Journal of Animal and Veterinary Advances 10 (1): 29-32, 2011
ISSN: 1680-5593

A Typing System Based on Ligase Detection Reaction for Myogenin Gene Polymorphisms in Chicken

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Abstract: Myogenin is a gene belonging to the MyoD family which codes for the bHLH transcription factor playing a key role in myogenesis. It affects the processes of differentiation and maturation of myotubes during embryogenesis. The parallel typing system based on ligase detection reaction was established in the present study and to detect the polymorphisms of MYOG gene which is candidate gene for chicken meat quality traits.

Key words: Myogenin gene, ligase detection reaction, polymorphism, meat quality, chicken, MyoD family

INTRODUCTION

Myogenin (MYOG) together with MyoD1, MYF-5 and MYF-6 (MRF4, herculin) belongs to the MyoD family genes. These genes code for the four bHLH transcription factors which control the processes of myogenesis induce an expression of muscle specific genes (Lassar *et al*., 1989) and can convert various nonmuscle cells into muscle (Weintraub *et al*., 1989). The myogenin gene is the only gene of MyoD family that undergoes expression in all skeletal muscle cell lines. The protein coded by the gene is necessary for regulation of skeletal muscles development during embryogenesis (Kitzmann *et al*., 1998).

The induction of the MYOG expression is associated with a rapid set-out of the myoblast differentiation program and start of specific muscle genes expression (Montarras *et al*., 1991; Buckingham, 1992). The knock-out experiments on murine embryos revealed a crucial role of MYOG in myogenesis.

The null myogenin-mutants in the homozygous state were stillborn and showed several muscle and skeletal defects. Muscle tissue was dramatically reduced, mononucleated myoblasts were present instead of muscle fibers, occasional myofibers showed a lowered density, disorganized structure and various stages of maturation (Hasty *et al*., 1993; Nabeshima *et al*., 1993).

These results suggest the role of MYOG in the differentiation and maturation of myoblasts and focused the attention of scientists on myogenin as a possible gene affecting the muscle phenotype in chicken. Wang *et al*. (2007, 2008) reported that the mutations in the promoter region of *MYOG* gene were associated with muscle fiber traits and some carcass traits.

Qingyuan partridge chicken or Qingyuan chicken is an important indigenous breed among them. It mainly distributes in Qingyuan city, Guangdong province, P.R. China and got its name by the fact that there are lots of sesame-like pocks interspersing the back feathers of hens. Qingyuan chicken is a light-body type breed with good meat quality which is famous for its 3 yellow, two thin and one partridge morphology features, i.e., yellow beak, shanks and skin, thin head and bone, partridge feather.

In the present study, we describe a new, sensitive assay for the detection of *MYOG* 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.

Two reported polymorphisms in the promoter region of chicken *MYOG* gene (T-618C and G-224A) (Wang *et al*., 2007, 2008) were genotyped in Recessive White and Qingyuan partridge chicken breeds by PCR-LDR and the distribution of different genotypes in different chicken breeds were analyzed to give some guidance to select chickens for meat quality traits in the future.

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MATERIALS AND METHODS

Experimental population: Blood samples of 169 Recessive White chickens (RW) and 193 Qingyuan partridge chickens (QY) were randomly collected from National Gene Pool for Indigenous Chicken Breeds (Yangzhou, China) and Guangdong Tiannong Food Co. Ltd., (Guangzhou, China), respectively.

DNA extraction and PCR amplification: DNA isolation was performed from whole blood using the Purgene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MI). Two pairs of primers were designed to amplify two fragments including T-618C and G-224A mutations. The primer sequences for T-618C were forward: 5' TTCTTTCCACTGACCGATCC 3', reverse 5' ACTGGGGACGTCAGAAACC 3' and the primer sequences for G-224A were: forward 5' AGAGTGTGGGAGGCTCAGG 3', reverse 5' CTGAGCAATGGAGTGCAGAA 3'.

PCR was carried out in 20 μL volume containing 1 μL genomic DNA, 0.4 μL primer mixture, 2μL 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: For each SNP, three probes were designed, one common probe and two discriminating probes for the two types (Table 1). 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.

Table 1: Probe sequences of LDR

Probe name	Probe sequences (5'-3')
MYOG-618T/C_modify	P-GATTCCAGCGGAGGGATCGGTCA GTTTTTTTTTTTTTTTT-FAM
MYOG-618T/C _C	TTTTTTTTTTTTTTTTCTCCTCCT ATTCCTGCTCTGGGCCG
MYOG-618T/C _T	TTTTTTTTTTTTTTTTTTCTCCTCCT ATTCCTGCTCTGGGCCA
MYOG-224G/A_modify	P-TCAGTGCTCCCCGCTGCTGCCCTGA TTTTTTTTTTTTT-FAM
MYOG-224G/A_G	TTTTTTTTTTTTTTTTAATGGAGTGC AGAATCCAGTGCTGC
MYOG-224G/A_A	TTTTTTTTTTTTTTAATGGAGTGCAG AATCCAGTGCTGT

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 Volts. Fluorescent ligation products were analyzed and quantified using the ABI Gene Scan 672 software.

