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Analysis of the genetic effects of *CAPN1* gene polymorphisms on chicken meat tenderness

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ABSTRACT. The micromolar calcium-activated neutral protease gene (*CAPN1*) is a physiological candidate gene for meat tenderness. Four previously identified single nucleotide polymorphism (SNP) markers located within the *CAPN1* gene were evaluated for their associations with variation in the meat tenderness of a Chinese indigenous chicken breed, a higher meat quality breed (i.e., Qingyuan partridge chicken), and the commercial Recessive White chicken breed. Warner-Bratzler shear force measurements were used to determine tenderness phenotypes for all animals; intramuscular fat (IMF) content and rate of water loss in the breast muscles were also measured. Genotyping was performed by the polymerase chain reaction-ligase detection reaction method. Polymorphisms were identified for all markers, except CAPN1 2546. The frequency of allele T was zero, and allele C was fixed for CAPN1 2546 in the studied populations. The SNP CAPN1 3535 in the *CAPN1* gene was significantly associated with tenderness and other meat quality traits, where animals inheriting the AA genotype had smaller shear force values, lower water loss rates, and higher IMF contents. Moreover, H1 (AAA) was the most advantageous haplotype for meat tenderness. The results of this study confirm some previously documented associations. Furthermore, novel associations have been

identified that, following validation in other populations, could be incorporated into breeding programs to improve meat quality.

Key words: Calpain (CAPN1); Ligase detection reaction; Chicken; Polymorphism; Meat tenderness

INTRODUCTION

Meat quality is of great importance in the broiler industry because consumers are willing to pay more money for superior products (Shackelford et al., 2001). Variation in meat tenderness has significant effects on consumer satisfaction with regard to chicken meat, and there has been significant interest in genetic selection to minimize problems associated with variation in meat tenderness. However, tenderness is a complex trait for breeding programs; variability in meat tenderness has not diminished, in part because of the inability to accurately select for increased tenderness. Thus, molecular marker information can be of great use in the identification of animals with particular genetic traits associated with the desired tenderness, and the selection process can be conducted on young animals, even before birth.

Calpains [i.e., intracellular Ca^{2+}-dependent cysteine proteases (EC 3.4.22.17)] involvement in muscle growth and development has been previously demonstrated. Furthermore, they are proenzymes that are regulated by Ca^{2+} binding and autoproteolytic modification (Goll et al., 2003). The calpains were discovered because the Z-disks in the muscle strips incubated in a Ca^{2+}-containing solution disappeared in the absence of other ultrastructurally detectable changes (Dayton et al., 1981). Four calpain genes (i.e., μ-calpain gene, *CAPN1*; m-calpain gene, *CAPN2*; p94 gene, *CAPN3*; and μ/m-calpain, *CAPN1.5*) are expressed ubiquitously in chickens (Okumura et al., 2005). Among the calpain family members, *CAPN1* degrades myofibrillar proteins under postmortem conditions and appears to be the primary enzyme in the postmortem tenderization process (Koohmaraie, 1992, 1994, 1996). Regulation of *CAPN1* activity has been correlated with variation in meat tenderness; thus, *CAPN1* is a good candidate gene for tenderness (Geesink and Koohmaraie, 1999).

Polymorphisms within the *CAPN1* gene have been previously investigated in association studies for meat quality traits, mainly for tenderness and marbling (Page et al., 2002; Okumura et al., 2006; Zhang et al., 2007a,b, 2008; Ribeca et al., 2013). In chickens, 4 polymorphisms, 3 synonymous single nucleotide polymorphisms (SNPs) (i.e., C2546T, G3535A, and C7198A), and one SNP within the 3'-UTR (G9950A) of the *CAPN1* gene have been found to have significant effects on meat tenderness (Zhang et al., 2007a,b, 2008). However, a publicly available evaluation on the association of these SNPs in Qingyuan partridge (QY) chicken is not yet available. QY chicken is an important indigenous breed distributed in Qingyuan, China. It is a light-body-type breed with good meat quality that is famous for its 3 "yellow", 2 "thin", and one "partridge" morphological features (i.e., yellow beak, shanks, and skin; thin head and bone; and partridge feathers).

