

Characterization of the Goose *CAPN3* Gene and its Expression Pattern in Muscle Tissues of Sichuan White Geese at Different Growth Stages

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Calpain 3 (*CAPN3*), also known as p94, is associated with multiple production traits in domestic animals. However, the molecular characteristics of the *CAPN3* gene and its expression profile in goose tissues have not been reported. In this study, *CAPN3* cDNA of the Sichuan white goose was cloned, sequenced, and characterized. The *CAPN3* full-length cDNA sequence consists of a 2,316-bp coding sequence (CDS) that encodes 771 amino acids with a molecular mass of 89,019 kDa. The protein was predicted to have no signal peptide, but several *N*-glycosylation, *O*-glycosylation, and phosphorylation sites. The secondary structure of *CAPN3* was predicted to be 38.65% α -helical. Sequence alignment showed that *CAPN3* of Sichuan white goose shared more than 90% amino acid sequence similarity with those of Japanese quail, turkey, helmeted guineafowl, duck, pigeon, and chicken. Phylogenetic tree analysis showed that goose *CAPN3* has a close genetic relationship and small evolutionary distance with those of the birds. qRT-PCR analysis showed that in 15-day-old animals, the expression level of *CAPN3* was significantly higher in breast muscle than in thigh tissues. These results serve as a foundation for further investigations of the function of the goose *CAPN3* gene.

Key words: *CAPN3* gene, clone, expression, goose

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Introduction

Goose meat is rich in protein and several trace elements, such as calcium, phosphorus, potassium, and sodium, but low in fat and cholesterol. Thus, goose meat is a nutritional and healthy food. However, the tenderness of goose meat restricts consumer acceptance; therefore, candidate genes that affect goose meat tenderness have received great research interest.

Previous studies have shown that the calpain system is closely related to meat quality traits in pigs (Gandolfi *et al.*, 2011), sheep (Grochowska *et al.*, 2017), and chickens (Zhang *et al.*, 2009). Calpains are Ca^{2+} -dependent intracellular cysteine proteases that are involved in cell motility (Glading *et al.*, 2002), and apoptosis regulation (Liu *et al.*, 2004), muscle atrophy (Richard *et al.*, 1995), myoblast fusion (Honda *et al.*, 2008), and muscle growth and develop-

ment (Sultan *et al.*, 2000; Zhang *et al.*, 2009). To date, 15 isoforms have been identified in humans (Sorimachi and Ono, 2012), and in vertebrates, calpain 1 and calpain 2 have been widely studied among calpain isoforms (Macqueen *et al.*, 2010; Zhang *et al.*, 2017). Among the calpain family members, calpain 3 (*CAPN3*, previously named p94) is particularly interesting, because in humans, limb-girdle muscular dystrophy type 2A (LGMDA2) is mainly caused by loss-of-function mutations of *CAPN3* (Richard *et al.*, 1995). In mammals, *CAPN3* is the most highly expressed in skeletal muscles, especially in the fast (type II) fibers (Jones *et al.*, 1999), but *CAPN3* mRNA has also been detected in the heart (Fougerousse *et al.*, 2000). In chickens, Zhang *et al.* (2012) and Sorimachi *et al.* (1995) reported that *CAPN3* is abundantly expressed in the skeletal muscle and is also expressed in the liver, heart, and brain. Subsequent studies by Ilian *et al.* (2001, 2004) and Felício *et al.* (2013) revealed significant correlations between meat quality and the expression or polymorphism of *CAPN3*. However, the gene and cDNA sequences of the goose *CAPN3* gene have not been published in GenBank. The expression level and pattern of *CAPN3* in goose are less clear, and its precise physiological functions are not well characterized in goose.

In this study, we first cloned the cDNA of the Sichuan

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White goose *CAPN3* gene, predicted the corresponding protein sequence, and performed phylogenetic and structural analyses. Then, we detected its expression patterns in muscle tissues of different growth stages using quantitative reverse transcription PCR (qRT-PCR). By comparing the cDNA sequences and mRNA expression patterns in goose breast and thigh muscle tissues, we sought to unravel potential roles of CAPN3 in goose muscle tissues. The results of this analysis will aid in understanding the function of the goose *CAPN3* gene.

Materials and Methods

Ethics Statement

All geese were fed according to Chinese local goose breeding standards. All experimental protocols were reviewed and approved of by the Sichuan Agricultural University Institutional Animal Care and Use Committee in College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, China (DKY-B201000805). Sampling procedures used strictly complied with the guidelines on Ethical Treatment of Experimental Animals.

