed from 2 ng of total RNA. The resulting cDNA was subjected to real-time quantitative PCR in the presence of SYBR Green Mix (Applied Biosystems) along with an ovalbumin gene-specific primer set (forward: 5′-CAA CCT GAT TTA CCA GCA GAA GTC-3′; reverse: 5′-CAC CAC AAA GCC TGG TCA TG-3′) using an Applied Biosystems 7300 Real-Time PCR System as recommended by the manufacturer. For normalization, complementary DNA from *18s* ribosomal RNA was amplified (using the following primer set: forward: 5′-GGA CGC GTG CAT TTA TCA GA-3′; reverse: 5′- AGT TGA TAG GGC AGA CAT TCG AA-3′) and relative quantification was performed from curves generated for ovalbumin and *18s* ribosomal RNA genes. Details of the method used are described by Huang et al. [24] and at http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_053412.pdf.

For Western blot analysis, cell extracts prepared from the magnum epithelium of the high and low hatchability birds were used. The equal protein levels of the cell extracts were adjusted according to the method of Bradford (with BSA as a reference standard), resolved by 15% SDS-PAGE, and transferred to polyvinylidene difluoride membranes (NEN Life Science Products, Boston, MA, USA) using SemiPhor semidry transfer units (Hoefer Inc., Holliston, MA, USA). Membranes were then probed with monoclonal antibodies specific to ovalbumin (ab17291; Abcam, Cambridge, UK) or actin (ab11003; Abcam). Unbound antibodies were washed and blots were incubated with goat anti-mouse IgG, horseradish peroxidase-conjugated antibody (ab6789; Abcam). Antibody bindings were visualized by a chemiluminescent substrate (Immobilon Western, Millipore, Billerica, MA, USA) for localization of the horseradish peroxidase, and one-dimensional image analysis software (TotalLab version 1.11; Ultra-Lum, Claremone, CA, USA) was then used to quantify band density.

2.3. Genomic DNA isolation

Genomic DNA was isolated from the blood of 187 laying Tsaiya ducks using the method described by Wu et al. [25] for SNP genotype analysis. Briefly, the saline-washed whole blood sample was centrifuged and resuspended in 3 mL of lysis buffer (10 mM TRIS-HCl, 150 mM NaCl, 10 mM EDTA) and 300 μL of 10% NH_4Cl, mixed well, then frozen and tha-wed twice. Subsequently, 75 μL proteinase K (10 mg/mL), 25 μL collagenase (3.8 IU/mL), and 200 μL 10% wt/vol SDS was added to the sample, which was then incubated at 55 °C for 24 hours with gentle agitation. The DNA was purified using a phenol chloroform extraction, followed by isopropanol precipitation and washing with 70% ethanol; it was then dried and dissolved in double distilled water for PCR.

2.4. Identification of SNP

For SNP identification, the 506-base pair (bp) fragments of the ovalbumin gene were amplified by PCR from the genomic DNA templates of 10 Tsaiya ducks with the primers TovaF (5′-AGT GGA TGC TGC AAG CGT CT -3′) and TovaR (5′-CCA CAA AGC CTG GTC ATG CT-3′). Sequencing was performed using an ABI 3100 DNA sequencer using ABI Primes BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems) according to the manufacturer's instructions. Sequences were screened against the National Center for Biotechnology Information nucleotide database using the BLASTN program (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome) and aligned using the GCG software alignment program version 3.1 (Genetics Computer Group, Madison, WI, USA). Finally, nucleotide sequences were analyzed using Vector NTI software version Advance 9 (Life Technologies, Grand Island, NY, USA) to depict the amino acid sequence of the PCR-amplified DNA fragment of the ovalbumin gene.

2.5. Genotyping by PCR restriction fragment length polymorphism and minisequencing

Genomic DNA from 187 ducks was used for PCR restriction fragment length polymorphism (RFLP) and minisequencing. The primer pair of TovaF1 (5′-ACA GCA