In the present study, we describe a new, sensitive assay for the detection of the *CAPN1* gene based on polymerase chain reaction-ligase detection reaction (PCR-LDR). LDR was originally developed for discriminating single-base mutations or polymorphisms (Barany and Gelfand, 1991). It utilizes the ability of DNA ligase to preferentially seal adjacent oligonucleotides hybridized to target DNA in which there is perfect complementation at the nick junction. The objective of this study was to identify 4 reported polymorphisms in the *CAPN1* gene

and assess the associations between the *CAPN1* polymorphisms and meat quality variation in QY and Recessive White (RW) chickens.

MATERIAL AND METHODS

Experimental animals

All animal studies were conducted in accordance with an animal use protocol approved by the animal use committee of the Chinese Ministry of Agriculture. RW chickens (Jiang-13 strain of white Plymouth Rock) and a preserved population of QY chickens were used in this study. All breeds were reared with the same energy (12.97 MJ/kg) and protein (17.01%) levels of diet under the same management system from the National Gene Pool for Indigenous Chicken Breeds (Yangzhou, China). All birds were kept in a stacked cage raising system, with one cage for each bird. Two hundred female chickens were randomly selected from each breed using a random-sampling method. Slaughter was conducted at the age of 16 weeks. All animals were measured and samples were collected. Meanwhile, 0.4 mL blood was collected from the ulnar vein of each individual, with heparin as an anticoagulant.

Phenotypic traits

After slaughter, 3 steaks of breast muscles on the same side of each carcass were sawed longitudinally in the middle; for the tenderometer, intramuscular fat (IMF) percentage, and water loss rate tests, samples were 2-3, 1-2, and 1 cm thick, respectively. For tenderometer testing, steaks were chilled for 24 h at 2° ± 1°C. Subsequently, they were placed in a water bath at 100°C until the center of the sample reached 82°C. Samples were left to cool to 7°C; then, tenderness was measured by Warner-Bratzler shear force, which determines the relative force required to pass a blunt blade through a section of meat. The measurements of IMF percentage and water loss rate were performed as described by Perry et al. (2001).

DNA extraction and genotyping

DNA was extracted from whole blood samples using the Puregene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MI, USA). The PCR-LDR method was used to perform genotyping. Four pairs of specific primers for CAPN1 (i.e., C2546T, G3535A, C7198A, and G9950A) were designed according to the genomic sequence of the *CAPN1* gene in the GenBank database (accession No. NC_006090.1; Table 1). PCR was conducted on the ABI 9600 (Applied Biosystems, Foster City, CA, USA) in a system with a total volume of 20 µL, which contained 50 ng genomic DNA, 5 pM primer mixture, 20 mM of each dNTP, 100 mM Mg^{2+}, 5X Q-Solution, and 5 U/µL Taq DNA polymerase. The amplification protocol was comprised of an initial denaturation and enzyme activation phase at 95°C for 15 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 1 min, extension at 72°C for 1 min; and a final extension at 72°C for 7 min. PCR products were checked on a 3% agarose gel stained with ethidium bromide to verify the amount added in the LDR.

For each SNP, 3 probes were designed, including one common probe and 2 discriminating probes for the 2 types (Table 2). The common probe anneals to the PCR-amplified template immediately downstream of the nucleotide in question. One allelic probe has at its 3'-end

the nucleotide corresponding to the wild-type allele. The other has at its 3'-end the nucleotide corresponding to the variant allele. These 2 allelic probes compete to anneal to the template adjacent to the common probe. This generates a double-stranded region containing a nick (i.e., missing phosphodiester bond) at the nucleotide position to be tested. Only an allelic probe with perfect complementation to the template is ligated to the common probe via DNA ligase.

Table 1. Sequences and PCR conditions for each pair of primers.

Primers	Sequence of the primer	Length of the product	Tm (°C)
2546C/T-forward	5'-ACCTCACGTGCCTCTCTCAC-3'	214	56
2546C/T-reverse	5'-AGCGGAACACTTACGTCGAT-3'		
3535G/A-forward	5'-TCACCTGAGGTTTGCATGTT-3'	190	56
3535G/A-reverse	5'-AGCCATCAAATGCAGGAAGT-3'		
7198C/A-forward	5'-GGTTCAGCAGGTTGTGCTTT-3'	216	56
7198C/A-reverse	5'-AGAGAGCCGAGCCCTAGTTC-3'		
9950G/A-forward	5'-CCCCTCTGTCCCACCATAG-3'	242	56
9950G/A-reverse	5'-GGGGGAAAGAGGAAAACAGA-3'		

Tm = melting temperature.

