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Short Communication

Polymorphism Analysis of FMO3 Gene in Egg Quail

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ABSTRACT

Flavin monooxygenase 3 (FMO3) is one member of flavin monooxygenase, FMO3 gene mutation has been proved the main causes of trimethylamine urine of human as well as fishlike smell of milk and eggs. In this study, polymorphism of FMO3 genes in Beijing white quail, China yellow quail and Korean quail was tested by PCR technology and hybrid DNA pool sequencing technology. Results can provide references for further exploring the action mechanism of FMO3 on fishlike smell of quail eggs. Research results show that, One SNPs mutation site (A66G) was detected from the exon 2 of FMO3, the A allele frequencies of A66G in Beijing white quail, China yellow quail and Korean quail were 0.603, 0.500 and 0.574, while G allele frequencies were 0.397, 0.500 and 0.426, respectively. One SNPs mutation site (C219T) was detected from the exon 9, C allele frequencies of C219T in Beijing white quail, China yellow quail and Korean quail were 0.125, 0.250 and 0.000, and the T allele frequencies were 0.875, 0.750 and 1.000, respectively. Two SNPs mutation sites (A39G and A41T) were detected from exon 7, A and G allele frequencies of A39G in Korean quail, Beijing white quail and China yellow quail were 0.811, 0.189, 0.605, 0.395, 0.491 and 0.509, A and T allele frequencies of A41T were 0.000, 1.000, 0.167, 0.833, 0.000 and 1.000, respectively. Two SNPs mutation sites (A139T and C187T) were detected from exon 4, A and T allele frequencies of A139T in Beijing white quail and Korean quail were 0.826, 0.174, 0.351 and 0.649, respectively, C and T allele frequencies of C187T were 0.370, 0.630, 0.283 and 0.717, respectively. According to clustering analysis, Beijing white quail has the closest genetic relationship with Japan quail, partridge, and mallard, which are clustered in one group.

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

Key words
Egg quail, FMO3 gene, Polymorphism Analysis, SNP, Phylogenetic tree

FMO3 is one member of flavin monooxygenase and it exists extensively in endoplasmic reticulum. Gene mutation of FMO3 has been proved the main cause of trimethylamine urine of human as well as fishlike smell of milk and eggs (Wang *et al*., 2013). Currently, researches on fishlike syndrome mainly focus on livestock, such as chicken, duck, goose, etc. Some studies are about human. Mo *et al*. (2011) detected 14 SNPs from FMO3 of quail, most of which were on the exon 7. SNP sites were detected from all 14 exons. SNPs on 3, 4, 5, 6, 7, 10 and 13 sites cause changes of the original encoded amino acids. These research conclusions provide technological supports to control fishlike smell in quail egg and selected breeding of quail egg quality from the perspective of genetics. Zhao *et al*. (2016) demonstrated that FMO3 exists in Taihang chicken group and individuals carrying FMO3 can be eliminated by molecular assisted marker. This can provide important references to increase and improve eggs of Taihang chicken. Based on experiment between fishlike smell and TMA content in yolk of duck egg, Li *et al*. (2018) discovered poor correlation between mutation sites and TMA content in yolk.

Recently, people pay more and more attentions to dietary quality and prefer green healthy foods. Quail egg and meats are highly appreciated by people due to the higher nutritive value than eggs and chicken. Hence, quail breeding becomes increasingly popular in China and foreign countries. People love quail eggs because of the higher nutritive value and tastes than eggs. 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 (Bai *et al*., 2016a, 2016b, 2016c, 2016d). The experimental values of quail in teaching and scientific studies are increasing gradually (Bai *et al*., 2017a, 2019). Some studies have

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proved that such fishlike smell syndrome of eggs is related with mutation of FMO3. Moreover, such mutation can be screened and eliminated through molecular assisted marker, thus enabling to assure quality of eggs. In this experiment, attentions were paid to explore polymorphism of FMO3 in three quail species, which was conducive to selected breeding of quail. Moreover, experimental results could provide references to improve overall quality of quail eggs and reduce production of fishlike smelling eggs.

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.

For PCR amplification primers at FMO3 gene were designed with references to the design of Mo *et al.* (2014), primers were synthesized by Beijing Dongguo Changsheng Biotechnology Co., Ltd, Primer details of FMO3 gene are shown in Table I. Pre-denaturation at 94°C for 4 min, then denaturation at 94°C for 40 s, annealing at 62-64°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°Cand finally the reaction was completed and cooled and preserved at 4°C.

Gene frequency (Bai *et al.*, 2016e, 2017b) was estimated according to the following formula: $F_1 = H_i / (H_1 + H_2)$ (i=1, 2), Where, F_1 is frequency of an allele at SNP site, H_1 and H_2 are heights of peak 1 and peak 2 of this SNP allele on the sequencing diagram.

Results and discussion

Agarose test results of FMO3 are shown in Figure 1, in all three Egg quail groups, the exon 4 of FMO3 has a clear band at 267bp. Exon 7 has a clear band at 279bp and exon 9 has a clear band at 337bp. In addition, exon 2 has two band s at 137bp and 270bp, the band at 137bp is the target band, with considerations to rubber cutting sequence, the impure band at 270bp may not influence sequencing.

