Expression analysis, single-nucleotide polymorphisms of the Myoz1 gene and their association with carcase and meat quality traits in chickens

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ABSTRACT
Previous studies reported that the Myoz1 gene plays a crucial role in signal transduction and muscle fibre type differentiation. This finding suggests that the Myoz1 gene is a potential candidate for affecting carcase and meat quality traits in animals. Therefore, this study aimed to detect genetic variations in Avian and Yellow Bantam chickens and to evaluate the effects of these variations on economically important carcase and meat-quality traits. Expression analysis using quantitative real-time PCR indicated that Myoz1 was broadly expressed in all four studied tissues (liver, heart, breast muscle and leg muscle). Expression of Myoz1 was significantly higher in both leg muscle and breast muscle compared to the liver and heart. Then, using direct sequencing, a total of five single nucleotide polymorphisms (SNPs) were identified within the Myoz1 gene in two breeds of chickens. These breeds included three non-synonymous SNPs (SNP1: g. 16022512 G > T, SNP3: g. 16022560 C > T and SNP5: g.16023903 A > G) corresponding to A103S, H119Y and S189G, respectively. Statistical analyses indicated that SNP2 (g. 16022529 T > C), SNP3, SNP4 (g.16023878 A > C) and SNP5 significantly influenced some carcase and meat quality traits. Meanwhile, we did not find any association between the constructed haplotype and carcase traits in the Avian nor Yellow Bantam chickens. In conclusion, SNPs in the Myoz1 gene could be used for marker-assisted selection in chicken breeding.

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Introduction
In the past decade, the layers and broiler poultry industry has developed rapidly, and breeding goals for broiler chickens will evolve from increasing quantity to improving quality because meat quality has become one of the most important traits due to consumer preferences (Grunert 2006). There are many intrinsic and extrinsic factors that influence meat quality, including muscle properties, such as muscle diameter (Smith and Fletcher 1988), muscle growth (Ono et al. 1993) and muscle fibre type (Klont et al. 1998). Muscle fibre type can contribute to different metabolic rates in chickens due to different properties regarding oxidative ability, which have a large impact on the feed conversion ratio. Muscle fibre type is also associated with meat colour stability, tenderness and water-retention capacity. Myoz1 is a candidate gene associated with muscle fibre type (Frey et al. 2008).

Myoz1 belongs to a family that has three members, Myoz1 (FATZ1, CS2), Myoz2 (FATZ2, CS1) and Myoz3 (FATZ3, CS3) (Von et al. 2009). Three different groups reported this gene family independently over a short period of time. The first report was in 2000; Frey et al. (2000) found calsarcin-1 and 2 by using a calcineurin catalytic subunit in a yeast two-hybrid screen. They found a muscle-type-specific expression pattern for the CS family, in which CS1 is expressed specifically in cardiac and slow-twitch skeletal muscle and is expressed exclusively on the Z line. This finding was confirmed by Faulkner et al. (2000), who reported CS1 as FATZ and also found that CS1 was expressed in the Z line and bound to filamin/ABP-L, a-actinin and telethonin. One year later, Takada and his research teams...
found this gene using yeast two-hybrid assays with α-actinin and γ-filamin, and they named CS2 Myoz1 (Takada et al. 2001). Since Myoz1 was reported, it has emerged as a candidate gene for heart failure and hypertrophy. Myoz1 knock-out mice exhibit increased exercise ability due to muscle fibre type alterations that are regulated by enhanced calcineurin activity (Frey et al. 2008), which can induce the expression of NFAT and express slow-fibre-specific genes. Another study indicated that other members of the Myoz family can also be references for the function of Myoz1. Myoz2 protects against Angiotensin-II-induced cardiac hypertrophy (Frank et al. 2007), and Myoz2 knock-out mice suffer from accelerated cardiomyopathy (Frey et al. 2004) due to its negative role on calcineurin activities.

