Pakistan J. Zool., pp 1-9, 2020. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20181227051220

Genetic Association of Two Novel SNPs in CYP7A1 Gene with Lipid Traits of Tianzhu Black Muscovy (*Cairina moschata*)

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ABSTRACT

Cholesterol 7α-hydroxylase (CYP7A1) plays a crucial role in the synthesis of bile acid, fatty acid and cholesterol metabolism in human and rodent. However, a little was known in the literature on the question of the effect of CYP7A1 gene on poultry lipid homeostasis. This study was performed to investigate the effect of the polymorphisms of CYP7A1 gene on lipid traits in Tianzhu Black Muscovy (*Cairina moschata*). We detected two novel silent mutations, CDS 216 A>G and CDS 681 T>A in exon 2 and exon 3 of CYP7A1 gene, respectively, and both SNPs changed DNA single strand conformation. The A and T allele of CDS 216 A>G and CDS 681 T>A was dominant allele, and its frequency was 0.554 and 0.800, respectively. Each SNP resulted in three genotypes. The genotypic distribution of CDS 216 A>G and CDS 681 T>A was not deviated and deviated from Hardy-Weinberg equilibrium (HWE), respectively. The polymorphism information content (PIC) of CDS 216 A>G and CDS 681 T>A was 0.372 and 0.269, respectively, and both belonged to medium levels of genetic diversity. Association analysis revealed that two SNPs were significant association with at least four of seven lipid indexes. Allele A of CDS 216 A>G locus and allele T of CDS 681 T>A locus was favorable to improving meat quality, respectively. Three haplotypes and six diplotypes were identified by the combination of two SNPs. Diplotypes had dominantly affected on tested lipid indexes except for IMF. Diplotype H1H1 was advantageous for the improvement of meat quality. Therefore, our data suggested that two novel SNPs: CDS 216 A>G and CDS 681 T>A in CYP7A1 gene were potential candidate markers for improving meat quality. It also provides reference data for early breeding and selection of Tianzhu Black Muscovy.

Article Information
Received 27 December 2018
Revised 02 March 2019
Accepted 30 April 2019
Available online 09 June 2020

Authors' Contribution
YYZ is project leader, and conceived and designed the study. GHT carried out the experimental operation of the study and wrote the article. YYQ carried out the experimental work. LW and JZL analyzed the data.

Key words
Tianzhu Black Muscovy, CYP7A1 gene, SNPs, Lipid traits, Genetic association.

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INTRODUCTION

In avian special, fatty tissue deposition excess is disadvantage for productive benefits. Therefore, increasing intramuscular fat (IMF) and reducing body fat deposition is one of breeding goals in modern poulty production (Jiang *et al*., 2017). Lipids contain fatty acids, triglycerides, phospholipids, and cholesterol. Cholesterol, as a vital component in eukaryotic cell membranes, which modulates membrane permeability and fluidity, is the precursor of bile acid, citamin D, and steroid hormones (Smolle and Johannes Haybaeck, 2014; Helsley and Zhou, 2017). LXRs, PPARs, SRs, and SREBPs are considered as the critical regulatory factors in lipid metabolic pathways (Huang *et al*., 2018; Gbaguidi and Agellon, 2004). To accomplish appropriate physiological levels, the metabolism of fatty acids and cholesterol are accurately dominated at the transcriptional and posttranscriptional stage (Griffin, 2013; Li *et al*., 2010). At present, modern molecular technologies interacted with traditional methods is the best efficiency for decreasing lipid deposition in modern poultry breeding.