Animals and Sample Collection

In this study, 30 Sichuan white geese were kept in the Experimental Farm for Waterfowl Breeding at Sichuan Agricultural University, with *ad libitum* access to water and a commercial corn-soybean-based diet. For *CAPN3* cloning and mRNA expression analysis, 15 female geese at different ages (15 and 22 days) were randomly selected, of which 3 geese aged 22 days old were used for cloning, and the remaining 12 were used for mRNA expression testing. All of these geese were healthy and of moderate weight, and they were fed in their housing. In the feeding room, the temperature was 15–19°C, and the environment was exposed to natural light. All geese were terminally killed by bleeding within 10 min of capture, and breast muscle and thigh muscle tissues were removed (within 5–10 min after death), frozen immediately in liquid nitrogen, and stored separately at –80

°C until total RNA extraction.

RNA Extraction and Reverse Transcription

Total RNA was isolated from the collected tissues using TRIzol reagent (Takara Bio, Dalian, China) according to the manufacturer's instructions and treated with 30 µl DNase/RNase-Free water (Takara Bio) to remove DNA contamination. Before reverse transcription, RNA integrity and quality were checked by electrophoresis (1% agarose gels stained with ethidium bromide) and with a Nanodrop spectrophotometer (Nanodrop 2000C; Thermo Scientific, MA, USA), respectively. Reverse transcription was carried out using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara Bio) according to the manufacturer's instructions. Briefly, each 10 µl reaction contained 2 µl of 5× PrimeScript™ buffer, 0.5 µl of PrimeScript™ RT Enzyme Mix I, 0.5 µl of OligodT Primer, 0.5 µl of random hexamers, 5.5 µl of RNase-free water, and 1 µl of total RNA. The reaction was carried out at 37°C for 15 min, terminated by 5 s at 85°C, and the cDNA was stored at 4°C.

First-strand cDNA of the breast muscle was used to clone the full-length cDNA of *CAPN3*. First-strand cDNA of other tissues was used for gene expression analysis.

Molecular Cloning of the Goose CAPN3 cDNA

The goose *CAPN3* gene was amplified from cDNA using PCR. The specific PCR primers were designed by using Primer 5.0 and Oligo 6.0 software based on predicted *Anser cygnoides CAPN3* sequence (GenBank ID: XM_013192108). Primers used for cloning are shown in Table 1. The 10 µl PCR reaction mixture contained 5 µl of 2× MasterMix (Tiangen, Beijing, China), 0.8 µl of each primer (10 mM each), 1 µl of cDNA (50–500 ng), and 2.4 µl of ddH₂O. Thermal cycles were as follows: initial denaturation at 94°C for 5 min, 34 cycles of 94°C for 45 s, 60°C for 30 s, and 72°C for 1 min, and final elongation at 72°C for 10 min. The PCR fragments were purified with the Gel Extraction Mini Kit (Qiagen, Hilden, Germany) and were cloned into the pMD-19T vector (Takara Bio). The plasmid DNA was isolated

Table 1. Primers used in this study

No	Gene	Type	Sequence (5' -3')	Product size (bp)	Tm (°C)
1	CAPN3	Clone	F: CGCTTACAACCTTTGACTAG R: CTGGCTGTAGAAGAGGGA	390	56
2	CAPN3	Clone	F: ATCCTGATTCCCACCTA R: AACGTCCACCCAGTCTCC	229	55
3	CAPN3	Clone	F: GCTGGCATCTTTCACTTT R: CGTTCCATTCCACCTGTC	619	56
4	CAPN3	Clone	F: AGGTCACGCCTACTCTGT R: TGTCATCTCCTGCAATCT	869	56
5	CAPN3	Clone	F: AGTCTTTTCAGAGGAGGTTG R: TGGTGTTAGGCGTACATG	605	55
6	CAPN3	Expression	F: AAGCGACATTGGCGAACT R: CCAGCCCACAAGACATCC	152	60
7	β -actin	Expression	F: CAACGAGCGGTTTCAGGTGT R: TGGAGTTGAAGGTGGTCTCGT	92	60

using a Plasmid Mini Kit (Qiagen) and was sequenced by BGI (Beijing, China).