The Myoz family is also under intensive study in livestock. In porcine species, all three members of the Myoz family were cloned and characterised. Expression profiling revealed that Myoz1 is mainly expressed in heart, skeletal muscle and smooth muscle (Wang et al. 2006). Another study revealed that from embryonic 33 days to adult, Myoz1 showed a stepwise increase in the expression in porcine skeletal muscle but was not involved in cellular proliferation of differentiation of C2C12 cells (Liu et al. 2011). The potential of the Myoz family to be serving as molecular markers was also evaluated. In cattle, a synonymous mutation, g.16718C > A, is associated with backfat thickness and tenderness (Yang et al. 2009). However, a SNP of the Myoz3 gene in porcine species is not associated with any economic traits (Wang et al. 2007). To date, there has been no study of the chicken Myoz1 gene. In this study, we aimed to detect the expression pattern of the Myoz1 gene from the embryonic stage to 70 days of age in multiple tissues, and we investigated the occurrence and distribution of Myoz1 gene SNPs (single-nucleotide polymorphisms) within two typical chicken breeds and tested for associations with variations in several phenotypic traits. The objective of this study is to provide a fundamental understanding of the chicken Myoz1 gene; and provide a possible candidate gene for marker-assisted selection (MAS) in chicken-breeding and expanding our understanding of chicken genetics.

**Materials and methods**

**Ethics statement**

The Committee on Experimental Animal Management of Sichuan Agricultural University approved all of the animal experiments, which were carried out strictly according to the Regulations for the Administration of Affairs Concerning Experimental Animals of the State Council of the People’s Republic of China. The chickens involved in this study were executed as painlessly as possible to reduce their suffering.

**Animal materials**

In this study, two broiler lines were used, Avian and Yellow Bantam (YB). The first line was a fast-growing white broiler (Avian), which had undergone long time selection by the Avian international poultry breeding Co., Ltd. The second line was the YB chicken, a Chinese local breed with a good performance traits and high meat quality tailored to Chinese tastes. It is mainly distributed in Guangzhou and its surrounding areas. Three hundred fertile eggs of each line were used to obtain embryos. Fertile eggs of the Avian line were purchased from the Wenjiang Zheng Da Corporation (Chengdu, Sichuan), and fertile eggs of the YB line were purchased from Jinling Animal Husbandry (Nanning, Guangxi). All the chicken eggs were hatched on the experimental farm for poultry breeding at Sichuan Agricultural University (Ya’an Sichuan), after the eggs hatched, 44 males and 44 females from each line were selected randomly. All chickens were managed in cages in an environmentally controlled room with continuous white light and allowed ad libitum access to food and water. They were fed the same commercial maize-soybean diet, containing 11.5 MJ/kg of ME and 190 g/kg CP from 1 to 56 days, then 12.0 MJ/kg of ME and 170 g/kg CP after 56 days.

Three embryos on embryonic day 15 (E15) and six chickens (3 males and 3 females) on day 1 (D1), day 40 (D40) and day 70 (D70) after hatching from each line were sacrificed for their liver, heart, breast muscle and leg muscle (Table 1). As soon as the tissue samples were severed from their bodies, they were stored in liquid nitrogen until RNA extraction.

**Phenotypic measurements and genomic DNA extraction**

At 70 days of age, we randomly selected 70 chickens (35 males and 35 females) from each breed (Table 1). Their body weight (BW) was weighed and body indexes, including femoral diameter (FD), shank diameter (SD), metatarsal diameter (MD), femoral length (FL), shank length (SL), metatarsal length (ML), femoral circumferenece (FC), shank girth (SG) and metatarsal circumference (MC), were measured at 70 days. Then, all chickens were slaughtered, and blood samples were collected from the 70 individuals and stored at −80 °C. The carcase traits, including semi-evisceration weight.
(SEW), eviscerated weight (EW), breast muscle weight (BMW), leg muscle weight (LMW), wing weight (WW), heart weight (HW), liver weight (LW), gizzard weight (GW), proventriculus weight (PW), abdominal fat weight (AFW) and femoral weight (FW) were measured. Meanwhile, partial meat quality traits, such as pH value, lightness (L*), redness (a*) and yellowness (b*) of breast and leg muscles, were measured. All of the detailed measuring methods were performed as described in ‘The poultry production performance terms and measurement statistics method’ (NY/T823-2004).

Genomic DNA was isolated from the blood samples by the standard phenol/chloroform method (Albariño and Romanowski 1994), and the concentration and purity were determined by a Nanodrop 2000 (Thermo Scientific, USA). The appropriate amount of TE buffer was added to the DNA samples to achieve a concentration of 100 ng/µL. Then, the DNA samples were preserved at −20 °C until use.

