Transcriptional co-activator PGC-1α gene is associated with chicken skeletal muscle fiber types

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ABSTRACT. The peroxisome proliferators-activated receptor-γ coactivator-1α (PGC-1α) is a candidate gene for meat quality traits because of its prominent role in muscle fiber type switching and determination. We investigated the effects of the PGC-1α gene on chicken skeletal muscle fiber type switching and on other meat quality traits. Single nucleotide polymorphisms were detected by PCR-SSCP and DNA sequencing, and then genotyping was performed by PCR-ligation detection reaction methods. Skeletal muscle fiber types, intramuscular fat content, shear forces, and water loss rate of the gastrocnemius lateralis muscle were measured in Qingyuan Partridge chickens and Recessive White chickens. Four SNPs, C171T in exon2, C384T in exon3, G646A in exon5, and A948G in exon8 were detected. Marker-trait association analysis indicated that G646A polymorphism was associated with skeletal myofiber type and that H1 (CCAA) was the most advantageous haplotype for skeletal myofiber

type. We concluded that polymorphisms of the PGC-1α gene and their haplotypes are associated with chicken skeletal myofiber type traits.

Key words: PGC-1α gene; Ligase detection reaction; Polymorphism; Myofiber type; Chicken; SNPs

INTRODUCTION

As living conditions have improved, the needs not only for meat production but for meat quality have also increased, especially in some developing countries. In animal breeding, meat quality is a complex trait, referring to the compositional, visual, and sensory traits of a carcass, or its retail cuts. The edible quality of chicken meat is stressed by Chinese consumers, which refers to the sensory attributes of cooked products, i.e., tenderness, flavor, juiciness, and color (Jiang and Groen, 2000). It has been well documented that myofiber type composition can profoundly influence postnatal muscle growth and meat quality (Karlsson et al., 1999; Pette and Staron, 2001; Yuan et al., 2010).

Skeletal muscles of vertebrates contain myofibers that differ in contractile function, mitochondrial content and metabolic properties (Berchtold et al., 2000). Slow-twitch myofibers (SM, type I) are the smallest, oxidative fibers with red color, high lipid content and many mitochondria, whereas white fast-twitch fibers (FWM, type IIb) are the largest glycolytic fibers possessing high glycogen content and few mitochondria. Red fast-twitch myofibers (FRM, type IIa) are intermediate oxidative-glycolytic fibers that are similar to SM in color but resemble FWM in their contractile property, possessing both aerobic and anaerobic metabolic capabilities. These different myofiber types also synthesize distinct arrays of contractile proteins, enzymes involved in intermediary metabolism, and regulatory proteins that act together to maintain a specific fiber phenotype (Booth and Thomason, 1991; Berchtold et al., 2000).

Studies of pathways downstream of neural activity have implicated calcium signaling through calcineurin, a calcium-calmodulin (CaM)-dependent protein phosphatase, and CaM kinase in the control of type-I-fiber-specific contractile proteins (Dutka et al., 2011; Hollingworth et al., 2012). However, the nuclear targets ultimately responsible for the marked and stable changes in gene expression and biological function are not well understood.

The peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), which was originally identified through its functional interaction with peroxisome proliferator-activated receptor-gamma, is an important regulator of many metabolic pathways, including adaptive thermogenesis, fatty acid β-oxidation, adipocyte differentiation, hepatic gluconeogenesis, muscle fiber specialization, and glucose uptake (Puigserver et al., 1998; Wu et al., 1999; Vega et al., 2000; Michael et al., 2001; Lin et al., 2002; Walter and Seebacher, 2007). Recent work has implicated PGC-1α as a principal factor regulating muscle fiber type determination (Lin et al., 2002).

Qingyuan partridge chicken is an important indigenous breed distributing in Qingyuan, Guangdong Province, China. It is a light-body type breed with good meat quality, which is famous for its three "yellow", two "thin", and one "partridge" morphology features, i.e., yellow beak, shanks and skin, thin head and bone, and partridge feathers. However, there are few studies investigating the polymorphisms of candidate genes related to myofiber type in poultry.