Table 2. Probe sequences of LDR.

Probe name	Probe sequences (5'-3')
2546C/T_modify	5' P-AGCGACTCGTAGCAGCCGTTCAGCCTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM 3'
2546C/T_C	5' TTTTTTTTTTTTTTTTTTTTTTTTTTAAAGCCCTCAGTGGTGCCCCCTGAG 3'
2546C/T_T	5' TTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAGCCCTCAGTGGTGCCCCCTGAA 3'
3535G/A_modify	5' P-TTCACCAGCTTCTTGAAGGTCACTGTTTTTTTTTTTTTTTTTTTTTTTT-FAM 3'
3535G/A_A	5' TTTTTTTTTTTTTTTTTTTTTTTTTTGAAGGCGGTGACAGAGGCATGGCCT 3'
3535G/A_G	5' TTTTTTTTTTTTTTTTTTTTTTTTTTGAAGGCGGTGACAGAGGCATGGCCC 3'
7198C/A_modify	5' P-CCATCCGTCTTCAGATCTTTGTCTGTTTTTTTTTTTTTTTTTT-FAM 3'
7198C/A_C	5' TTTTTTTTTTTTTTTTTTGTTGCGGCAGGAGTCCAGACTGAAG 3'
7198C/A_A	5' TTTTTTTTTTTTTTTTTTTTTGTTGCGGCAGGAGTCCAGACTGAAT 3'
9950G/A_modify	5' P-GTGCATAGGATGAGAGGAGCACGATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM 3'
9950G/A_A	5' TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAGGGTGTAGTGGTACTGCTGTGT 3'
9950G/A_G	5' TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAGGGTGTAGTGGTACTGCTGTGC 3'

For each PCR product, the ligation reaction was performed in a final volume of 10 µL containing 1 µL buffer, 1 µL probe mix, 0.05 µL Taq DNA ligase (New England Biolabs, USA), 1 µL PCR product, and 6.95 µL deionized water. The LDR parameters were as follows: an initial heating at 94°C for 2 min, followed by 35 cycles of 30 s at 94°C and 2 min at 60°C. The LDR products were analyzed on the ABI 3100 DNA Sequencer (Applied Biosystems). To confirm the accuracy of the PCR-LDR genotyping method, direct DNA sequencing of randomly selected PCR products was performed. The proportion of the sequencing samples were ~5%. The results of the PCR-LDR genotyping showed 100% concordance to direct DNA sequencing of the randomly selected PCR products.

Statistical analyses

Haplotypes were inferred by the PHASE 2.0 program (Stephens et al., 2001). Association analyses of single polymorphisms or haplotypes with meat quality were determined by ANOVA using a general linear model (GLM) and type III sums of squares performed by the SAS 9.0 software. The model was calculated as follows:

$$Y_{ij} = \mu + B_i + G_j + e_{ij}$$

where Y_{ij} is an observation on the traits, μ is the overall population mean, B_i is the effect of breed, G_j is the effect of genotype or haplotype, and e_{ij} is the residual error. Multiple comparisons were analyzed with the least square means (LSM), and for a given trait, if its effect had a P value >0.05 it will be excluded from the model. The minimum diplotype frequency was set at 2%. All values are reported as LSM ± SE.

RESULTS

Gene and genotype frequencies

The first objective of this study was to test whether SNPs associated meat quality and carcass traits in other chicken populations are polymorphic in QY chickens. The results showed that the markers *CAPN1* 3535, *CAPN1* 7198, and *CAPN1* 9950 were polymorphic both in RW and QY chickens because fixed alleles were not observed and the allele frequencies for all markers were >1% (Table 3). However, for *CAPN1* 2546, animals that did and did not contain TT and CT, respectively, were observed; the frequencies of alleles T and C were zero and fixed, respectively (Table 3). The electrophoretic profile for the PCR-LDR analyses of the C2546T, G3535A, C7198A, and G9950A sites are shown in Figures 1-4. There were large breed differences in allelic frequencies of 2 SNPs (i.e., G3535A, G9950A). At the G3535A and G9950A sites, the A allele was more frequent than the G allele in QY chickens, while the G allele was more frequent than the A allele in RW chickens. At the C7198A site, the allelic frequency distribution was in agreement among breeds; the A allele was more frequent than the C allele.

Table 3. Allelic frequencies of the 4 sites in the 2 chicken populations.