Sequence, Structure, and Phylogenetic Analyses

The obtained nucleotide sequence of the goose *CAPN3* gene was analyzed at NCBI (<http://blast.ncbi.nlm.nih.gov/>) and was compared to the sequence database using the BLAST server (<http://www.ncbi.nlm.nih.gov/blast>). The open reading frame was predicted with the NCBI/ORF Finder program (<http://www.ncbi.nlm.nih.gov/forf/gorf.html>). Protein domains were predicted using the Conserved Domain Architecture Retrieval Tool at NCBI (<http://www.ncbi.nlm.nih.gov/BLAST>) and the ClustalW software (<http://align.genome.jp>) was used for multiple alignment. The fundamental characteristics of the predicted protein including molecular weight (Mw) and isoelectric point (pI) were analyzed with ProtParam (<http://www.expasy.org/tools/protparam.html>). TMpred (http://www.ch.embnet.org/software/TMPRED_form.html) and TMHMM (<http://www.cbs.dtu.dk/services/TMHMM-2.0>) were used to predict transmembrane domains. Signal peptides were predicted by the SignalP4.0 server (<http://www.cbs.dtu.dk/services/SignalP>). NetOGlyc 3.1 (<http://www.cbs.dtu.dk/services/NetOGlyc>) and NetNGlyc 1.0 (<http://www.cbs.dtu.dk/services/NetNGlyc>) were used to predict potential *O*- and *N*-glycosylation sites, respectively. Disulfide bonds were predicted using the SCRATCH protein predictor (<http://scratch.proteomics.ics.uci.edu>). Phosphorylation sites were predicted using NetPhos 2.0 (<http://www.cbs.dtu.dk/services/NetPhos>). Secondary structures of deduced amino acid (aa) sequences were predicted by using the HNN method (<http://npsa-pbil.ibcp.fr/>). The protein 3D conformation was predicted using the SWISS-MODEL server (<http://www.expasy.org/swissmod/SWISSMODEL.html>). A phylogenetic tree was constructed using the MEGA 7.0 software. GenBank accession numbers of *CAPN3* from different species for the phylogenetic analysis are listed in Table 2.

qRT-PCR

qRT-PCR was used to evaluate changes in *CAPN3* expression in muscle tissues of Sichuan white geese in different growth stages. The primers used for qRT-PCR are listed in Table 1. The housekeeping gene β -actin was selected as the endogenous control gene. qPCR was performed on a CFX-96 qPCR Real-Time PCR Detection System (Bio-Rad) and was carried out in a total volume of 25 μ l, with 2.0 μ l cDNA, 0.8 μ l of each specific primer, 12.5 μ l SYBR Premix EX TaqTM (Takara Bio), and 8.9 μ l of ddH₂O. The thermal protocol included one cycle of 95°C for 10 s, followed by 40 cycles of 95°C for 10 s and 60°C for 15 s. A 45-cycle-based melting curve was generated, starting at a temperature of 55°C and increasing by 0.5°C every 10 s, to determine primer specificity. Each sample was analyzed in triplicate. mRNA levels were normalized to β -actin mRNA and expressed as a fold change relative to the expression level in the control by using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

Means were compared by one-way ANOVA using the SAS 6.12 software, a multiple comparison test was performed using Duncan's method, and $P < 0.05$ was considered statistically significant. Means \pm SEM were plotted using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA).

Results

Cloning and Characteristics of Goose *CAPN3* cDNA

A 2,453-bp sequence of goose *CAPN3* was obtained by cloning and splicing using cDNA generated from RNA isolated from breast muscle of Sichuan White goose as the template, which is in line with the predicted *CAPN3* gene sequence of *A. cygnoides* (GenBank: XM_013192108). The cDNA contains a 2,316-bp ORF, encoding a 771 aa protein.

The deduced aa sequence of Sichuan White goose *CAPN3* was compared to the *CAPN3* sequences from 14 other

Table 2. Accession numbers of *CAPN3* nucleic acid and protein sequences used for multiple sequence alignments and for reconstruction of the phylogenetic tree in this study

Species	Accession no. (nucleic acid)	Accession no. (protein)
<i>Anser cygnoides domesticus</i>	XM_013192108	XP_013047562
Duck (<i>Anas platyrhynchos</i>)	XM_005021679	XP_005021736
Chicken (<i>Gallus gallus</i>)	NM_001004405	NP_001004405
Cattle (<i>Bos taurus</i>)	NM_174260	NP_776685
Medium ground-finch (<i>Geospiza fortis</i>)	XM_005419401	XP_005419458
House mouse (<i>Mus musculus</i>)	NM_007601	NP_031627
Human (<i>Homo sapiens</i>)	NM_000070	NP_000061
Japanese quail (<i>Coturnix japonica</i>)	XM_015864332	XP_015719818
Macaque (<i>Macaca fascicularis</i>)	NM_001287701	NP_001274630
Norway rat (<i>Rattus norvegicus</i>)	NM_017117	NP_058813
Helmeted guineafowl (<i>Numida meleagris</i>)	XM_021403511	XP_021259186
Pigeon (<i>Columba livia</i>)	XM_021287237	XP_021142912
Pig (<i>Sus scrofa</i>)	NM_214171	NP_999336.2
Sheep (<i>Ovis aries</i>)	NM_001009212	NP_001009212
Turkey (<i>Meleagris gallopavo</i>)	XM_019615582	XP_019471127

