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A Novel Method Based on Ligase Detection Reaction for *PGC-1α G646A* Gene Polymorphism and its Genetic Effect on Meat Quality in Chicken

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Abstract: The Peroxisome Proliferators-activated receptor-γ Coactivator-1α (PGC-1α) was investigated as a candidate gene for meat quality and fatness traits in chicken because of its prominent role in muscle fiber specialization and adipogenesis. Variations of PGC-1α+646G/A may associate with chicken meat quality traits. The objective of the current research was to investigate the association of this single nucleotide polymorphisms in *PGC-1α* gene with meat quality traits in Recessive White chickens and Qingyuan Partridge chickens. Genotyping was performed by Polymerase Chain Reaction-Ligation Detection Reaction (PCR-LDR) Method. Marker-trait association analysis indicated that there were significant associations between G646A genotypes and the trait of water loss rate. Researchers putatively drew the conclusion that this SNP in *PGC-1α* gene could be used as the potential molecular marker for meat quality trait in chicken.

Key words: *PGC-1α* gene, ligase detection reaction, polymorphism, meat quality, chicken, China

INTRODUCTION

As the living conditions improved, the needs not only for meat production but for meat quality are 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 upon by Chinese consumers which refers to the sensory attributes of cooked products, i.e., tenderness, flavor, juiciness and color (Jiang and Groen, 2000). Unfortunately, traditional selection techniques have not been effective in increasing the edible quality of chicken meat which is attributed to the fact that most meat quality traits cannot be generically evaluated in breeding animals and the progeny test is slow, expensive and often impractical (Dikeman *et al*., 1998). However, identification of the genes and/or polymorphisms underlying quantitative/qualitative traits and an understanding of how these genes/polymorphisms interact with the environment or with other genes affecting economic traits might be the keys to successful application of marker-assisted selection in the commercial animal population. Although, traditional selection depending on the phenotypic value of the broiler has made significant improvement in growth rate during the later half of the last century, poultry geneticists face additional challenges today because of negative correlation between production performance and tenderness traits. Accompanying selection for rapid growth, meat quality of chicken shows a decreasing trend (Rance *et a1*., 2002). The Peroxisome proliferators-activated receptor-γ Coactivator-1α (PGC-1α) which was originally identified through its functional interaction with peroxisome proliferators-activated receptor-γ 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). In chicken a Single Nucleotide Polymorphism (SNP) from G-A at position 646 of the open reading frame of *PGC-1α* gene causing an Asp216Asn amino acid substitution was identified and further study revealed that this SNP was associated with abdominal fatness (Wu *et al*., 2006). An *et al.* (2008) reported that birds with AG genotype at G646A loci had the better meat quality in White plymouth rock.

Qingyuan partridge chicken is an important indigenous breed distributing in Qingyuan city, Guangdong province, P.R. 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;

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partridge feather. In the present study, researchers 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. G646A mutation in PGC-1α was genotyped in Recessive White and Qingyuan Partridge chicken breeds by PCR-LDR and its genetic effect on chicken meat quality was also evaluated.

MATERIALS AND METHODS

Experimental animals: Blood samples of 100 (50 male, 50 female) Recessive White chickens (RW) and 100 (50 male, 50 female) Qingyuan Partridge chickens (QY) were randomly collected from National Gene Pool for Indigenous Chicken Breeds (Yangzhou, China). Breast muscles were collected from each individual and then muscle IMF content, shear forces and water loss rate (WHC) were measured after slaughter at the age of marketing.

DNA extraction and PCR amplification: DNA isolation was performed from whole blood using the Purgene DNA Isolation kit (Gentra Systems, Inc., Minneapolis, MI). One pair of primers was designed to amplify a fragment including G646A mutation. PCR product length was 212 bp. The primer sequences were: forward 5'G GAGCAAT AAAGCGAAGAGC 3', reverse 5' CTGTGCTCCCACAC CTACCT 3'.