### Total RNA extraction and cDNA preparation

Total RNA was extracted from liquid nitrogen-frozen tissue samples as described above using Trizol reagent (Invitrogen, Carlsbad, CA) and was dissolved in RNase-free H2O (Tiangen Biotech Co., Ltd, Beijing, China). The integrity of the RNA was evaluated via electrophoresis on 1% agarose gels, and the concentration and purity of RNA were detected with a Nanodrop 2000 at a 260/280 nm absorbance ratio (Thermo Scientific, USA).

cDNA was synthesised using the PrimeScript1 RT Reagent Kit (Perfect Real-Time) (TaKaRa Biotechnology Co., Ltd, Dalian, China) according to the manufacturer’s instructions. The reaction was performed using the Gene Amp PCR System 9700 (Bio-Rad, USA) in a volume of 10 µL containing 2 µL of 5 × PrimeScript™ buffer, 0.5 µL of PrimeScript™ RT Enzyme Mix I, 0.5 µL of Oligo dT Primer, 0.5 µL of random hexamers, 5.5 µL of RNase-free water and 1 µg of total RNA. The reactions were performed as follows: 42 °C for 2 min, 37 °C for 15 min and 85 °C for 5 s. According to the manufacturer’s instructions, the products of the reaction were stored at 4 °C.

### RT-qPCR analysis of Myoz1 expression

Chicken mRNA sequences of GAPDH (NM_204305.1) and Myoz1 (XM_421619.5) were retrieved from GenBank. The primers were designed by using Primer Premier 5 (Table 2). The expression levels of the chicken Myoz1 gene were detected by using the SYBR Green I assay on the PCR touch T960 (Hangzhou Jingle Scientific instrument Co., Ltd.). Each sample was run in duplicate and used an 11 µL mixture containing 6 mL of SYBR premix Ex Taq™ (Takara Biotechnology Co., Ltd, Dalian, China), 1 µL of cDNA, 0.5 µL of forward primer, 0.5 µL of reverse primer and 3 µL of RNase-free H2O (Tiangen Biotech Co., Ltd, Beijing, China) with the following procedure: 95 °C for 10 s; 40 cycles at 95 °C for 5 s and 60 °C for 30 s. The Myoz1 mRNA expression was analysed by the 2−ΔΔCt method after testing the amplifying efficiency of the primers.

### Myoz1 genetic variant identification

Based on the sequence of chicken Myoz1 (EMBL ID: ENSGALG000000005226), five pairs of primers (Table 2) were designed and synthesised by TsingKe Biological Technology (Chengdu, Sichuan) to amplify all five exons of Myoz1. Seventy DNA samples of each line were divided into 3 DNA pools (30, 30, 10) to detect which exon of Myoz1 contained SNPs. We used the Gene Amp PCR System 9700 (Bio-Rad, USA) to perform PCR in a 10 µL mixture containing a 1 µL pool of DNA, 0.5 µL (10 pmol/µL) of each primer, 3 µL of ddH2O and 5 µL of 2 × Mastermix (including Mg2+, dNTPs, Taq DNA polymerase; TsingKe Biological Technology, Chengdu, Sichuan). PCR was run with the following
Data were analysed with the Generalize Linear Model (GLM) procedures of SPSS 22.0. The model is as follows:

\[ Y_{ijk} = \mu + S_i + G_j + B_k + (G \times S) \times B_k + E_{ijk} \]

where \( Y \) represents the traits measured on chickens; \( \mu \) represents the population mean; \( S_i \) represents the fixed effects of sex; \( G_j \) represents the fixed effects of genotype; \( B_k \) represents the fixed effects of breed; \( G \times S \) represents the interaction among genotype, sex and breed; and \( E_{ijk} \) represents the random error.

The differences in Myoz1 mRNA expression were examined by one-way ANOVA. Data are presented as the mean ± SEM, and all statistically significant \( p \) values were less than .05.

### Results

**Expression profile of Myoz1**

Understanding the expression pattern of Myoz1 is one of the most important step towards understanding its function. Through real-time PCR, we are able to profile its expression patterns (Figure 1).