animals using DNAMAN. The coding sequence of Sichuan White goose *CAPN3* was 99.87% identical to *A. cygnoides* *CAPN3*, and 93.42%, 93.16%, 93.16%, 92.92%, 89.18%, 88.94%, 86.65%, 71.79%, 71.51%, 70.99%, 70.87%, 70.43%, 70.17%, and 70.10% identical to that of helmeted guinea-fowl, Japanese quail, turkey, duck, chicken, pigeon, medium ground-finch, macaque, human, Norway rat, pig, house mouse, cattle, and sheep, respectively. The aa sequence was 100%, 97.23%, 96.85%, 96%, 93.41%, 92.57%, 92.21%, 89.11%, 74.11%, 74.05%, 73.93%, 73.93%, 73.73%, 73.02%, and 72.66% identical to that of *A. cygnoides*, Japanese quail, turkey, helmeted guineafowl, duck, pigeon, chicken, medium ground-finch, macaque, Norway rat, human, house mouse, pig, cattle, and sheep, respectively (Table 2 and Fig. 1).

Analysis of the AA Sequence of Goose *CAPN3* and Phylogenetic Analysis

The physicochemical properties of the Sichuan White goose *CAPN3* protein were predicted using ProtParam software (Table 3). The results showed that the *CAPN3* protein molecular formula is $C_{3971}H_{6105}N_{1075}O_{1185}S_{36}$, and the molecular weight is 89,019 kDa. The theoretical pI is 5.54, and the instability index is 36.75. The grand average of hydropathicity of the protein was -0.531 . The Sichuan White goose *CAPN3* protein was predicted to be non-secretory, as it does not contain signal peptide sequences, and a transmembrane domain was not predicted. In addition, goose *CAPN3* was predicted to have 13 *O*-glycosylation sites (Thr^{13,21,22,24,25,26,30,34,310} and Ser^{17,20,588,591}), four *N*-glycosylation sites (Asn⁷⁸, Asn¹¹², Asn²¹⁸, and Asn⁴⁰⁹), 66 phosphorylation sites (Table 4), and no disulfide bonds. The hydrophobicity profile of the protein was calculated by ProtScale of ExPASy (Fig. 2). The ordinate represents the hydrophobic score—the higher the score, the more hydrophobic, the lower the score, the lower the hydrophobicity. The abscissa represents aa position. As shown in Fig. 2, the aa at position 474 is highly hydrophobic and that at position 484 is highly hydrophilic. Moreover, the protein has substantially more hydrophilic than hydrophobic amino acids.

Secondary structural analysis indicated that the putative goose *CAPN3* protein comprised 38.65% alpha helix, 18.55% extended strand, and 42.80% random coil. The fully automatic procedure on the SWISS-MODEL server was used to construct a 3D structural model of a segment of the goose *CAPN3* sequence (aa 47–770). The result showed that the segment was similar to that of the calpain-2 catalytic subunit 3bow.1.A, which is the template of this 3D model in the SWISS-MODEL library (Arnold *et al.*, 2006; Benkert *et al.*, 2011; Biasini *et al.*, 2014) (Fig. 3). Meanwhile, in the predicted 3D structure, the QMEAN score was -2.23 , and the GMQE score was 0.73.

A phylogenetic tree was constructed from the deduced Sichuan White goose *CAPN3* and *CAPN3* sequences from other animals by the Neighbor Joining (NJ) method in MEGA 7.0 (Fig. 4). As shown in Fig. 4, the Sichuan White goose *CAPN3* protein evaluated in this study had a position close to *A. cygnoides* and *Anas platyrhynchos* *CAPN3* pro-

teins, while it was distant from *Bos taurus* and *Ovis aries* *CAPN3* proteins.

Expression Profile of Sichuan White Goose *CAPN3* mRNA

The mRNA levels of *CAPN3* in the breast muscle and thigh muscle of Sichuan white geese at 15 and 22 days of age were determined by qRT-PCR. As shown in Table 5, *CAPN3* mRNA expression in thigh tissue increased from the 15-day to the 22-day stage, but there was not significant difference ($P > 0.05$). However, in the breast muscle, the expression of *CAPN3* was significantly higher in 15-day-old than in 22-day-old geese ($P < 0.05$). Meanwhile, at 15 days, the *CAPN3* mRNA level in thigh muscle was lower than that in breast muscle, although the difference was not significant ($P > 0.05$). In contrast, at 22 days, the expression of *CAPN3* mRNA in thigh muscle was significantly higher than that in breast muscle ($P < 0.05$) (Table 5 and Fig. 5).