In the present study, we describe a new, sensitive assay for the detection of PGC-1α 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 objectives of the present study were to identify polymorphisms of the PGC-1α gene, to develop a PCR-LDR method for genotyping, and to evaluate the associations of the polymorphism of this gene with myofiber type and other meat quality traits in Qingyuan partridge chicken and Recessive White chicken.

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.

Recessive White chicken (RW, Jiang-13 strain of white Plymouth Rock) and preserved population of Qingyuan partridge chicken (QY) 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. Two sides of the leg gastrocnemius lateralis were collected from each individual after slaughter at the age of 16 weeks; one was for measuring IMF content, shear forces and water loss rate, and another was taken for measuring the muscle fiber diameter, muscle fiber density and densities of SM, FRM, and FWM with myosin ATPase staining. Meanwhile, 0.4 mL blood was collected from the ulnar vein of each individual with heparin as an anticoagulant.

Histochemical analysis

Myosin ATPase staining was used to identify myofiber type and to measure myofiber size. Briefly, muscle blocks were excised perpendicular to the direction of the myofibers. Freshly excised muscles were mounted on corks coated with gum tragacanth, rapidly frozen in liquid N, and then stored at -80°C. Serial tissue sections of 10 mm thickness were prepared with a cryostat at -80°C. Sections were washed twice for 1 min each with 18 mM $CaCl_2$ and 100 mM Tris-HCl (pH 7.3; pre-rinse solution) and pre-incubated for 5 min at pH 10.4 (100 mM glycine, 1% $CaCl_2$, 3.7% formaldehyde) at room temperature. The sections were then washed twice for 30 s each time with pre-rinse solution prior to incubation in 18 mM $CaCl_2$, 20 mM glycine, and 0.025 mM ATP, pH 9.4, for 1 h 45 min at 37°C. Afterwards, sections were washed four times in 1% $CaCl_2$ wash solution and then rinsed in 2% $CoCl_2$ for 4 min at room temperature. Subsequently, sections were washed in distilled water four times for 20 s each and then stained in a 1% azure stain for 30 s. The stained sections were finally rinsed continuously under tap water for 10 min and washed in distilled water for 5 min, then dehydrated in ascending series of ethanol concentrations, delipidated in a solution of xylene - absolute ethanol (1:1, v/v) and coverslipped. Six to seven fascicles (containing about 400-500 myofibers)

were randomly selected from five serial sections of each sample using a light microscope (Olympus BH-2, Tokyo, Japan) with camera (JVC, Yokohama, Japan) at 200X magnification, and the mean percentages of SM, FRM, and FWM myofibers were calculated using image processing software (Image-Pro Plus 4.5, Silver Spring, MD, USA). The fiber type was determined as described by Wegner et al. (2000). FWM are stained dark blue, and FRM are stained light blue, whereas SM are nonstained (Hoogenraad et al., 1979).

SNP analysis and PCR amplification

DNA was isolated from whole blood using the Purgene DNA Isolation kit (Gentra Systems, Inc., Minneapolis, MN, USA). Seventeen pairs of PCR primers (data not shown) were designed from chicken genomic and cDNA sequences to amplify the coding region of the PGC-1α gene. Mutations were detected by PCR-SSCP using pooled DNA in the two chicken breeds. SNPs in genomic DNA were detected by direct gene sequencing, according to the PCR-SSCP result.

PCR was then carried out in a 20-μL volume containing 1 μL genomic DNA, 0.4 μL primer mixture, 2 μL dNTP, 0.6 μL Mg^{2+}, 2 μL buffer, 4 μL Q-Solution and 0.2 μL Taq DNA polymerase. The amplification protocol comprised 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 56°C for 1 min, and extension at 72°C for 1 min, and then a final extension at 72°C for 7 min. PCR products were checked on 3% agarose gels stained with ethidium bromide to ensure the amount added in LDR.