The expression pattern of Myoz1 showed a high tissue preference. The expression level of Myoz1 was significantly higher in both leg muscle and breast muscle compared to the liver and heart despite sex, breed and time (Figure 2). Although time is also considered to be one of the most important factors that influence the expression of Myoz1 in leg muscle and breast muscle, these two tissues, which had high Myoz1 expression, had different expression profiles. At E15, Myoz1 was expressed at a moderate level in both tissues and rose significantly in breast muscle after hatching until D40; then Myoz1 expression began to slowly increase to a plateau. Conversely, expression of Myoz1 in leg muscle after hatching showed a decreasing trend, and the decrease between D1 and D70 reached a significant level. Sex and breed are also key factors that influence the expression of Myoz1, and both breeds showed sex-dependent expression patterns at different time points. On D1, the Myoz1 expression level in leg muscle was significantly (\( p < .05 \)) higher among males than females in the Yellow Bantam line. On D40, the Myoz1 expression level in breast muscle was significantly (\( p < .001 \)) among females than males in the Avian

### Table 2. Primers used to check the expression level and identify SNPs in chicken Myoz1 gene.

| Gene name | Sequence of primer | Function | Production size | Annealing temperature, °C |
|-----------|--------------------|----------|-----------------|---------------------------|
| GAPDH    | F: 5'-CCGGAACATCACCCGCGTC-3' R: 5'-GCCAGCACTCGTTCCAACCAAA-3' | Real-time PCR | 136 | 60 |
| Myoz1    | F: 5'-TCCTAACAGCTTGGTTAGGATTTG -3' R: 5'-ACCACTCTGCATCAGCACTT-3' | Real-time PCR | 138 | 60 |
| P1       | F: 5'-GCCATCTCCGATCCGACCT-3' R: 5'-CTACCCCTTCGACGCCC-3' | PCR | 236 | 60 |
| P2       | F: 5'-GCATCTTGGCCCATACAAGCG-3' R: 5'-GCATCTTGGCCCATACAAGCG-3' | PCR | 451 | 60.5 |
| P3       | F: 5'-GGACACAAAAGCTGCAATGGT-3' R: 5'-GCCAGATAGAGTGCTATGGAAG-3' | PCR | 379 | 62 |
| P4       | F: 5'-GGCAAGATAGATGTTCAATAGCAAG-3' R: 5'-GCCAGATAGAGTGCTATGGAAG-3' | PCR | 467 | 60 |
| P5       | F: 5'-TGTCACCGAAATGGTGGGAGG-3' R: 5'-GCCAGATAGAGTGCTATGGAAG-3' | PCR | 480 | 60 |

P1 to P5 stands for the 5 exons of Myoz1 gene respectively.
breed, whereas there was no difference of expression detected in leg muscle.

**Identification of SNPs in the chicken Myoz1 gene**

A total of five primer pairs were designed to cover exons 3 and 4. After PCR amplification and direct Sanger-sequencing of the five amplicons from all of the DNA pools, the SNPs were identified and are listed in Table 3. In brief, we are able to identify 5 SNPs, g.16022512 G > T (SNP1), g.16022529 T > C (SNP2), g.16022560 C > T (SNP3), g.16023878 A > C (SNP4) and g.16023903A > G (SNP5). Three of them are non-synonymous, including SNP1, SNP3 and SNP5, which correspond to A103S, H119Y and S189G, respectively.

**Genotypic and allele frequencies**

The genotype and allele frequencies as well as the population genetic diversity parameters of the two chicken populations are shown in Table 3. For SNP1, only two genotypes were identified and were denominated as the GG and GT genotypes. The frequencies of the most frequent allele G in SNP1 were 0.99 (Avian) and 0.83 (YB), respectively. The second allele, T, was comparatively rare in Avian and YB chickens, which were the two breeds that showed no deviation from Hardy-Weinberg equilibrium (p > .05). For SNP2, the C allele was the most abundant allele and showed frequencies of 0.89 in Avian and 0.83 in YB. The populations of Avian and YB chicken were both in Hardy-Weinberg equilibrium (p > .05). For SNP3, SNP4 and SNP5, the C, A and A alleles were more abundant in both breeds. Meanwhile, at the SNP3 site, the homozygous state for the T allele was extremely rare in Avian (0.07) and was not observed in YB chickens (0), whereas a higher frequency of homozygous CC genotypes was observed in both breeds. For SNP4 and SNP5, the frequency of homozygous AA was higher than the CC and GG genotypes, respectively.