PCR was carried out in 20 uL volume containing 1 μL genomic DNA, 0.4 μL primer mixture, 2 μL dNTP, 0.6 μL Mg2+, 2 μL Buffer, 4 μL Q-Solution and 0.2 μL Taq DNA polymerase. The amplification protocol 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 sec, annealing at 56°C for 1 min, extension at 72°C for 1 min and then a final extension at 72°C for 7 min. PCR products were checked in 3% agarose gels stained with ethidium bromide to ensure the amount added in LDR.

Ligase detection reaction: 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.

Table 1: Probe sequences of LDR

Probe name	Probe sequences (5'-3')
G646A_modify	P-TCGGGCATCGGGGAAGGGCTGGCGTTTT TTTTTTTTTTTTTTTTTTTTTTT-FAM
G646A_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTGCCT CCTGGGGCGGAGGGGTGCCG
G646A_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTGC CTCCTGGGGCGGAGGGGTGCCA

One allelic probe has at its 3' end the nucleotide corresponding to the wildtype 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 shown as following: an initial heating at 94°C for 2 min, followed by 35 cycles of 30 sec 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 of 0.5 mM EDTA. Aliquots of 1 μL of the reaction products were mixed with 1 μL of 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.

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 to direct DNA sequencing of the randomly selected PCR products (Table 1).

Statistical analyses: Association analysis of single polymorphisms with meat quality were determined by ANOVA using General Linear Model (GLM) and type III sums of squares performed by SAS 9.0 software. The model is:

$$Y_{ijkn} = \mu + a_i + b_j + c_k + (ab)_{ij} + (ac)_{ik} + (bc)_{jk} + (abc)_{ijk} + e_{ijkn}$$

Where:

Y_{ijkn} = Observation on the traits
μ = Overall population mean
a_i = Effect of breed
b_j = Effect of the sex
c_k = Effect of genotype
$(ab)_{ij}$ = Interaction effect of the breed and sex
$(ac)_{ik}$ = Interaction effect of the breed and genotype
$(bc)_{jk}$ = Interaction effect of the genotype and sex
$(abc)_{ijk}$ = Interaction effect of the breed, genotype and sex
e_{ijkn} = Residual error

RESULTS AND DISCUSSION

Genotype and allele frequencies: The electrophoretic profile of PCR-LDR analysis of G646A site is shown in Fig. 1. Three genotypes (GG, GA and AA) were found at this site. Allele frequencies and genotype frequencies in different chicken breeds and Chi-square test are shown in Table 2. Recessive White chickens and Qingyuan Partridge chickens were all in Hardy-Weinberg equilibrium (p>0.05) and the G allele was more frequent than the A allele in both breeds.

Table 2: Gene frequencies and genotype frequencies of different breeds

		Genotype frequency			Allele frequency		
Breed	No.	GG	GA	AA	G	A	χ^2
QP	100	0.365	0.490	0.144	0.611	0.389	0.05
RW	100	0.708	0.234	0.057	0.825	0.175	3.23

$\chi^2_{0.05\,(2)} = 5.99$, $\chi^2_{0.01\,(2)} = 9.21$

Table 3: Variance analysis of PGC-1α G646A

	IMF		Shear forces		Water loss rate	
Effect	F-value	p-value	F-value	p-value	F-value	p-value
Genotype	0.064	0.938	1.673	0.190	4.509	0.012
Breed x genotype	0.522	0.720	0.460	0.765	3.739	0.006
Sex x genotype	0.728	0.484	1.435	0.240	0.589	0.556
Breed x sex x genotype	1.257	0.290	0.333	0.802	1.535	0.206

Association of single SNP with chicken meat quality: Statistical analysis was applied to test the significance of the difference of breed effect, sex effect, genotype effect and the interaction effects, respectively. Only significant effect on water loss rate existed in the two breed (p<0.05) and only the interaction between breed and genotype was significant (p<0.01) (Table 3) so, researchers analyzed genotypic effect in separate breed. The result was shown in Fig. 2.

