Pakistan J. Zool., vol. 52(3), pp 1193-1196, 2020 DOI: https://dx.doi.org/10.17582/journal.pjz/20190613010643

Short Communication

Association between *MyoG* Gene Polymorphism and Egg Quality of Egg Quail

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Article Information
Received 13 June 2019
Revised 30 July 2019
Accepted 11 September 2019
Available online 17 March 2020

Authors' Contribution
JYB conceived and designed the study and conducted the lab work. SY and HDF analyzed the data and wrote the article. YZP and XHW helped in sampling. XYF, HC and KPS helped in analysis of data.

Key words
Egg quail, MyoG Gene, Egg quality, Association analysis, SSCP

ABSTRACT

Quail is extensively cultured in China for characteristic of quick growth, small fodder consumption, early sexual maturity, big egg output and short production period. To recognize molecular markers of egg quality of quail, SNP in control regions of cytogenin gene (MyoG) 5' in China yellow quail, Beijing white quail, Korean quail detected by PCR-SSCP method in this study. Moreover, correlations of control regions of MyoG 5' with egg quality of quail were analyzed. Results demonstrated that: In egg quail group, six genotypes were detected in Locus A in the control region of MyoG 5', including AA, BB, CC, AB, AC and BC. The C allele frequency at Locus A in China yellow quail, Beijing white quail and Korean quail was the highest (0.461, 0.528 and 0.579). A total of 9 genotypes were detected in locus B in egg quail group, which were AA, BB, CC, DD, EE, AB, AC, AD and AE. The C allele frequency at Locus B in China yellow quail, Beijing white quail and Korean quail was the highest (0.612, 0.623 and 0.633). The egg shape index and yolk index of egg quail are significantly correlated with locus B ($P<0.05$). However, locus A has no significant impacts on egg quality of egg quail ($P>0.05$).

Myogenin (MyoG) gene is a kind of myogenic regulatory factor and it regulates muscle growth together with myogenic determination gene, myogenic regulatory factor 4, myostatin and myogenic factor 5. Both MyoD and MyoG are myostatin. The former one determines myogenesis, while the later one induces terminal differentiation of skeletal muscle (Brooks *et al*., 1999). Weintraub *et al*. (1991) discovered form an experiment that mice with MyoG knockout alone might die for defects in skeletal muscle development, but mice with all 3 myogenic regulator genes knockout didn't show any defects in skeletal muscle development. Based on abundant studies, MyoG, a myocyte specific transcription factor, not only can regulate expression of MyoG, but also can interact with other myogenic factors and regulate expression of muscle-specific genes. As a transcriptional regulatory factor, MyoG triggers synthesis of a series of skeletal muscle specific embryonal receptor and contractile proteins. Therefore, MyoG is the only one irreplaceable myogenic regulatory factor (Hasty *et al*., 1993). Subsequently, abundant studies concerning the relationship between mutation of MyoG and human diseases have been reported in the whole world (Knapp *et al*., 2006). Recently, there are extensive studies on MyoG in China. However, most of them concentrate in formation mechanism of muscles as well as genetic expression and regulation of MyoG (Biressi *et al*., 2013).

At present, quail breeding is more and more popular in poultry, and quail is smaller than other poultry, so quail can be used as a good new experimental animal. Quail can be used as a research animal in many subjects such as poultry reproduction, histology, nutrition, endocrinology, embryology, physiology, pharmacology and so on (Li *et al*., 2019; Bai *et al*., 2016a, 2016b, 2019).The experimental values of quail in teaching and scientific studies are increasing gradually (Bai *et al*., 2016c, 2016d, 2017). Therefore, correlations of MyoG with egg quality of quail were discussed in the present study, which provided references for marker assisted selection of quail.

Materials and methods

In this experiment, egg quails were 80 China quails with yellow feathers, 80 Beijing quails with white feathers and 80 Korean quails. All egg quails were females. Blood samples (5ml each) were collected at vein in wings and stored in heparin sodium anticoagulant tubes which were then kept in a refrigerator under -20 °C. DNA was extracted by poultry whole blood DNA kit and kept under -20 °C. Egg quality indices included egg weight, egg shape index, yolk index, yolk weight, shell thickness and egg white height.