Ligase detection reaction

According to the result of SNP scanning, four SNPs were detected in the coding region of PGC-1αgene. For each SNP, three probes were designed, one common probe and two discriminating probes for the two types (Table 1). The common probe anneals to the PCR amplified template immediately downstream of the nucleotide in question. The common probes contained a phosphate in the 5' terminal position and a 6-carboxyfluorescein (FAM) fluorophore at its 3' end. 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 two allelic probes compete to anneal to the template adjacent to the common probe. This generates a double stranded region containing a nick (missing phosphodiester bond) at the nucleotide position to be tested. Only the allelic probe with perfect complementation to the template will be ligated to the common probe by the DNA ligase.

LDR reactions were carried out in a 10-μL mixture 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 reaction program was 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.

Reactions were stopped by chilling the tubes in an ethanol-dry ice bath and adding 0.5 mL 0.5 mM EDTA. Aliquots of 1 μL reaction products were mixed with 1 μL loading buffer (83% formamide, 8.3 mM EDTA, and 0.17% Blue Dextran) and 1 μL ABI GS-500 Rox-Fluorescent molecular weight marker, denatured at 95°C for 2 min., chilled rapidly on ice prior to loading on an 5 M urea-5% polyacrylamide gel, and electrophoresed on an ABI 3100 DNA sequencer at 3000 V. Fluorescent ligation products were analyzed and quantified using the ABI Gene Scan 672 software.

Table 1. Probe sequences of LDR.

Probe name	Probe sequences (5'-3')
Exon 2_modify	P-CAACCCTGTGTCAGCCCCTCAGCTGTTTTTTTTTTTTTTTTTTTTT-FAM
Exon 2_C	TTTTTTTTTTTTTTTTTTTTCACTGAGCCAAGTGCTGGGGACACG
Exon 2_T	TTTTTTTTTTTTTTTTTTTTTCACTGAGCCAAGTGCTGGGGACACA
Exon 3_modify	P-CTGTACCACTTGAGCCCCCCCAGGATTTTTTTTTTTTTTTTTTTTTTTT-FAM
Exon 3_C	TTTTTTTTTTTTTTTTTTTTTTTTGCTGGAGATGACCTCAGACTGGTCG
Exon 3_T	TTTTTTTTTTTTTTTTTTTTTTTTTGCTGGAGATGACCTCAGACTGGTCA
Exon 5_modify	P-TCGGGCATCGGGGAAGGGCTGGCGTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM
Exon 5_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCTCCTGGGGCGGAGGGGTGCCG
Exon 5_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCTCCTGGGGCGGAGGGGTGCCA
Exon 8_modify	P-GTTCGTAGTCAGATATTTGAGAAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM
Exon 8_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTGGTTTGGTCTGAGGAGGGTCATC
Exon 8_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTGGTTTGGTCTGAGGAGGGTCATT

Sequencing

To confirm the accuracy of PCR-LDR genotyping method, direct DNA sequencing of randomly selected PCR products was performed. The proportion of the sequencing samples were about 5%. The results of the PCR-LDR genotyping showed 100% concordance with 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 analysis of single polymorphisms or haplotypes with meat quality were determined by ANOVA using general linear model (GLM) and type III sums of squares performed by the SAS 9.0 software. The model is $Y_{ij} = \mu + B_i + G_j + e_{ij}$, 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 the e_{ij} is the residual error. The minimum diplotype frequency was set at 2%. All values are presented as least square means ± standard error (SE).

RESULTS

Characteristics of the study population

Two hundred Recessive White chickens (RW) and 200 Qingyuan partridge chickens were enrolled in this study. The meat quality characteristics of these two breeds are summarized in Table 2. All meat quality traits except for water loss rate were significantly different between Recessive White chickens and Qingyuan partridge chickens (P < 0.05). Qingyuan partridge chickens had a significantly higher slow-twitch myofiber ratio and significantly lower white fast-twitch myofiber ratio than did Recessive White chickens, which corroborated the sensory test results reported earlier (Chen et al., 2000). The breed with better meat quality had a higher slow-twitch myofiber ratio, which also supported the notion that myofiber types play a major role in meat quality traits.