Figure 1. Overall presentation of the profiled expression pattern of Myoz1. The relative expression level of Myoz1 mRNA as analysed by RT-qPCR was processed by log10. AM: Avian male; AF: Avian female; YM: Yellow bantam male; YF: Yellow bantam female; E15: embryonic day 15; D1: 1 day after hatching; D40: 40 days after hatching and D70: 70 days after hatching.
Additionally, Avian and YB chickens both deviated significantly from Hardy–Weinberg equilibrium due to heterozygote deficiency ($p < .01$).

**Genetic diversity analysis**

It is generally known that PIC-values are indicative of the number of polymorphisms that are present in a gene; for example, $\text{PIC} < 0.25$ indicates a low number of polymorphisms, $0.25 < \text{PIC} < 0.50$ indicates an intermediate number of polymorphisms, and $\text{PIC} > 0.50$ indicates a high number of polymorphisms (Yuan et al. 2013). As shown in Table 4, Avian and YB chickens showed intermediate genetic diversity at the SNP3 and SNP4 loci, respectively, but the other SNPs all had a low number of polymorphism.

![Figure 2](image_url)

**Figure 2.** Expression profiling of *Myoz1*. (a) Expression profiling in different tissues at different times. E15: embryonic day 15; D1: 1 day after hatching; D40: 40 days after hatching; D70: 70 days after hatching; BM: breast muscle and LM: leg muscle. (b) Expression comparison between sexes on D1 in Yellow bantam breed. (c) Expression comparison between sexes on D40 in Avian bantam breed. *$p < .05$, **$p < .01$, ***$p < .001$.**

| SNP     | Breed | Number | Genotype frequency | Allele frequency | $p$ value |
|---------|-------|--------|--------------------|-----------------|-----------|
| SNP1 (G16022512T) | A     | 70     | GG 0.97, GT 0.03, TT 0.00 | G 0.99, T 0.01 | $p > .05$ |
|         | YB    | 70     | GG 0.66, GT 0.34, TT 0.00 | G 0.83, T 0.17 | $p > .05$ |
| SNP2 (T16022529C) | A     | 70     | CC 0.80, TC 0.19, TT 0.01 | C 0.89, T 0.11 | $p > .05$ |
|         | YB    | 70     | CC 0.66, TC 0.34, TT 0.00 | C 0.83, T 0.17 | $p > .05$ |
| SNP3 (C16022560T) | A     | 70     | CC 0.49, CT 0.44, TT 0.07 | C 0.71, T 0.29 | $p > .05$ |
|         | YB    | 70     | CC 0.79, CT 0.21, TT 0.00 | C 0.89, T 0.11 | $p > .05$ |
| SNP4 (A16023878C) | A     | 70     | AA 0.81, AC 0.04, CC 0.14 | A 0.84, C 0.16 | $p < .01$ |
|         | YB    | 70     | AA 0.71, AG 0.06, GG 0.23 | A 0.74, G 0.26 | $p < .01$ |
| SNP5 (A16023903G) | A     | 70     | AA 0.96, AG 0.00, GG 0.04 | A 0.96, G 0.04 | $p < .01$ |
|         | YB    | 70     | AA 0.94, AG 0.01, GG 0.04 | A 0.95, G 0.05 | $p < .01$ |

A: Avian line; AA: major allele homozygote; AB: heterozygote; BB: the minor allele homozygote; YB: Yellow Bantam line; $p$ value is the results of $\chi^2$ test of Hardy–Weinberg equilibrium.
Association analyses

After SNP identification, haplotype and diplotype was constructed, and we analysed the association between markers (SNPs, haplotype/diplotype) and carcase traits in both breeds through GLM as described above and applied a cut-off value of $p < 0.05$. We did not find any association between the constructed haplotype/diplotype and carcase traits in the Avian nor Yellow Bantam chickens (data are not shown). The results of the association between SNPs and carcase traits are listed separately for two breeds (Tables 5 and 6). In the Yellow Bantam line, we found that SNP2 was strongly correlated with the shank bone length ($p = 0.06$) and lightness of breast muscle ($p = 0.01$), and SNP3 was significantly correlated with 8 carcase traits, including semi-evisceration weight ($p = 0.046$), evisceration weight ($p = 0.035$), breast muscle weight ($p = 0.037$), leg muscle weight ($p = 0.033$), heart weight ($p = 0.034$), metatarsal length ($p = 0.06$), and the pH value of breast muscle ($p = 0.021$). SNP5 was correlated with 5 carcase traits, including leg muscle weight ($p = 0.043$), femoral diameter ($p < 0.001$), metatarsal weight ($p = 0.049$), and shank bone length ($p = 0.036$). In the Avian line, SNP3 was associated with liver weight ($p = 0.031$), SNP4 was associated with heart weight ($p = 0.002$) and SNP5 was associated with 5 carcase traits, including leg muscle weight ($p = 0.031$), gizzard weight ($p < 0.001$), proventricular weight ($p = 0.031$), shank bone length ($p < 0.001$), shank bone diameters ($p = 0.037$), the $a^*$-value of breast muscle ($p < 0.001$) and the leg muscle ratio ($p = 0.028$).