Discussion

CAPN3 is involved in multiple important functions (Zhang *et al.*, 2017) and has drawn great research interest because of its three specific regions (N-terminal, IS1 and IS2) (Ono *et al.*, 2016). For example, defects IS1/2 in *CAPN3* lead to limb girdle muscular dystrophy type 2A (LGMD2A) symptoms and other disease (Beckmann and Spencer, 2008). Meanwhile, the rapidity of *CAPN3* auto-degradation was also thought to through regulate its activity in N-terminal (Ono *et al.*, 2014). Although it has also been demonstrated to be a candidate protein for meat tenderness in mammals, including cattle (Robinson *et al.*, 2012; Natrass *et al.*, 2014) and pigs (De Smet *et al.*, 2003), its roles, particularly its physiological activities, in geese have not been sufficiently examined. Therefore, this work will have the potential to increase our understanding of the functional roles of *CAPN3* gene during improving goose meat quality provides a molecular basis. This study is the first to report the full-length cDNA of Sichuan White goose *CAPN3*. It contained a 2,316-bp coding sequence (CDS) that encodes 771 aa. Sequence alignment revealed that the aa sequence of the Sichuan white goose *CAPN3* protein was highly similar to those of species including Japanese quail, turkey, helmeted guineafowl, duck, pigeon, and chicken (greater than 90% sequence identity for all analyzed bird species). This result is consistent with the zoological classification and suggests that *CAPN3* protein is relatively evolutionarily conserved. Meanwhile, consistent with *CAPN3* protein in other animals, such as cattle and chickens (Zhang *et al.*, 2012; Pan *et al.*, 2013; Wang *et al.*, 2016), the Sichuan white goose *CAPN3* protein has no signal peptide.

Previous research has shown that disulfide bonds play an important role in maintaining the stability of protein conformations (Hatahet and Ruddock, 2009), and the instability coefficient is used as an indicator of protein stability. Usually, when the instability coefficient is greater than 40, the protein structure is considered unstable (Emanuel and Marco, 2011). In this study, the Sichuan white goose *CAPN3* was predicted to lack disulfide bonds, and its instability coefficient was less than 40, indicating that *CAPN3* is