SNP screen results

Among the 17 fragments amplified by 17 primer pairs, four fragments exhibited poly-

morphisms. Sequence analysis revealed a C/T substitution at position 171 in exon 2, a C/T substitution at position 384 in exon 3, a G/A point mutation at position 646 in exon 5 and an A/G point mutation at position 948 in exon 8 of the open reading frame of the chicken PGC-1α gene. However, only the G646A mutation in exon 5 caused the alteration of the corresponding amino acid from Asp to Asn, while the other three mutations were all silent mutations.

Table 2. Myofibre types and other meat quality indexes in lateral gastrocnemius in the two chicken breeds.

| Breeds[1] (N)	Slow-twitch myofiber ratio (%)	Red fast-twitch myofiber ratio (%)	White fast-twitch myofiber ratio (%)	Shear forces (kg)	Water loss rate (%)	IMF (%)
RW (200)	38.86 ± 4.15[a]	20.55 ± 3.12[a]	51.99 ± 3.98[a]	2.606 ± 0.075[a]	31.9 ± 0.008	1.253 ± 0.086[a]
QY (200)	55.52 ± 7.43[b]	10.97 ± 3.6[b]	33.51 ± 4.35[b]	2.341 ± 0.093[b]	31.8 ± 0.009	1.174 ± 0.112[b]

[a,b]Means with the different superscripts within the same column differs significantly (P < 0.05). [1]RW and QY represent Recessive White chicken breeds and Qingyuan partridge chicken, respectively.

Genotypic and allelic frequencies

The electrophoretic profile of PCR-LDR analysis of C171T, C384T, G646A, and A948G sites are shown in Figures 1 to 4. Three genotypes (GG, GA, and AA) were found at the G646A site, while at the other three sites only two genotypes (one homozygote and one heterozygote) were found. The genotypic and allelic frequencies of the four polymorphisms in the two chicken breeds are listed in Table 3. The frequencies of the C171 and C384 alleles were 1.0 in Recessive White chickens.
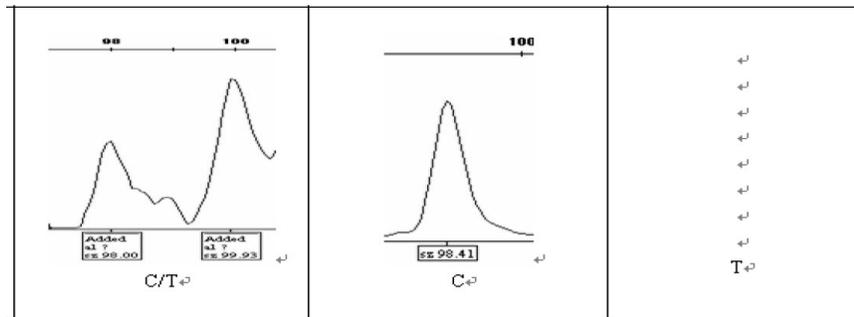
Figure 1. Genotype result of PGC-1α C171T. sz = size.
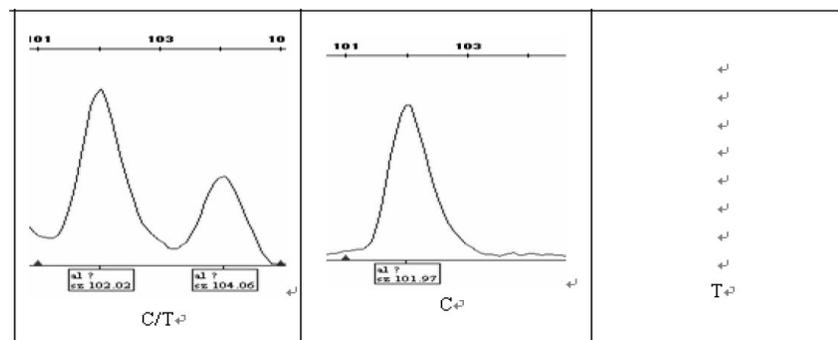
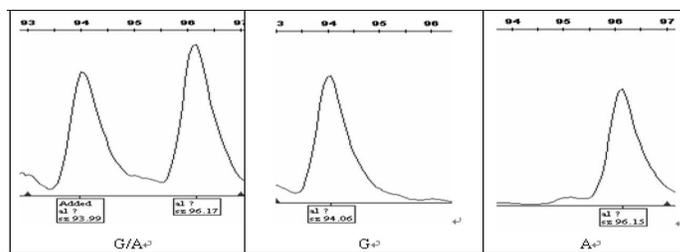**Figure 2.** Genotype result of PGC-1α C384T. sz = size.