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0030-9923/2020/0003-1193 $ 9.00/0

Table I. Primer sequence information.

Primer	Primer sequence (5'-3')	Annealing temperature (°C)	Fragment size
A locus	F:GGTGGGTGTGGGGAATGTGCT R:CCGGCTTTGCTCTTAACTCT	61.9	203bp
B locus	F:AAACCCACTCCATTGTGC R:CACTACTTGGCTCCTCTAGTT	57.2	236bp

Table II. Polymorphism of *MyoG* gene in egg quail.

Polymorphism		A loci			B loci		
		China yellow quail	Beijing white quail	Korean quail	China yellow quail	Beijing white quail	Korean quail
Genotype frequency	AA	0.292	0.211	0.238	0.493	0.385	0.406
	BB	0.062	0.014	0.048	0.030	0.000	0.054
	CC	0.323	0.366	0.444	0.052	0.015	0.000
	DD	-	-	-	0.090	0.031	0.039
	EE	-	-	-	0.095	0.092	0.047
	AB	0.046	0.085	0.000	0.060	0.108	0.188
	AC	0.246	0.239	0.270	0.045	0.000	0.000
	AD	-	-	-	0.060	0.092	0.094
	AE	-	-	-	0.075	0.277	0.172
	BC	0.031	0.085	0.000	-	-	-
Allele frequency	A	0.438	0.373	0.373	0.612	0.623	0.633
	B	0.101	0.099	0.048	0.060	0.054	0.148
	C	0.461	0.528	0.579	0.075	0.015	0.000
	D	-	-	-	0.119	0.077	0.086
	E	-	-	-	0.134	0.231	0.133
Heterozygosity	He	0.585	0.572	0.523	0.584	0.549	0.552
Number of effective alleles	Na	2.412	2.338	2.098	2.405	2.219	2.234
Polymorphism information content	PIC	0.496	0.487	0.428	0.552	0.500	0.513

For PCR amplification, primers at loci A and B in the control region of MyoG 5' were designed with references to the design of Wang *et al*. (2007) which were listed in Table I. Primers were synthesized by Beijing Dongguo Changsheng Biotechnology Co., Ltd. Pre-denaturation at 94°C for 4 min, then denaturation at 94°C for 40 s, annealing at 57-60°C for 1 min, annealing at 72°C for 20 seconds, denaturation, annealing and elongation were carried out for 35 cycles, then elongation at 72°C and finally the reaction was completed and cooled and preserved at 4°C.

For SSCP analysis in this experiment, 5μL denatured upper sample buffer and 5μL PCR products were mixed evenly, which was degenerated for 10 min under 98°C. The mixture was treated by electrophoresis with 10% acrylamide gel for 15 min under 220V, and then treated by electrophoresis for 6h under 90V. Finally, the mixture was developed by silver staining under lights. Pictures were taken and stored.

For data analysis, analytical model used was $Yijkl=\mu+B_i+W_j+M_k+e_{ijkl}$, where Y_{ijkl} is the phenotype value of traits, μ is the total mean value, B_i is the first variety effect (i = 1, 2, 3), W_j is the j age effect(j=9, 10, 11, 12, 13,14, 15, 16, 17, 18), M_k is the k genotype effect, e_{ijkl} is the residual effect.

Results and discussion

The SNPs in the control region of MyoG 5' are shown in Figure 1. The target fragment gained from amplification of locus A is 203bp and the target fragment gained from amplification of locus B is 236bp. These agree with expectations and show good specificity. These target fragments can be applied in subsequent experiments.

Figure 2 shows genotype test results at loci A and B of the control region of MyoG 5' of three egg quails. A total of 6 genotypes are discovered at locus A, which are AA, BB, CC, AB, AC and BC. AB and BC are not observed in Korean quail. Nine genotypes are detected at loci B, namely, AA, BB, CC, DD, EE, AB, AC, AD and AE. BB and AC are not detected in Beijing white quails, and CC and AC are not detected in Korean quail.