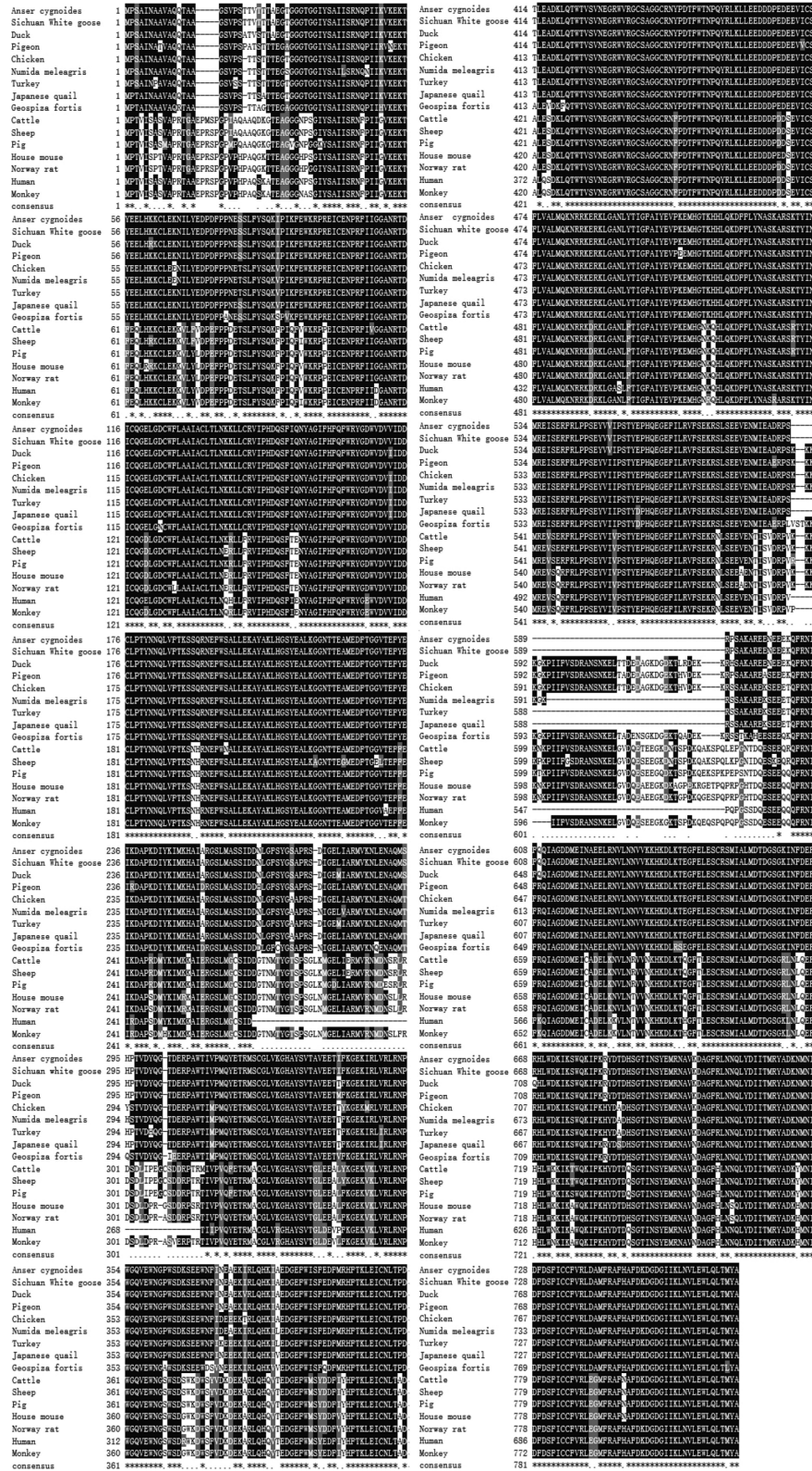


Fig. 1. Multiple alignment of deduced aa sequences of CAPN3 from Sichuan White goose and the indicated species. Absolutely conserved aa are highlighted in dark; highly conserved sequences are highlighted in gray. GenBank accession numbers of the CAPN3 sequences are listed in Table 2.

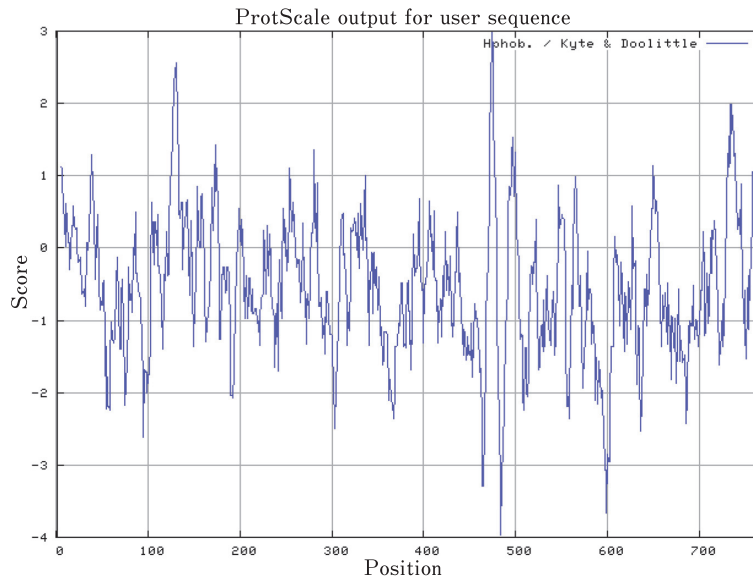


Fig. 2. Hydrophobicity profile of Sichuan White goose CAPN3 generated with the ProtScale program.

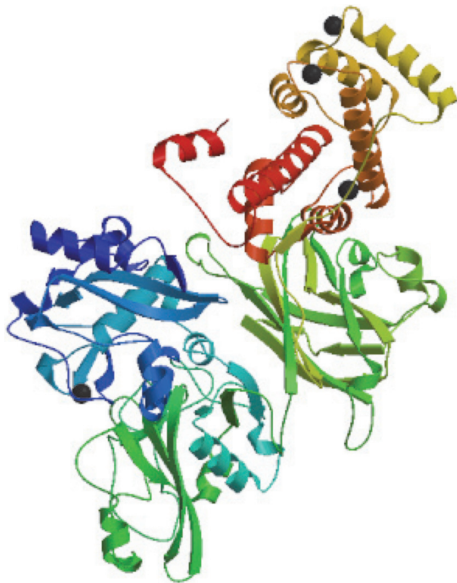


Fig. 3. 3D structure of Sichuan White goose CAPN3 based on homology modeling using SWISS-MODEL.

relatively stable.

Hägglund *et al.* (2004) reported that *N*-glycosylation occurs in the rough endoplasmic reticulum and the Golgi apparatus, whereas *O*-glycosylation occurs only in the Golgi apparatus. Our results indicated that goose CAPN3 might be regulated by glycosylation. Because goose CAPN3 was not predicted to have signal peptides, it likely cannot enter the rough endoplasmic reticulum to be glycosylated. Currently, there are no reports on the glycosylation of CAPN3 protein;

Table 3. Physiochemical characteristics of the Sichuan White goose CAPN3 protein

Physiochemical characteristics	CAPN3
Amino acids	771
Number of atoms	12,372
Molecular weight (Da)	89,019.81
Isoelectric point	5.54
Asp + Glu	116
Arg + Lys	97
Extinction coefficients	136,095
Aliphatic index	73.90
Instability index	36.75
Grand average of hydropathicity	-0.531

thus, we speculate that CAPN3 is translated and synthesized on the ribosomes of the rough endoplasmic reticulum and then enclosed in vesicles that merge with the Golgi apparatus, allowing glycosylation. This hypothesis regarding the process of CAPN3 glycosylation requires verification.