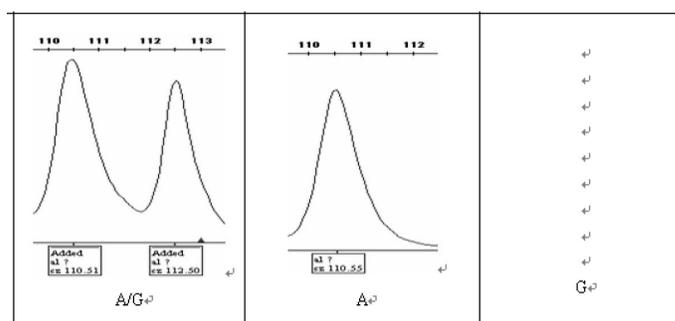**Figure 3.** Genotype result of PGC-1α G646A. sz = represents size.

Figure 4. Genotype result of PGC-1α A948G. sz = represents size.

Table 3. Allelic frequencies of the four polymorphhic sites in two chicken breeds.

Breed[1] (N)	171		384		646		948	
	C	T	C	T	G	A	G	A
RW (200)	1.00	0	1.00	0	0.71	0.29	0.90	0.10
QY (200)	0.87	0.13	0.96	0.04	0.86	0.14	0.91	0.09

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

Haplotypes and their frequencies

Haplotypes constructed based on four SNPs and their frequencies in the two breeds are shown in Table 4. Seven haplotypes were indentified in 400 chickens, in which the two most abundant ones, designated H1 (CCAA) and H3 (CCGA), accounted for about 95% of the genetic information. Haplotype H3 was predominant in both the Recessive White breed and Qingyuan partridge breed.

Table 4. Haplotypes constructed with 4 single nucleotide polymorphisms (SNP) and frequencies in the two chicken breeds.

Haplotype	Site				Breed[1]	
	171	384	646	948	QY	RW
h1	C	C	A	A	0.099	0.328
h2	C	C	A	G	0.025	-
h3	C	C	G	A	0.683	0.602
h4	C	C	G	G	0.054	0.070
h5	T	C	A	G	0.020	-
h6	T	C	G	A	0.084	-
h7	C	T	G	A	0.035	-

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

Marker-trait association analysis

Statistical analysis was applied to test the significance of the difference in breed effect, genotype effect and the interaction between the genotype effect and the breed effect between different genotypes of the two loci, respectively. The breed effect and the interaction between the genotype effect and the breed effect were not significant. There was a significant genotypic effect only in the G646A polymorphic locus, and therefore, we further analyzed the genotypic effect of G646A site with the combination of the two breeds (Table 5). With regard to the myofiber type trait, individuals with the AA genotype had a significantly higher slow-twitch myofiber ratio than did those with the GG and GA genotypes (P < 0.05), and GA genotype birds also had significantly higher slow-twitch myofiber ratio compared to GG genotype birds (P < 0.05). Meanwhile, AA and GA genotype birds had significantly lower white fast-twitch myofiber ratio than did GG genotype birds (P < 0.05), while AA genotype birds also had a little lower white fast-twitch myofiber ratio than did GA genotype birds, but the difference was not significant. The associations between genotypes and diameter and density of muscle fibers and IMF content were also significant. AA and GA genotype birds had significantly lower muscle fiber diameter and muscle fiber density compared to GG genotype birds (P < 0.05). The IMF content of individuals with the AA genotype was significantly higher than that of individuals with GG and GA genotype (P < 0.05). However, there was no significant association between genotypes and red fast-twitch myofiber ratio, shear forces and water loss rate.