Gene frequency and genotype frequency at loci A

and B in the control region of MyoG 5' in three egg quail groups are shown in Table II. Obviously, The C allele frequency of China yellow quail, Beijing white quail and Korean quail is the highest, valuing 0.461, 0.528 and 0.579, respectively. In short, genetic polymorphism of China yellow quail, Beijing white quail and Korean quail is relatively abundant (He>0.5). All three quail groups are moderate polymorphism (0.25<PIC<0.5). For locus B, A allele frequency of China yellow quail, Beijing white quail and Korean quail is the highest, valuing 0.612, 0.623 and 0.633, respectively. Genetic polymorphism of China yellow quail, Beijing white quail and Korean quail is high (He>0.5). All three quail groups are highly polymorphism (PIC>0.5).

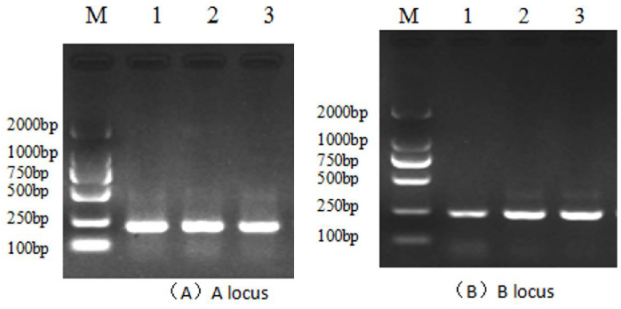

Fig. 1. Agar detection of *MyoG* gene.
M is Marker DL2000, 1 is Chinese yellow quail, 2 is Beijing white quail, 3 is Korean quail.

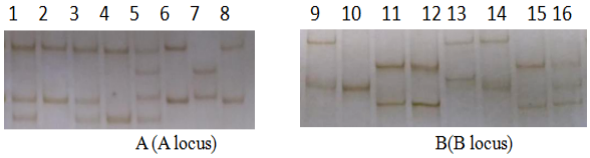

Fig. 2. SSCP results of *MyoG* gene.
A:1, 3 are AC genotype; 2, 6, 8 are CC genotype; 4 are AA genotype; 5 are AB genotype and 7 are BB genotype.
B: 9, 14 are DD genotypes; 10 are BB genotypes; 11, 12, 15 are AA genotypes; 13 are EE genotypes; and 16 are AB genotypes.

Association analysis between MyoG and egg quality of egg quail is obvious in Table III. It can be seen, that for locus A, there's no significant difference among AA, AB, AC, BB, BC and CC (P>0.05). For locus B, egg shape index of CC is significantly higher than that of EE (P<0.05), CC and EE show similar egg shape index with AA, AB, AC, AD, AE, BB and DD (P>0.05). Yolk index of AC and CC is significantly higher compared with that of AB and BB (P<0.05), Yolk index of BB is greatly lower compared with those of other genotypes (P<0.05). Egg weight, yolk weight, shell thickness and egg white height are similar among different genotypes (P>0.05).

Discussion

Wei *et al.* (2014) found 2 mutation sites and 6 genotypes in the third exon of MyoG of Bian chicken.

Table III. Association of *MyoG* gene polymorphism with egg quality in egg Quails.