In addition to glycosylation, phosphorylation is considered an important modification. Huttlin *et al.* (2010) and Cohen-Kaplan *et al.* (2012) both considered that protein phosphorylation affects various biological processes, such as enzyme-activity regulation, cell division, and signal transduction. In this study, the goose CAPN3 protein was predicted to contain several phosphorylation sites, and the number of serine phosphorylation sites was larger than that in other species, such as cattle (Wang *et al.*, 2016). The different results might be due to the species being different.

To better understand the expression pattern of the goose *CAPN3* gene, *CAPN3* mRNA levels were analyzed in breast

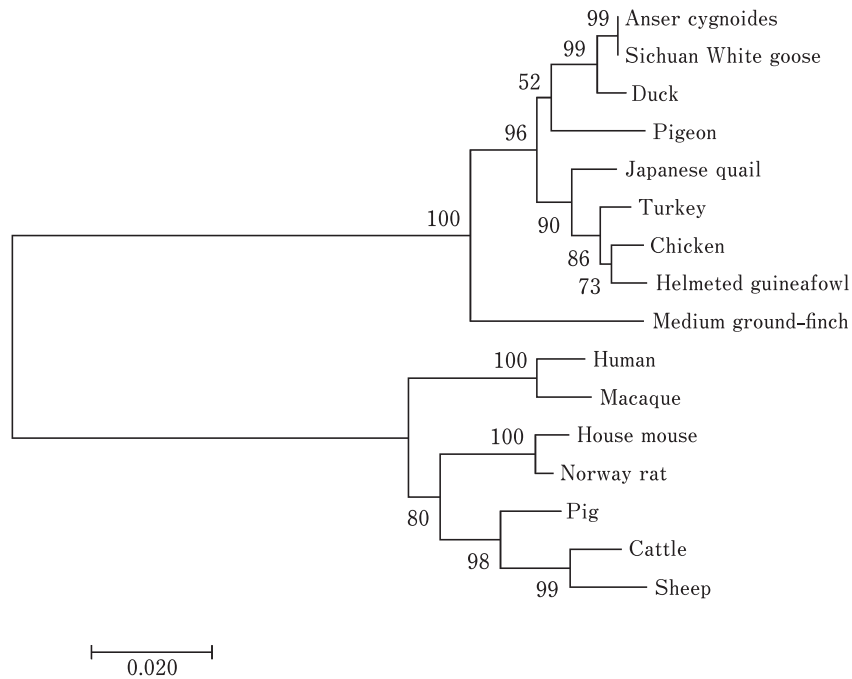


Fig. 4. **Phylogenetic tree based on aa sequences of CAPN3 from goose and the indicated species.** UPGMA analysis based on the Poisson correction model was performed using Mega 7.0 software. Numbers at each branch indicate the bootstrap percentage.

Table 4. **Putative phosphorylation sites identified in the goose CAPN3 protein**

Putative phosphorylation sites	Quantity	Position
Serine	33	Ser3, Ser17, Ser20, Ser85, Ser189, Ser190, Ser210, Ser255, Ser259, Ser260, Ser268, Ser271, Ser275, Ser321, Ser331, Ser364, Ser367, Ser394, Ser426, Ser437, Ser524, Ser528, Ser538, Ser546, Ser553, Ser569, Ser573, Ser575, Ser588, Ser591, Ser659, Ser676, Ser694
Threonine	22	Thr24, Thr25, Thr30, Thr34, Thr55, Thr134, Thr220, Thr231, Thr310, Thr333, Thr338, Thr403, Thr411, Thr422, Thr424, Thr450, Thr495, Thr530, Thr554, Thr639, Thr686, Thr717
Tyrosine	11	Tyr56, Tyr70, Tyr211, Tyr234, Tyr244, Tyr316, Tyr531, Tyr548, Tyr684, Tyr695, Tyr713

and thigh muscle tissues collected from Sichuan white geese. Our results showed that *CAPN3* was expressed in both muscle tissues, which is consistent with a report that the level of *CAPN3* gene expression in the breast muscle and leg muscle was significantly higher than that in other tissues in chickens (Zhang *et al.*, 2012). Wu *et al.* (2015) reported high levels of *CAPN3* expression in the muscle atrophic phase after denervation. The expression level of *CAPN3* in skeletal muscle is reportedly associated with carbohydrate

oxidation and insulin concentrations (Walder *et al.*, 2002). In the current study, *CAPN3* mRNA was abundantly expressed in goose muscle at the two developmental stages examined, and the mRNA levels tended to increase at the later growth stage (22 days) in thigh muscle and maintain a high level at the earlier growth stage (15 days) in breast muscle. This finding is in partial accordance with previous findings in duck, in which the *CAPN3* mRNA level is low at the embryonic stage, but increases at neonatal stages (7 days)