In Recessive White chickens, individuals with GG genotype had significant lower water loss rate than those with AA (p<0.01) and GA (p<0.05) genotypes, GA genotype birds also had a little lower water loss rate than AA genotype birds but the difference was not significant. While in Qingyuan partridge chickens, individuals with AA genotype had significant higher water loss rate than GA and GG genotype individuals (p<0.01) GG genotype birds also had a little higher water loss rate than GA genotype birds but the difference was not significant. In the present study, researchers 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 a minimum of background noise. It has been used in the detection of some virus, oncogenes and tumor-suppressor genes (Khanna *et al*., 1999; Rondini *et al*., 2008).

Because of its critical function in activating many nuclear hormone receptors in regulating energy

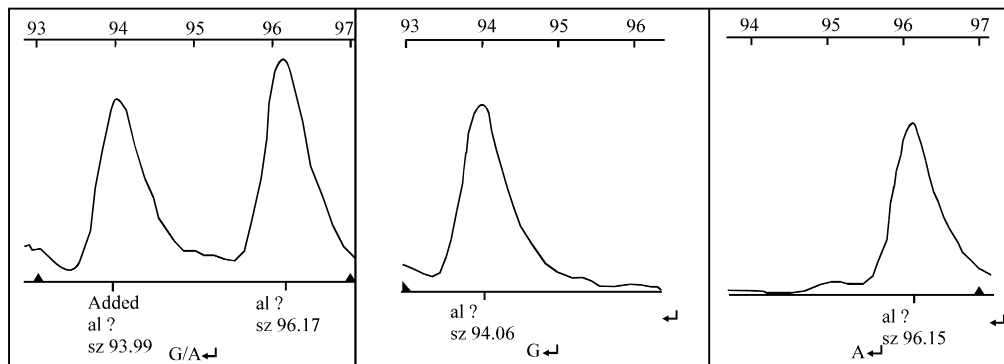

Fig. 1: Genotype result of PGC-1α G646A

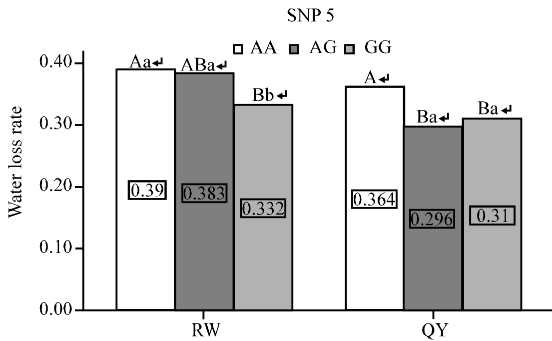

Fig. 2: Th e simple effect of PGC-1α G646A on water loss rate

homestasis, thermal regulation and glucose metabolism in liver, fat tissue and muscle, the chicken *PGC-1α* gene is a potential candidate gene for meat quality. Wu *et al.* (2006) reported that a G-A polymorphism at position 646 of the open reading frame in chicken *PGC-1α* gene was significantly related with AFW and %AFW 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.

The result showed that birds with AG genotype had the better meat quality. In the present study, PCR/LDR method was used to genotype single nucleotide polymorphism of PGC-1α+646G/A in an important Chinese indigenous chicken breed with better meat quality-Qingyuan partridge chicken and one commercial breed-Recessive White chicken.

The genotyping results were concordance to direct DNA sequencing indicating the accuracy of PCR-LDR method. The distribution of allelic frequencies revealed that the G allele was the most frequent in both breeds which was concordance with that in White Plymouth Rock (Wu *et al*., 2006).

CONCLUSION

The associations detected by the analysis suggest that there was significant association between G646A genotypes of *PGC-1α* gene and the trait of water loss rate.

GG genotype individuals had significant lower water loss rate than AA genotype individuals indicating that birds with GG genotype might had better meat quality. Meanwhile, the effect in both breeds was similar so, researchers could conclude that there may be a relationship between this SNP site and meat quality traits in chickens.

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