Table 5. Association of G646A genotypes with myofiber types and other meat quality traits in chicken breeds.

Traits	GG	GA	AA
Slow-twitch myofibers ratio (%)	40.3 ± 3.05[a]	44.9 ± 2.12[b]	49.8 ± 3.13[c]
Red fast-twitch myofibers ratio (%)	13.55 ± 2.10	21.25 ± 3.25	15.3 ± 2.24
White fast-twitch myofibers ratio (%)	48.05 ± 1.25[a]	39.95 ± 1.32[b]	38.15 ± 1.57[b]
Muscle fiber diameter (um)	53.44 ± 3.25[a]	46.13 ± 2.32[b]	47.55 ± 3.48[b]
Muscle fiber density	415.25 ± 3.45[a]	316.15 ± 2.56[b]	302.13 ± 4.11[b]
Shear forces (kg)	2.369 ± 0.048	2.359 ± 0.091	2.393 ± 0.210
Water loss rate (%)	0.328 ± 0.013	0.310 ± 0.006	0.300 ± 0.005
IMF (%)	1.038 ± 0.049[a]	1.106 ± 0.092[a]	1.375 ± 0.212[b]

[a,b]Means with the different superscripts within the same row differs significantly (P < 0.05).

According to the results of the PHASE 2.0 software, seven haplotypes (H1: CCAA, H2: CCAG, H3: CCGA, H4: CCGG, H5: TCAG; H6: TCGA, H7: CTGA) were constructed. Nine diplotypes with frequencies higher than 2% (4 of H1H1, 22 of H1H3, 6 of H1H6, 6 of H2H3, 94 of H3H3, 18 of H3H4, 6 of H3H5, 26 of H3H6 and 10 of H3H7) were observed in Qingyuan partridge chickens, and five diplotypes (10 of H1H1, 92 of H1H3, 58 of H3H3, 22 of H3H4, and 4 of H1H4) in Recessive White chickens, so a total of 400 individuals with ten diplotypes (14 of H1H1, 114 of H1H3, 6 of H1H6, 6 of H2H3, 152 of H3H3, 40 of H3H4, 6 of H3H5, 26 of H3H6, 10 of H3H7 and 4 of H1H4) were used in the association analysis, and significant association (P < 0.05) of the haplotypes with myofiber types was observed (Table 6). Among the ten diplotypes, H1H1 had a significantly higher (P < 0.05) mean value of slow-twitch myofiber ratio compared to the other diplotypes, while H3H4 had a significantly higher (P < 0.05) mean value of white fast-twitch myofiber ratio compared to the other diplotypes.

Table 6. Least square means ± standard errors of myofiber types according to haplotype combinations[1].

Trait	Slow-twitch myofibers ratio (%)	red fast-twitch myofibers ratio (%)	white fast-twitch myofibers ratio (%)
H1H1 (14)[2]	48.2 ± 3.2[a]	14.5 ± 1.4	39.3 ± 3.1[a]
H1H3 (114)	43.5 ± 2.9[b]	15.3 ± 1.9	40.2 ± 2.4[a]
H1H6 (6)	44.5 ± 2.6[b]	14.8 ± 1.5	40.7 ± 2.5[a]
H2H3 (6)	44.2 ± 2.1[b]	14.5 ± 1.7	41.3 ± 2.2[a]
H3H3 (152)	42.5 ± 2.7[b]	15.3 ± 2.2	42.2 ± 2.5[a]
H3H4 (40)	41.2 ± 2.2[b]	14.3 ± 1.6	44.5 ± 2.1[b]
H3H5 (6)	44.8 ± 2.4[b]	13.6 ± 1.9	41.6 ± 2.5[a]
H3H6 (26)	43.9 ± 2.5[b]	14.1 ± 1.8	42.0 ± 2.1[a]
H3H7 (10)	43.8 ± 2.1[b]	14.6 ± 1.5	41.6 ± 2.3[a]
H1H4 (4)	44.9 ± 2.6[b]	14.8 ± 1.9	40.3 ± 2.3[a]

[a,b]Means with the different superscripts within the same column differs significantly ($P < 0.05$). [1]Only the haplotype combinations for which the observed number was no less than 4 were listed. [2]The number in parentheses is the observed number for a particular haplotype combination.