Loci	Genotype	Egg weight/g	Egg shape index	Yolk index	Yolk weight/g	Shell thickness/mm	Egg white height/mm
A loci	AA	10.445±0.148^{a}	1.277±0.009^{a}	0.291±0.011^{a}	3.407±0.079^{a}	0.172±0.004^{a}	1.559±0.093^{a}
	AB	10.286±0.238^{a}	1.284±0.030^{a}	0.301±0.051^{a}	3.386±0.140^{a}	0.160±0.004^{a}	1.671±0.206^{a}
	AC	10.603±0.176^{a}	1.282±0.009^{a}	0.287±0.008^{a}	3.541±0.054^{a}	0.170±0.003^{a}	1.525±0.075^{a}
	BB	10.386±0.354^{a}	1.264±0.022^{a}	0.294±0.025^{a}	3.343±0.069^{a}	0.171±0.004^{a}	1.814±0.187^{a}
	BC	10.343±0.368^{a}	1.261±0.024^{a}	0.316±0.016^{a}	3.471±0.130^{a}	0.168±0.005^{a}	1.837±0.264^{a}
	CC	10.585±0.129^{a}	1.287±0.007^{a}	0.299±0.007^{a}	3.467±0.055^{a}	0.173±0.003^{a}	1.729±0.075^{a}
B loci	AA	10.510±0.105^{a}	1.281±0.007^{ab}	0.305±0.007^{ab}	3.412±0.048^{a}	0.169±0.002^{a}	1.614±0.062^{a}
	AB	10.420±0.290^{a}	1.286±0.009^{ab}	0.254±0.022^{b}	3.593±0.123^{a}	0.168±0.006^{a}	1.541±0.151^{a}
	AC	11.233±0.713^{a}	1.285±0.027^{ab}	0.327±0.038^{a}	3.367±0.176^{a}	0.184±0.014^{a}	1.943±0.309^{a}
	AD	10.347±0.246^{a}	1.278±0.013^{ab}	0.277±0.011^{ab}	3.447±0.076^{a}	0.178±0.005^{a}	1.636±0.125^{a}
	AE	10.575±0.206^{a}	1.277±0.015^{ab}	0.301±0.012^{ab}	3.460±0.074^{a}	0.170±0.003^{a}	1.632±0.100^{a}
	BB	11.300±0.077^{a}	1.273±0.005^{ab}	0.191±0.005^{c}	4.100±0.032^{a}	0.165±0.002^{a}	1.270±0.045^{a}
	CC	10.250±0.484^{a}	1.331±0.018^{a}	0.330±0.027^{a}	3.350±0.210^{a}	0.169±0.006^{a}	1.900±0.415^{a}
	DD	10.575±0.550^{a}	1.311±0.016^{ab}	0.298±0.021^{ab}	3.400±0.100^{a}	0.181±0.011^{a}	1.750±0.191^{a}
	EE	10.600±0.203^{a}	1.257±0.012^{b}	0.302±0.016^{ab}	3.531±0.109^{a}	0.175±0.004^{a}	1.828±0.185^{a}

Note: In the table, there are significant differences between different lowercase letters on the shoulder of the same column (P < 0.05), but there are no significant differences between the same letters (P > 0.05).

Tang *et al*. (2013, 2015) discovered 1 mutation site at exon 1 and exon 3 of MyoG of Jinghai yellow chicken, which involved 3 genotypes. Zhao *et al*. (2016) discovered 1, 2 and 3 mutation sites in exons 1, 2 and 3 of MyoG of three ear duck. Wang *et al*. (2007) discovered 1 mutation site and 3 genotypes at locus A in the control region of MyoG5' of broiler chicken, and found 3 SNPs loci and 6 genotypes at loci B. In this study, polymorphism in 3 egg quail groups at loci A and B in the control region of MyoG 5' was detected. Six genotypes were found at locus A and 9 genotypes were found at locus B. These revealed that MyoG had rich polymorphism in egg quail groups, which was similar with polymorphism research results on other poultries.

In studies on correlation between MyoG polymorphism and production performance of poultries, Zhao *et al*. (2016) concluded that two mutations of MyoG could influence breast muscle rate, weight and carcass net weight of duck significantly ($P<0.05$). Wang *et al*. (2007) discussed correlation of MyoG polymorphism with slaughter traits and meat quality of broiler chicken, finding significantly positive correlation between MyoG and muscle fiber growth of chicken ($P<0.05$). Tang *et al*. (2013) discovered 3 genotypes (AA, AB and BB) on exon 1 of MyoG of Jinghai yellow chicken, the body weight of BB at 2nd, 4th and 6th week was far higher than those of AB, and the body weight of BB at 8th week was higher than that of AA, showing significant differences ($P<0.05$). Wei *et al*. (2014) found 2 same sense mutation sites on MyoG, polymorphisms of these two mutation sites were correlated with growth performance of Bian chicken ($P<0.05$). This study significant correlations of locus B in the control region of MyoG 5' with egg shape index and yolk index of egg quail are discovered ($P<0.05$), however, locus A has no significant impacts on egg quality of egg quail ($P>0.05$). Correlation between loci A and B in the control region of MyoG 5' with egg quality of egg quail has to be further studied.

Acknowledgements

Sincere gratitude goes to the sponsor of National Natural Science Foundation (31201777) and Industry-University-Research Cooperation Project in Henan Province (152107000095.0).

Statement of conflict of interest

The authors declare that there is no conflict of interset.

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