Cholesterol 7α-hydroxylase (CYP7A1) also called cytochrome P450 7A1, which is a vital rate-limiting enzyme in bile acid biosynthesis, catalyzing cholesterol to 7α-hydroxycholesterol (Chen, 2015). CYP7A1 is a target in LXRs signaling pathway (Baranowski, 2008). LXRs with retinod X receptors (RXRs) form obligate heterodimers, and trigger the expression of SREBP-1c and cholesterol efflux-related genes, and finally cause triglyceride accumulation through fatty acid synthase (FAS) (Pan *et al*., 2018; Cai *et al*., 2018; Song *et al*., 2015). The hydroxylation of cholesterol is modulated by bile acid derivatives and hormones via a negative feedback mechanism (Chiang, 2009). Oxycholesterol combines with LXRα, and the liganded LXRα with RXR forms a heterodimer and interacts with liver X receptor (LXR) response element (LXRE) in the CYP7A1 promoter, consequently raising

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0030-9923/2020/0001-0001 $ 9.00/0
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the transcriptional level of CYP7A1. Upregulation of CYP7A1 accelerates bile acid synthesis (Wang *et al*., 2018; Meng *et al*., 2018). In addition, small heterodimer partner (SHP) which is a transcriptional repressor interacts with a transactivator LRH-1 and commands it against activating its target genes CYP7A1 and SHP (Mercer *et al*., 2018; Lin, 2015; Zhong *et al*., 2018). Therefore, SHP suppresses the transcription of CYP7A1 and the gene coding itself. In humans and mammal, the lack of CYP7A1 could lead to reduce bile acid production and accumulate cholesterol in the liver, resulting in downregulation of LDL receptors and following hypercholesterolemia (Moon *et al*., 2016; Al-Aqil *et al*., 2018). The latest results demonstrated that some miRNAs (such as miR-34a, miR-122, miR-17, miR-383, and miR-146b *etc*.) and lncRNAs (such as lnc-HC, lncLSTR, and hnRNPA2B1 *etc*.) are the regulators of CYP7A1 in cellular cholesterol and fatty acid metabolism (Zeng *et al*., 2018; Lan *et al*., 2016; Gong *et al*., 2018; Cui *et al*., 2018; Zinkhan *et al*., 2018; Xia *et al*., 2017). According to previous reports, CYP7A1 shows to paly a crucial role in lipid deposition in the liver and hypercholesterolemia. However, CYP7A has been little known about the effect on poultry lipid metabolism. In avian species, CYP7A1 gene include 6 exons. The encoded amino acid sequence of exon 2 and exon 3 contains fifteen function domains and regulates CYP7A1 mRNA transcription level and lipid transport. Thus, the overall aim of this study was to detected whether the SNPs of CYP7A1 gene exon 2 and exon3 are closely related with lipid traits in Tianzhu Black Muscovy (*Cairina moschata*). To test this hypothesis, we genotyped two novel SNPs of CYP7A1 gene and identified two SNPs that are associated with lipid traits.

MATERIALS AND METHODS

Experimental animal

Bood samples were collected from 195 females of Tianzhu Black Muscovy (*Cairina moschata*) which is a native breed in China. All the birds were incubated on the same day, and consequently raised in the same environment and management conditions in poultry research institute of Guizhou University, Guizhou, China. Blood samples were achieved through the wing vein, and were euthanized that the specific implementation plan was taken all experimental ducks placed in an operating room filled with a mixture of 90% argon and 10% nitrogen at 70 days of age, and then all of ducks were quickly bled, and collected blood samples and dissected after they were unconscious. All animal experiments were performed according to the Laboratory animal—Guideline for ethical review of animal welfare of China (permit number: GB/T 35892-2018) that was issued by China Laboratory Animal Standardization Technology Committee (SAC/TC 281).

Samples and data collection

Chest muscles and abdominal fat was determinated using slaughter segmentation method (Lin *et al*., 2018), respectively. Intramuscular fat (IMF) content of chest muscles was measured using the Soxhlet extraction method (Li *et al*., 2018). The percentage of abdominal fat (AFP) was calculated according to Poultry Production Terms and Measurement Method of Ministry of Agriculture of China (NY/T 823-2004). Serum was isolated at 4°C, 3500 rpm for 10 min from blood that was stewed at room temperature for 1 h. The contents of serum total cholesterol (TCH) and triglycerides (TG) were detected using qualified laboratory methods (He *et al*., 2018). Unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), and essential fatty acid (EFA) in chest muscle was detected as described by Han *et al*. (2013), respectively. Genomic DNA was extracted from blood using QIAamp DSP DNA Blood Mini Kit (Shanghai Labpal Co. Ltd., China), and then measured its concentration and purity using ND-2000 spectrophotometer (Thermo Fisher Scientific Inc.), and finally stored at -20°C for further experiments.