DISCUSSION

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, therefore reducing the chance of false positive reactions. Any low-level polymerase errors remain unselected, and thus contribute only minimal background noise. It has been used in the detection of some viruses, oncogenes and tumor-suppressor genes (Khanna et al., 1999; Rondini et al., 2008).

Recently, myofiber-type composition of skeletal muscle has attracted great attention both in human health and animal production, owing to its close association with insulin sensitivity in mammals (Hickey et al., 1995; Korach-André et al., 2005) and its significance for lean-mass deposition and meat quality in meat-producing animals (Maltin et al., 2003), respectively. Adult skeletal muscle displays plasticity that allows the conversion of different fiber types in response to chronic change in contractile demands (Hood, 2001; Schiaffino and Reggiani, 2011). Recent work has implicated the transcriptional coactivator PGC-1a in muscle fiber type switching and determination (Lin et al., 2002; Liang et al., 2009).

The genetic effect of PGC-1α gene on myofiber type determination has not been studied to date. Wu et al. (2006) reported that a G to A polymorphism at position 646 of the open reading frame in the chicken PGC-1α gene was significantly related to abdominal fat weight, and had no effects on growth traits, so this SNP could be a molecular marker for the selection of abdominal fat. An et al. (2008) further studied the genetic effect of this SNP on meat quality in White Plymouth Rock and found that birds with the AG genotype had better meat quality. In the present study, PCR/LDR method was used to genotype single nucleotide polymorphisms of the coding region of the PGC-1α gene in an important Chinese indigenous chicken breed with better meat quality, Qingyuan partridge chicken, and one commercial breed, Recessive White chicken. Four SNPs (C171T, C384T, G646A, and A948G) were detected, and the genotyping results were in concordance with direct DNA sequencing, indicating the accuracy of the PCR-LDR method. Although the meat quality traits between the two chicken breeds were significantly different, the allele frequencies of the two sites in both breeds were close. The frequencies of C171 and C384 allele were 1.0 in Recessive White chickens, and the G allele was the most frequent in both breeds, which agreed with that in White Plymouth Rock (Wu

et al., 2006). Only G646A genotypes of the PGC-1α gene showed a significant association with myofiber types and other meat quality traits when further association studies were carried out at each polymorphism. Moreover, the breed effect and the interaction effect between breeds and genotypes were not significant, and therefore, it was concluded that there may be a relationship between this SNP site and meat quality traits in chickens. Meanwhile, breeders can reduce abdominal fat significantly by selecting AA birds without affecting the continued improvement of growth rate (Wu et al., 2006).

Haplotype analysis is very important in the mapping of complex-disease genes because of the abundance of SNPs and the limited power of conventional single-site analyses (Cardon and Abecasis, 2003; Lin, 2004). In this study, haplotypes were constructed with the 4 SNPs, and the association of haplotype combinations with myofiber type and other meat quality traits was analyzed. The H1H1 diplotype was found to be associated with a high ratio of slow-twitch myofiber compared to other diplotypes (H1H3, H1H6, H2H3, H3H3, H3H4, H3H5, H3H6, H3H7, and H1H4), while H3H4 had a significantly higher ratio of white fast-twitch myofiber than did other diplotypes. The difference between H1 (CCAA), H3 (CCGA) and H4 (CCGG) haplotypes existed at the G646A and A948G sites, and therefore, these two sites may have a greater genetic effect, and H1 may be the most advantageous haplotype for myofiber types because of nucleotide substitution interaction (Putt et al., 2004).

The results obtained in current study showed that polymorphisms of the PGC-1α gene and their haplotypes are associated with chicken myofiber type as well as other meat quality traits, where the H1H1 diplotype could be a potential marker for myofiber type trait.

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