Breed (N)	2546		3535		7198		9950	
	C	T	G	A	C	A	G	A
QY (200)	1.00	0.00	0.14	0.86	0.38	0.62	0.30	0.70
RW (200)	1.00	0.00	0.72	0.28	0.31	0.69	0.61	0.39

RW and QY represent Qingyuan partridge chicken and Recessive White chicken breeds, respectively.

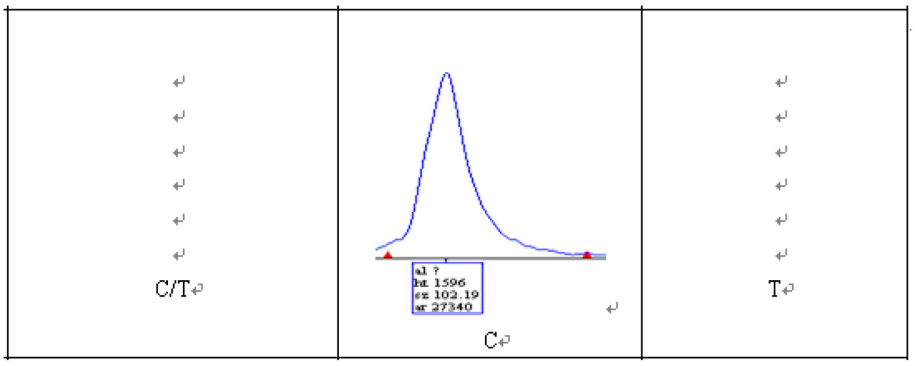

Figure 1. Genotype results for *CAPN1* C2546T.

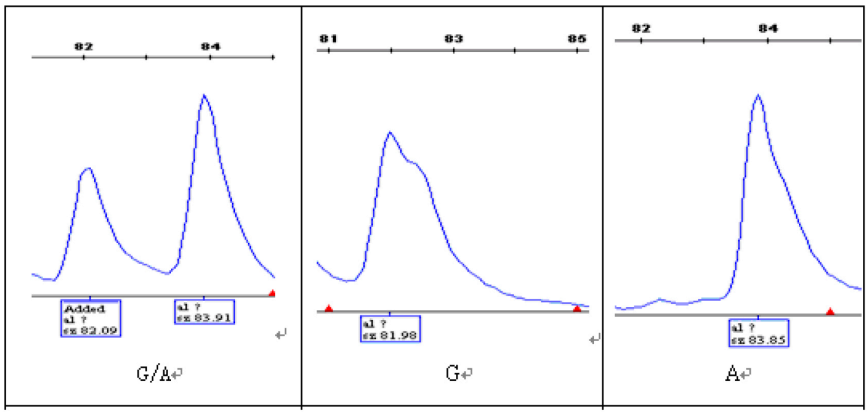

Figure 2. Genotype results for *CAPN1* G3535A.

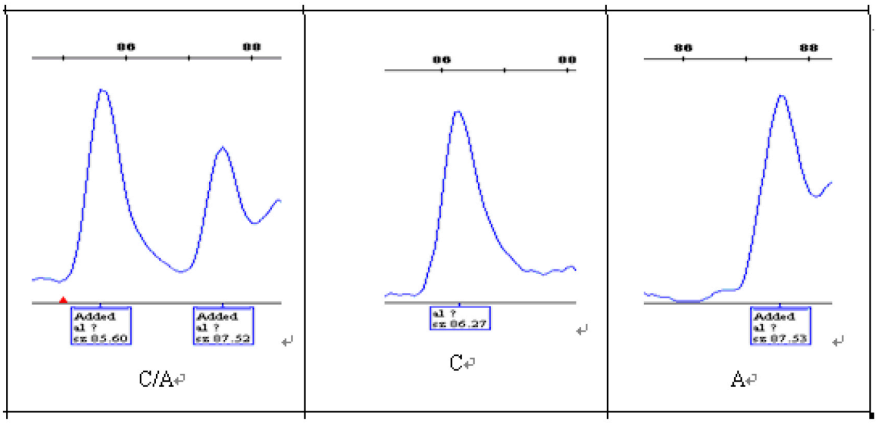

Figure 3. Genotype results for *CAPN1* C7198A.