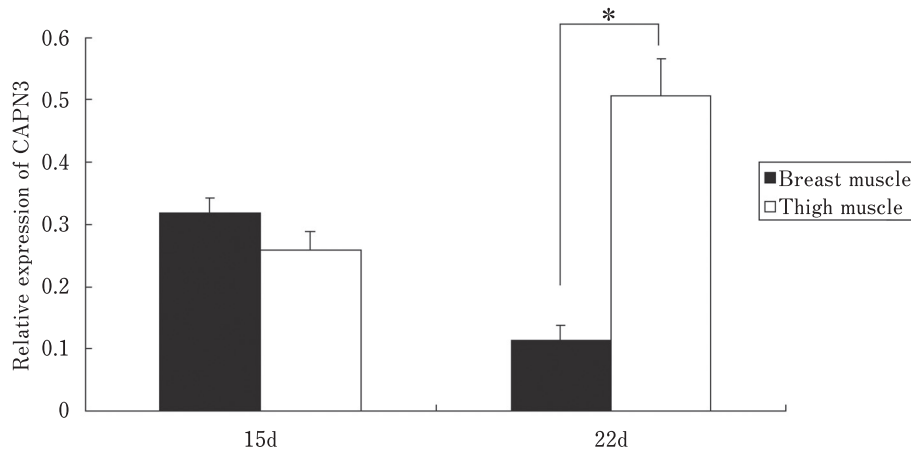


Fig. 5. mRNA expression levels of *CAPN3* in two different muscle tissues at the indicated growth stages. Error bars show the SEM of triplicate measurements. * $P < 0.05$.

Table 5. *CAPN3* mRNA expression levels in muscle tissues during different growth stages

Animal age	Number	Breast muscle	Thigh muscle
15 days	6	0.319 ± 0.025 ^a	0.259 ± 0.028
22 days	6	0.113 ± 0.025 ^b	0.506 ± 0.031 ^A

^{a, b} Means within a column with no common superscript differ significantly ($P < 0.05$);

^{A, B} Means within a row with no common superscript differ significantly ($P < 0.05$).

The results are shown as the mean ± SEM.

(Zhu *et al.*, 2014). Presumably, the abundant expression of *CAPN3* in developing goose muscle suggests that *CAPN3* is involved in the development and functions of goose muscle, such as controlling skeletal satellite cell proliferation/differentiation. Interestingly, the *CAPN3* expression level in thigh muscle was lower than that in breast muscle at 15 days, while at 22 days, the level in thigh muscle was higher than that in breast muscle. We speculated that through long-term breeding, the goose has lost its ability to fly, and therefore, the thigh muscle, primarily used for moving and swimming, has a higher level of activity, resulting in high *CAPN3* expression. *CAPN3* is not inhibited by calpastatin (Ono *et al.*, 2004) and its expression pattern has been a matter of debate. In chickens, the *CAPN3* mRNA level in breast muscle is higher than that in thigh muscle (Zhang *et al.*, 2012). Yang *et al.* (2012) reported that the *CAPN3* mRNA level in *longissimus* muscles of pigs was correlated with tenderness. Conversely, tenderization processes were not affected in *CAPN3* knockout mice (Geesink *et al.*, 2005). Additionally, Richard *et al.* (1995) found that inactivating mutations in human *CAPN3* cause recessive limb girdle muscular dystrophy type 2. These results suggest that *CAPN3* expression might have an effect on the tenderness of skeletal muscles, but unknown roles of *CAPN3* in different muscle types should be investigated further.

In conclusion, a full-length cDNA of *CAPN3* from the Sichuan white goose (2,316 bp long, encoding a 771 aa protein) was cloned and characterized. The protein was predicted to have 4 *N*-glycosylation, 13 *O*-glycosylation, and several phosphorylation sites. Homology analysis revealed that the aa sequence of goose *CAPN3* protein was highly similar to those of other species. qRT-PCR analysis showed that *CAPN3* expression was higher in breast muscle in 15-day-old than in 22-day-old animals. These results provide an important theoretical basis for further research into the functions and regulatory mechanism of *CAPN3* in geese.

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