Statistical analyses: Haplotypes were inferred by the PHASE 2.0 software http://www.stat.washington.edu/stephens/software.html (Stephens *et al*., 2001).

RESULTS AND DISCUSSION

Genotype and allele frequencies: 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 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). The electrophoretic profiles of PCR-LDR analysis of T-618C and G-224A site are shown in (Fig. 1 and 2). Three genotypes were found at each site.

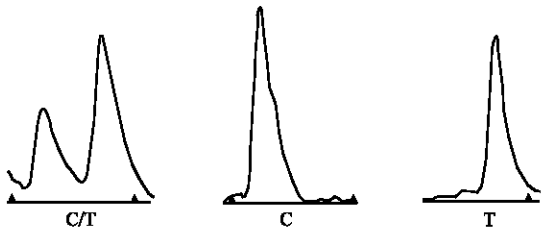

Fig. 1: Genotype result of MYOG T-618C

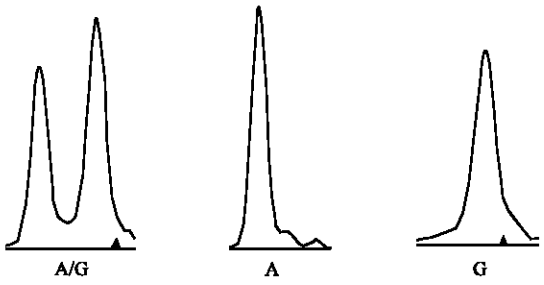

Fig. 2: Genotype result of MYOG G-224A

Table 2: Gene frequencies and genotype frequencies of different SNPS in different breeds

			Genotype frequency			Allele frequency		
Breed	Number	SNP	CC/AA	TC/GA	TT/GG	C/A	T/G	χ^2
QY	193	T-618C	0.073	0.424	0.503	0.285	0.715	0.23
		G-224A	0.257	0.529	0.215	0.522	0.478	0.38
RW	169	T-618C	0.243	0.533	0.225	0.510	0.490	0.43
		G-224A	0.314	0.426	0.260	0.527	0.473	1.72

χ^2 0.05 (2) = 5.99, χ^2 0.01 (2) = 9.21

Table 3: Haplotype and diplotype frequencies in different breeds

	Haplotype				Diplotype								
Breed	H1	H2	H3	H4	H1H1	H1H2	H1H3	H1H4	H2H2	H2H4	H3H3	H3H4	H4H4
QY	0.403	0.315	0.120	0.162	0.166	0.259	0.062	0.254	0.083	0.104	0.031	0.016	0.026
RW	0.438	0.098	0.119	0.345	0.168	0.030	0.144	0.317	0.018	0.078	0.000	0.078	0.168

Allele frequencies and genotype frequencies of each site in different chicken breeds and Chi square test are shown in Table 2. In the two sites, recessive white chickens and Qingyuan partridge chickens were all in Hardy-Weinberg equilibrium ($p>0.05$). At T-618C site, the T allele was more frequent than the C allele in Qingyuan partridge chickens while C was the superior allele in Recessive White chickens; at G-224A site, the A allele was more frequent than the G allele in both breeds.

Haplotype frequencies: As far as haplotypes were concerned, four haplotypes (H1: TA, H2: TG, H3: CA, H4: CG) were constructed and there were 9 diplotypes (H1H1, H1H2, H1H3, H1H4, H2H2, H2H4, H3H3, H3H4, H4H4) in Qingyuan partridge chickens and 8 diplotypes (H1H1, H1H2, H1H3, H1H4, H2H2, H2H4, H3H4, H4H4) in Recessive White chickens. H3H3 was the unique diplotype in Qingyuan partridge chickens. Haplotype and diplotype frequencies in different chicken breeds were shown in Table 3. The distribution of different haplotypes and diplotypes in different chicken breeds were significant different ($p>0.01$), this result supported the result of Wang *et al.* (2008). In Qingyuan partridge chickens, H1 was the most frequent haplotype followed by H2 and then H4 and H3 while in Recessive White chickens, H1 was the most frequent haplotype followed by H4 and then H3 and H2.

This may explain why significant meat quality difference existing in the two chicken breeds, the two mutations in the promoter region of *MYOG* gene may relate to the meat quality traits in chicken. Nevertheless, further study should be conducted in larger population size and the meat quality traits should be tested to confirm the genetic effects of *MYOG* gene. It will make it possible to distinguish the *MYOG* gene as potentially useful in MAS of meat quality traits in chickens.

CONCLUSION

The genotypes of T-618C and G-224A in the promoter region of MYOG gene were identified in Qingyuan partridge chickens and Recessive White chickens and the results were coincidence with direct sequencing. The genotype and haplotype distribution in different breeds was significant different. The results indicated that these two SNPs might relate to chicken meat quality trait.

ACKNOWLEDGEMENTS

This research was supported by the Earmarked Fund for Modern Agro-Industry Technology Research System, Natural Science Foundation of Jiangsu Province (BK2009177) and Doctorial funds of Poultry Institute of Jiangsu province (JQ2009001).

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