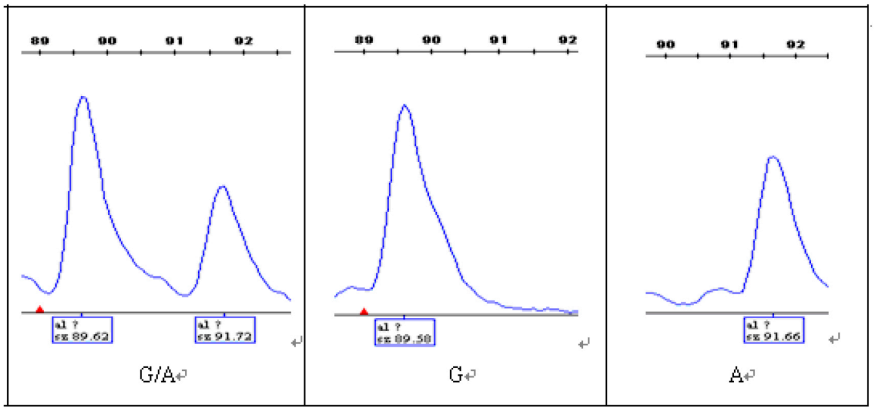

Figure 4. Genotype results for *CAPN1* G9950A.

Haplotypes and frequencies

Haplotypes constructed based on 3 SNPs and their frequencies in the 2 breeds are shown in Table 4. Seven haplotypes were identified in 400 chickens. We identified 2 major haplotypes, including H1 (AAA, 43%) and H7 (GCA, 41%); and 5 moderate haplotypes, including H2 (AAG, 28%), H3 (ACA, 23%), H4 (ACG, 21%), H5 (GAA, 30%), and H6 (GCG, 14%). H1 was a unique haplotype in QY chickens, while H7 was a unique haplotype in RW chickens.

Table 4. Haplotypes constructed with 3 single nucleotide polymorphisms (SNPs) and frequencies in the 2 populations.

Haplotype	Site			Breed	
	3535	7198	9950	QY	RW
H1	A	A	A	0.43	-
H2	A	A	G	0.12	0.16
H3	A	C	A	0.18	0.05
H4	A	C	G	0.15	0.06
H5	G	A	A	0.08	0.22
H6	G	C	G	0.04	0.10
H7	G	C	A	-	0.41

RW and QY represent Qingyuan partridge chicken and Recessive White chicken breeds, respectively.

Association of SNPs with chicken meat quality traits

Statistical analysis was applied to test the significance of differences in the breed effect, genotype effect, and interaction between the genotype and breed effects among different genotypes of the 3 loci. The breed effect and interaction between the genotype and breed effects were not significant. The only significant genotypic effect was identified in the G3535A polymorphic locus; therefore, we further analyzed the genotypic effect of the G3535A site using a combination of the 2 breeds (Table 5). In summary, AA genotype individuals had significantly smaller shear force values, lower water loss rates, and higher IMF contents than those of the GG genotype individuals ($P < 0.05$). AA and GA genotype birds had significantly smaller shear force mean value than that of the GG genotype birds (-0.09 and -0.14, respectively), and there was no significant difference between the AA and GA genotypes. Similar results were obtained for the water loss rate trait; AA and GA genotype birds had significantly lower mean water loss rate than that of the GG genotype birds (-0.015 and -0.020, respectively), and there was no significant difference between the AA and GA genotypes. Individuals with the AA genotype had significantly higher mean IMF content than those of the GA (+0.077) and GG (+0.090) genotype individuals, and there was no significant difference between the GA and GG genotypes.

Association of *CAPN1* haplotypes with chicken meat quality traits

With regard to the haplotypes, 9 diplotypes with frequencies >2% (i.e., 36 of H1H1, 24 of H1H2, 26 of H1H3, 26 of H1H4, 14 of H1H5, 16 of H1H6, 10 of H3H3, 10 of H3H4, and 10 of H3H5) were observed in QY chickens, and 8 diplotypes (i.e., 42 of H2H7, 12 of H3H5, 12 of H3H7, 14 of H4H7, 12 of H5H5, 46 of H5H7, 14 of H6H7, and 28 of H7H7)