**Discussion**

Temporal and spatial expression patterns are central characteristics of a gene. In mice and humans, the Myoz1 gene is expressed in skeletal muscle, specifically in fast twitch muscle (Frey et al. 2000). In porcine species, CS-2 mRNA is most abundant in skeletal muscle,

| Table 4. The genetic information of SNP sites. |
|---|
| **Species** | **SNP** | **Ho** | **He** | **PIC** |
| Avian | SNP1 | 0.343 | 0.284 | 0.0196 |
| | SNP2 | 0.343 | 0.284 | 0.1766 |
| | SNP3 | 0.214 | 0.191 | 0.3270 |
| | SNP4 | 0.057 | 0.366 | 0.2327 |
| | SNP5 | 0.014 | 0.095 | 0.0739 |
| Yellow Bantam | SNP1 | 0.029 | 0.028 | 0.2424 |
| | SNP2 | 0.186 | 0.191 | 0.2424 |
| | SNP3 | 0.443 | 0.414 | 0.1766 |
| | SNP4 | 0.043 | 0.275 | 0.3108 |
| | SNP5 | 0.000 | 0.082 | 0.0905 |

Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphism information content.

| Table 5. Genotype and associated carcase trait of Yellow Bantam. |
|---|
| **Markers** | **Genotype** | **Traits** | **p value** |
| SNP2 | L$^*$ value of breast muscle | TT (7) | 43.730 ± 3.31$b$ |
| | Semi-evisceration weight, kg | CC (34) | 1.154 ± 0.054$b$ |
| | Evisceration weight, kg | 0.894 ± 0.039$b$ |
| | Breast muscle weight, g | 75.551 ± 3.364$b$ |
| | Leg muscle weight, g | 99.361 ± 6.336$b$ |
| | Heart weight, g | 8.001 ± 0.704$b$ |
| | Shank bone circumference, cm | 3.921 ± 0.069$b$ |
| | Breast muscle PH value | 5.931 ± 0.041$b$ |
| SNP3 | Leg muscle weight, g | 108.787 ± 4.575$b$ |
| | Metatarsal length, cm | 7.870 ± 0.155$b$ |
| | Shank bone length, cm | 10.786 ± 0.183$b$ |
| SNP4 | Liver weight, g | 52.773 ± 1.275$b$ |
| | Heart weight, g | 40.767 ± 0.655$b$ |
| | Leg muscle weight, g | 266.211 ± 3.892$b$ |
| | Gizzard weight, g | 9.224 ± 0.058$b$ |
| | Proventriculus weight, g | 9.521 ± 0.219$b$ |
| | Femoral length, cm | 12.425 ± 0.058 |
| | Shank bone length, cm | 4.788 ± 0.058|
| | Leg muscle weight | 20.550 ± 1.836 |

$ab$: Values in the same row not sharing a common superscript differ significantly ($p < 0.05$).

| Table 6. Genotype and associated carcase trait of Avian. |
|---|
| **Markers** | **Genotype** | **Traits** | **p value** |
| SNP3 | Liver weight, g | 52.773 ± 1.275$b$ |
| | Heart weight, g | 40.767 ± 0.655$b$ |
| SNP4 | Leg muscle weight, g | 266.211 ± 3.892$b$ |
| | Gizzard weight, g | 9.224 ± 0.058$b$ |
| | Proventriculus weight, g | 9.521 ± 0.219$b$ |
| | Femoral length, cm | 12.425 ± 0.54 |
| | Shank bone length, cm | 4.788 ± 0.058 |