SNP detection results of FMO3 in egg quails are shown in Figure 2. Exon 2 of FMO3 has one SNPs mutation site (A66G). Exon 9 has one SNPs mutation site (C219T). Exon 4 has 2 SNPs mutation sites (A139T and C187T). Exon 7 has 2 SNPs mutation sites (A39G and A41T).

In this study, average contents of 4 basic groups of exon 2 of FMO3 are A=22.86%, T=20%, C=16.19% and G=40.95%. Obviously, FMO3 might be in the region with relatively higher CG content. Whether AT and CG contents in FMO3 can influence functions of gene has to be further proved by studies.

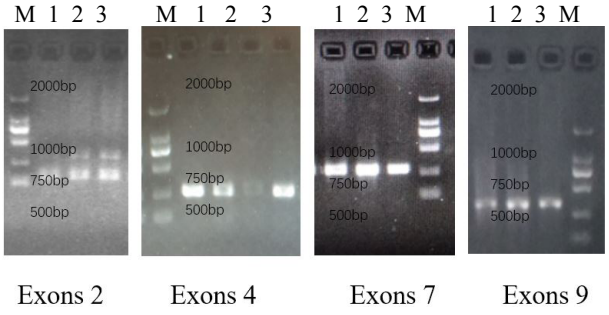

Fig. 1. Agarose electrophoresis results of FMO3 gene. M, DL2000 Mark; 1, Chinese yellow quail; 2, Beijing white quail; 3, Korean quail.

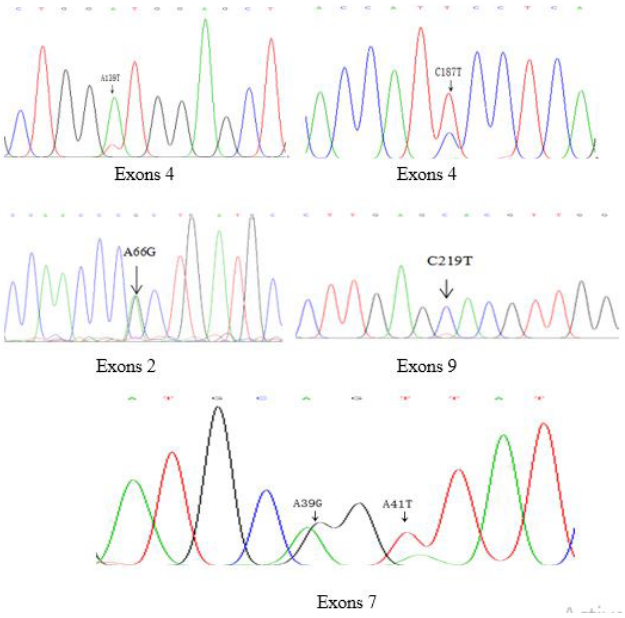

Fig. 2. SNP detection of FMO3 gene.

The gene frequencies of the FMO3 gene in the three egg mites population are shown in Table II, as can be seen from Table II, the A allele frequencies of A66G site of exon 2 in Beijing white quail, China yellow quail and Korean quail were 0.603, 0.500 and 0.574, while G allele frequencies were 0.397, 0.500 and 0.426, respectively. C allele frequencies of C219T site of exon 9 in Beijing white quail, China yellow quail and Korean quail were 0.125, 0.250 and 0.000, and the T allele frequencies were 0.875, 0.750 and 1.000, respectively. A and G allele frequencies of A39G site of exon 7 in Korean quail, Beijing white quail and China yellow quail were 0.811, 0.189, 0.605, 0.395, 0.491 and 0.509. A and T allele frequencies of A41T site of

Table I. Primer sequence of FMO3 gene.

FMO3 gene	Primer sequence (5'→3')	Annealing temperature	Product length
Exon 2	F:ATGGTGCGACGCGTGGCTGT	64°C	137bp
	R:TTCCGTGTAGCGCCAGAGCC		
Exon 4	F:GGGATAGAGAAAGTTTAAAGGTTG	65.4°C	267bp
	R:ACACGGCTCATCACCCAGGA		
Exon 7	F:GTGAAGGAATTCAGAGAAACA	62.0°C	279bp
	R:CTGAAACACCTTGACTGCC		
Exon 9	F:TGGTTCGGACAAGCAACAC	64°C	337bp
	R:TAGAGCACAGTGAGGAGGAG		

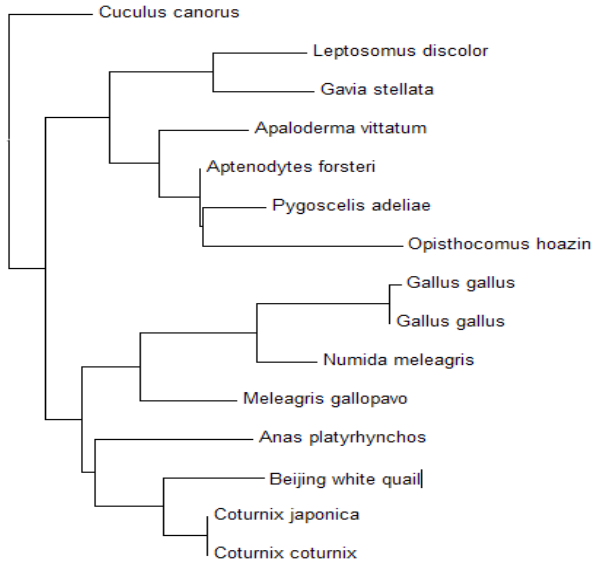

Fig. 3. Evolutionary tree of FMO3 gene in different species.