were observed in RW chickens; only H3H5 was a shared diplotype between the 2 chicken populations. Thus, a total of 352 individuals with 16 diplotypes (i.e., 36 of H1H1, 24 of H1H2, 26 of H1H3, 26 of H1H4, 14 of H1H5, 16 of H1H6, 10 of H3H3, 10 of H3H4, 22 of H3H5, 42 of H2H7, 12 of H3H7, 14 of H4H7, 12 of H5H5, 46 of H5H7, 14 of H6H7, and 28 of H7H7) were used in the association analyses, and a significant association ($P < 0.05$) between the haplotypes and meat quality was observed (Table 6). Among the 16 diplotypes, H1H1 had a significantly smaller ($P < 0.05$) mean value for shear force when compared to those of the H1H4, H2H7, H3H7, H5H5, H5H7, H6H7, and H7H7 diplotypes. H5H5 had a significantly higher ($P < 0.05$) mean value of water loss rate compared to those of the H1H5, H1H6 and H2H7 diplotypes. Moreover, the H1H1, H1H3, and H3H7 diplotypes had significantly higher ($P < 0.05$) mean values for IMF content when compared to those of the H1H2, H2H7, H3H3, H3H4, H3H5, H4H7, H5H5, H5H7, and H7H7 diplotypes.

Table 5. Association of G3535A genotypes with meat quality traits in chicken breeds.

Traits	GG	GA	AA
Shear force (kg)	2.463 ± 0.147^{a}	2.323 ± 0.093^{b}	2.373 ± 0.093^{b}
Water loss rate (%)	0.317 ± 0.012^{a}	0.297 ± 0.008^{b}	0.302 ± 0.009^{b}
IMF (%)	1.428 ± 0.141^{a}	1.441 ± 0.090^{a}	1.518 ± 0.092^{b}

[a,b]Means with the different superscripts within the same row differ significantly ($P < 0.05$). IMF = intramuscular fat.

Table 6. Least squares means ± SE of myofiber types according to haplotype combinations[1].

Trait	Shear force (kg)	Water loss rate (%)	IMF (%)
H1H1	1.820 ± 0.264^{a}	0.309 ± 0.010^{ab}	2.076 ± 0.127^{a}
H1H2	2.388 ± 0.153^{ab}	0.316 ± 0.013^{ab}	1.089 ± 0.147^{b}
H1H3	2.280 ± 0.147^{ab}	0.309 ± 0.012^{ab}	2.053 ± 0.254^{a}
H1H4	2.502 ± 0.147^{b}	0.305 ± 0.012^{ab}	1.708 ± 0.123^{ab}
H1H5	2.460 ± 0.216^{ab}	0.282 ± 0.018^{a}	1.886 ± 0.228^{ab}
H1H6	2.170 ± 0.200^{ab}	0.286 ± 0.017^{a}	1.815 ± 0.147^{ab}
H2H7	2.488 ± 0.132^{b}	0.301 ± 0.011^{a}	1.092 ± 0.120^{b}
H3H3	2.335 ± 0.264^{ab}	0.325 ± 0.022^{ab}	1.210 ± 0.254^{b}
H3H4	2.228 ± 0.264^{ab}	0.320 ± 0.022^{ab}	0.955 ± 0.254^{b}
H3H5	2.349 ± 0.187^{ab}	0.329 ± 0.016^{ab}	1.345 ± 0.180^{b}
H3H7	2.845 ± 0.264^{b}	0.308 ± 0.022^{ab}	2.378 ± 0.254^{a}
H4H7	2.185 ± 0.125^{ab}	0.320 ± 0.022^{ab}	1.325 ± 0.254^{b}
H5H5	2.488 ± 0.264^{b}	0.353 ± 0.022^{b}	0.972 ± 0.141^{b}
H5H7	2.486 ± 0.128^{b}	0.324 ± 0.011^{ab}	1.182 ± 0.141^{b}
H6H7	2.686 ± 0.237^{b}	0.314 ± 0.020^{ab}	0.982 ± 0.208^{b}
H7H7	2.662 ± 0.153^{b}	0.321 ± 0.013^{ab}	1.166 ± 0.192^{b}

[a,b]Means with the different superscripts within the same column differ significantly ($P < 0.05$). [1]Only the haplotype combinations for which the observed number was no less than 4 are listed. IMF = intramuscular fat.