$ab$: Values in the same row not sharing a common superscript differ significantly ($p < 0.05$).
with prominent expression detected in smooth muscle, the liver and the lung, and lower levels are detected in both the heart and kidney, with temporal expression patterns revealing a rise throughout the whole prenatal period and a gradual drop in post the prenatal period (Wang et al. 2006). Our results showed similar spatial patterns, with temporal expression patterns from porcine species. The skeletal muscle used for the study (Wang et al. 2006) is longissimus dorsi muscle, and the different temporal expression patterns between longissimus dorsi muscle and leg muscle in our study perhaps occurred due to distinctions between different skeletal muscles. Whereas both breast muscle and longissimus dorsi muscle are predominantly fast-twitch glycolytic muscle, differences between the two tissues still exist. This phenomenon reveals a different expression pattern of Myoz1 between birds and mammals, and therefore, we suspect that the mechanism of the Myoz1 gene may vary by species. Interestingly, expression of Myoz1 was barely detected in leg muscle at day 70. Although the slow muscle fibres in leg muscle account for a large proportion of the total fibre, there is still a considerable number of fast twitch fibres in which the Myoz1 gene was specifically expressed. We conclude that the Myoz1 gene is expressed specifically in fast twitch muscle, but not all fast twitch fibres express the Myoz1 gene. The mechanism for different myoz1 expression patterns for fast twitch fibres that come from two types of skeletal muscle requires further study.

Additionally, we found sex to be a key factor that influence chicken Myoz1 gene expression, and we speculated that the differences between male and female chickens were due to different muscle development patterns in skeletal muscle.

After analysing basic characteristics and expression profiles, we conducted variation analysis on the Myoz1 gene in two highly genetic diversify breeds. The SNPs were great candidates for molecular markers; 5 SNPs were found in an exon of the Myoz1 gene, including 3 non-synonymous SNPs in both breeds. In the avian line, 11 traits were found to be associated with SNPs, and one of 11 traits particularly caught our interest, the L* value of breast muscle, due to it being a sign of different muscle fibre components (Klont et al. 1998). Functional studies of Myoz1 have mainly focussed on its negative role in fast-to-slow muscle fibre transition, and the Myoz1 knock-out mouse has a better capacity for exercise and stamina due to excess slow muscle fibre resulting from the increase in the CaN/NFAT pathway activity (Frey et al. 2008), which influences muscle colour. Although SNP2 is not a non-synonymous SNP, a previous study suggested that synonymous SNPs influence the splicing and/or mRNA stability of mRNA (Chamary et al. 2006), and are associated with their expression level. Further study is needed to confirm this hypothesis. We also believe that SNP5 of the Avian breed, which is correlated with the redness (a*) value of leg muscle, supports our conclusion. Association analysis not only provides valuable evidence for MAS, but also provides insight into the function of the Myoz1 genes. Ever since it was first reported, the Myoz family has emerged as candidate genes for heart hypertrophy (Frank et al. 2007; Osio et al. 2007; Paulsson et al. 2010; Bang 2016). Our results show that SNP3 and SNP4 are associated with heart weight in the Yellow Bantam line and Avian line, respectively. As mentioned before, SNP3 is located inside the N-myristoylation site motif, which may influence the function of Myoz1 because post-translational regulation can contribute to cardiomyopathies (Paulsson et al. 2010), and generally, our results revealed the potential of Myoz1 gene variations to be markers for marker-assisted selections. However, before applying these finding to selection, more work should be done, including expanding the population for an association study and including more breeds in that study, meanwhile, collected the tissues from the phenotypic measured chicken for total RNA extraction and Myoz1 gene expression analysis in future research, to know whether there is a relationship among the the differential expression level of Myoz1 gene, SNPs and phenotypes may help to understanding the regulation and function of Myoz1 gene and further confirm our finding.

Conclusions

In general, we analysed the expression pattern of the chicken Myoz1 gene in both Avian and Yellow Bantam breeds and found that the Myoz1 gene is not expressed in all fast twitch fibres and that the Myoz1 expression pattern is correlated with tissue, sex and age. We suspect that expression of Myoz1 may have different patterns between mammals and birds. We also analysed the correlations of variations with multiple carcase traits. We found 5 SNPs of Myoz1 in the Avian breeds, and 3 of which is correlated with 10 traits, including the redness of leg muscle. In the Yellow Bantam breed, we also found 5 SNPs, and 3 of them were correlated with 11 carcase traits, including the redness of breast muscle. Further studies need to be complete to further validate our results that show that SNPs of Myoz1 are suitable use as meat quality markers.
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Disclosure statement

The authors declare that they have no conflicts of interest.

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