Table II. Gene frequencies of FMO3 gene.

FMO3 gene	SNPs	China yellow quail	Beijing white quail	Korean quail
Exon 2	A66G	A(0.500)	A(0.603)	A(0.574)
		G(0.500)	G(0.397)	G(0.426)
Exon 4	A139T	-	A(0.826)	A(0.351)
			T(0.174)	T(0.649)
	C187T	-	C(0.370)	C(0.283)
			T(0.630)	T(0.717)
Exon 7	A39G	A(0.491)	A(0.605)	A(0.811)
		G(0.509)	G(0.395)	G(0.189)
	A41T	A(0.000)	A(0.167)	A(0.000)
		T(1.000)	T(0.833)	T(1.000)
Exon 9	C219T	C(0.250)	C(0.125)	C(0.000)
		T(0.750)	T(0.875)	T(1.00)

exon 7 were 0.000, 1.000, 0.167, 0.833, 0.000 and 1.000, respectively. A and T allele frequencies of A139T site of exon 4 in Beijing white quail and Korean quail were 0.826, 0.174, 0.351 and 0.649, respectively. C and T allele frequencies of C187T site of exon 4 were 0.370, 0.630, 0.283 and 0.717, respectively.

A phylogenetic tree of FMO3 gene sequences of different species was constructed by DNA Star (Fig. 3). According to clustering analysis of egg quail, Beijing white quail had the closest genetic relationship with Japan quail, partridge, and mallard, which were clustered in one group. Jungle fowl, Agelastes meleagrides and turkey were clustered into the second group. Cuckoo was clustered into the fourth group independently, and the rest were clustered the third group.

Polymorphism of FMO3 has been studied a lot. Existing studies involve both people and animals. Polymorphism of FMO3 is discovered not only from animals, but also from human (Lattard *et al*., 2003). Zeng *et al*. (2003) found that mutation frequencies of FMO3 in 4 Dai communities in Yunnan Province were different. In addition, expression level of FMO3 was also related with many production performances. Tian *et al*. (2017) pointed out that expression level of FMO3 was positively correlated with fat deposition. FMO3 is also related with many diseases. Obstacle of trimethylamine is manifested by "trimethylaminuria" in human bodies. Recently, FMOs have certain functions in human brain. In the Rotenone-induced Parkinson model, activity of FMOs is inhibited by tapazole, which can strengthen activity of caspase 3 and decrease expression of Parkin protein. Hence, it is speculated that dysfunction of FMOs is one cause of dopaminergic apoptosis in the Rotenone-induced Parkinson model and it provides a new clue for studying the pathological model. Zhao *et al*. (2016) demonstrated that individuals with FMO3 susceptibility genes could be eliminated by molecular assisted marker by investigating

distribution of FMO3 sensitive sites in different chicken species. Tian *et al*. (2017) detected few gene frequencies of FMO3 mutation sites in Lhasa white chicken, but individuals with mutant genes still can be eliminated by molecular assisted marker. According to detection results of duck FMO3 in FATGY highly conservation region, Yang *et al*. (2016) found no mutation of basic groups. In other words, no gene mutation was detected at the FMO3 sensitive sites.

Zhong *et al*. (2017) discovered mutation of basic group in quail egg which was closely related with TMA content. This mutation weakened activity of FMO3 enzyme, thus causing fishlike smelling quail eggs. In addition, Mo *et al*. (2011) discovered a mutation site on exon 7 of FMO3 in quail. The basic group C was mutated to the basic group T, thus inducing transformation of threonine in highly conservation area of FATGY. Therefore, there's genetic effect of fishlike smelling quail eggs similar with chick eggs. Moreover, Mo *et al*. (2014). discovered that exon 4 of FMO3 had only one mutation site (A/G) and detected 6 mutation sites in exon 7. Among them, functions of amino acid at two sites were changed. In this study, two mutation sites (A139T and C187T) were found in exon 4 of FMO3, and only two mutation sites (A39G and A41T) were found in exon 7. These mutation sites disagreed with research results of Mo Fengtao. This study also demonstrated that there's one mutation site on exon 2 (A66G) and exon 9 (C219T) of FMO3, respectively. This conformed to the research conclusion of Mo Fengtao. There are few studies concerning quail. Most studies on FMO3 in animals mainly focused on chicken. Hence, whether gene mutation of FMO3 in quail can cause fishlike smelling quail eggs still has to be further studied.

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Statement of conflict of interest

The authors declare there is no conflict of interest.

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