DISCUSSION

Understanding the genetic basis of protein metabolism in chickens will provide an opportunity for genetic improvement in muscle growth traits. The study of candidate genes, based on related results that have been well characterized in humans and mice, is one of the primary methods used to determine whether specific genes are associated with economic traits in farm animals. In the present study, we describe the development of a new mutation detection method based on PCR-LDR, which is highly sensitive and quantitative. A distinguishing feature

of PCR-LDR is that misligations do not undergo subsequent amplification, thereby reducing the chance of false-positive reactions. Any low-level polymerase errors remain unselected; thus, background noise is minimized. This method has been used in the detection of some viruses, oncogenes, and tumor-suppressor genes (Khanna et al., 1999; Rondini et al., 2008).

In poultry, Maeda et al. (1991) measured muscle protein turnover rate and calpain activity in the muscle of 2 quail lines divergently selected for body size. Zhang et al. (2007a,b) found that 3 variants in the *CAPN1* gene were associated with breast muscle fiber density and some carcass traits in chickens. Thus, the primary objective of this study was to test previously identified associations between 4 SNPs from the *CAPN1* gene and economically important meat quality traits in an important Chinese indigenous chicken breed, a better meat quality chicken breed (i.e., QY chickens), and a commercial breed (i.e., RW chickens). Among the 4 reported SNPs, 3 (i.e., *CAPN1* 3535, *CAPN1* 7198, *CAPN1* 9950) were polymorphic; for *CAPN1* 2546, the C allele was fixed in the studied populations. In 8 meat-type chicken populations, including 5 purebred lines (developed from Chinese local breeds) and 3 crossbreeds, *CAPN1* 2546 was more polymorphic, as can be observed in Zhang et al. (2008). However, this observation may be contributed more to the genetic differences between breeds. Nevertheless, the genotyping results were in accordance with the direct DNA sequencing results, indicating the accuracy of the PCR-LDR method.

The allele frequencies in the 2 chicken breeds were different at the G3535A and G9950A sites; the A allele occurred more frequently than the G allele in QY chickens, and the G allele occurred more frequently than the A allele in RW chickens. Of all the markers on the *CAPN1* gene studied herein, *CAPN1* 7198 showed the best segregation. All genotypic classes were observed, and the class with the fewest animals (CC in QY chickens and AA in RW chickens) represented 15.8 and 12.0% of the total samples, respectively.

A significant association was observed only for the G3535A genotypes of the *CAPN1* gene and meat quality traits when further association studies were conducted for each polymorphism. The AA and GA genotypes were associated with increased tenderness when compared to the GG genotype; thus, the A allele was advantageous for chicken meat quality. The results obtained for the QY and RW chickens confirm the results from previous studies that documented the effect of the SNP *CAPN1* 3535 on meat tenderness in 5 purebred and 3 crossbred lines (Zhang et al., 2007a,b). Moreover, the breed and interaction effects between breeds and genotypes were not significant; therefore, *CAPN1* 3535 is an important SNP on the *CAPN1* gene because it explains the functional variation for tenderness in many chicken populations.

Haplotype or haplotype block analysis provided a practical solution for resolving the innate problems of a single-marker analysis, such as noisy, unsatisfied, and obscured important localization information (Daly et al., 2001). Both haplotype diversity and the method of SNP selection based on maximum haplotype diversity have typically been preferred (Huang et al., 2003). In this study, haplotypes were constructed with 3 SNPs, and we analyzed the associations between haplotype combinations and meat quality traits. The H1H1 diplotype was found to be associated with smaller shear force and higher IMF content, and H1 was a unique haplotype in QY chickens, indicating that the H1H1 diplotype may be the most advantageous haplotype for meat tenderness. However, only one of the SNPs from the *CAPN1* gene had a significant effect on any of the traits in the SNP analyses, thus incorporating haplotype information would not improve the performance of marker-assisted selection, primarily for the populations in the current study.

CONCLUSIONS

The results presented herein confirm some previously documented associations; for example, the association between the CAPN1 3535 genotype and tenderness, which is the most important qualitative trait for consumers. Furthermore, novel associations have been identified that, following validation in other populations, could be incorporated into breeding programs to improve meat quality. Finally, while some previously noted associations were not replicated in the current study, it is important to note that validation is dependent on the specific nature of the population screened, and genetic background may influence the magnitude of the effect of a polymorphism. Validation failure may be due to a lack of true associations between the trait and markers but could also be caused by differences in SNP frequencies, marker-causative mutation linkage phases, genotype-by-environment interactions, or epistasis as well as sample size effects and measurement. Nevertheless, for those associations confirmed in the current study, the additional validation instills confidence for the use of these markers in selection programs to improve meat quality.

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