Primer design and PCR amplification

Based on the mRNA sequence of duck CYP7A1 gene (GenBank accession: JQ922243) and *Anas platyrhynchos* reference genomic sequence (NW_004676497.1), three pairs of primers were designed using Oligo 6.22 software and synthesized in Invitrogen Co. Ltd. (Peking, China) (Table I). PCR amplification was accomplished in a total volume of 50 μL containing 2×Pfu Master Mix

Table I.- Primers information for PCR amplification.

Primers	Primers sequence(5'→3')	Position / bp	Annealing temperature (°C)
P1	F: AGGAGAGCCACCACTTGAAA	Exon 2	58
	R: GGGTCAGTGAGGAAATGAA	CDS 87-245 bp /159 bp	
P2	F: GGTAGCATTGACTCAGCAGA	Exon 3	60
	R: TCTCGGTCTCCTGCTTTGATA	CDS 334-619 bp /286 bp	
P3	F: AGGAGACCGAGAGAGCACATAT	Exon 3	60
	R: GTGGCAGGAATGGTGTTGGC	CDS 608-905 bp/ 298 bp	

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(Peking Cwbiotech Co. Ltd., China) 20 μL, each of forward and reverse primer (10 μmol/L) 2 μL, template genomic DNA (100ng/μL) 2 μL, and ddH_2O 24 μL. PCR protocol was implemented as below: 95°C for 10 min; 35 cycles at 94°C for 40 s, annealing for 40 s at the optimum primer annealing temperature, and at 72°C for 45 s; final extension at 72°C for 5 min.

Gene polymorphism analysis

Each of PCR products was purified and sequenced in Invitrogen Co. Ltd. (Beijing, China), and subsequently identified SNPs using software Align IR version 2. To further analyzed whether SNPs changed DNA conformation. SSCP analysis was performed using the following program: a total volume of 12 μL consisted of 4μL each of PCR products and 8 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% bromophenol blue and 0.025% xylene-cyanole), denatured at 98 °C for 15 min and then quickly chilled on -20°C for 1 min to acquire single-stranded DNA, and subsequently subjected to electrophoresis on 12% polyacrylamide gel (polyacrylamide : bisacrylamide = 29:1) which was run with 1× TBE buffer for 10 h at 140 voltage constantly. The gel was washed in 10% deionized ethanol for 8 min and then incubated 8 min in 0.1% silver nitrate, and finally visualized 6 min in the mixture of 0.5% (v/v) formaldehyde and 2% sodium hydroxide. The SSCP bands on the gel were photographed using BIO-RAD Gel Doc XR+ imaging system (BIO-RAD, USA). The PCR fragments from different SSCP bands were sequenced and further verified the discovered SNPs in CYP7A1 gene.

Statistical analysis

Genotype and allele frequency, pair-loci D'/r^2 value, haplotype and diplotype frequency, and chi-square ($\chi 2$) were calculated by SHEsis online software (http://analysis.bio-x.cn/). Heterozygosity (*He*), effective number of alleles (*Ne*), and polymorphism information content (*PIC*) were estimated using Cervus 3.0 software. Correlation analysis were performed using SPSS 16.0 software. The linear model was depicted as follow: Y =μ+ G + e, e - the random error, μ - the mean for each trait, G - the genotype effect or diplotype effect, and Y – a lipid trait. Data were demonstrated as mean ± SE.

RESULTS

SNPs identification

Three different primer pairs for CYP7A1 gene were investigated for SNPs in Tianzhu Black Muscovy through PCR products direct sequencing. Two single-base mutations were detected at the 216th (primer P1) and 681th (primer P3) bp of CDS region of CYP7A1 gene. Both of 216th bp A→G transition in exon 2 and 681th bp T→A transversion in exon 3 were silent mutation (Fig. 1). Furthermore, SSCP analysis indicated that both SNPs resulted in single-stranded DNA conformation changes (Fig. 2). The CDS 216 A>G mutation produced three genotypes AA, AG and GG. The CDS 681 T>A mutation also led to three genotypes TT, TA and AA.

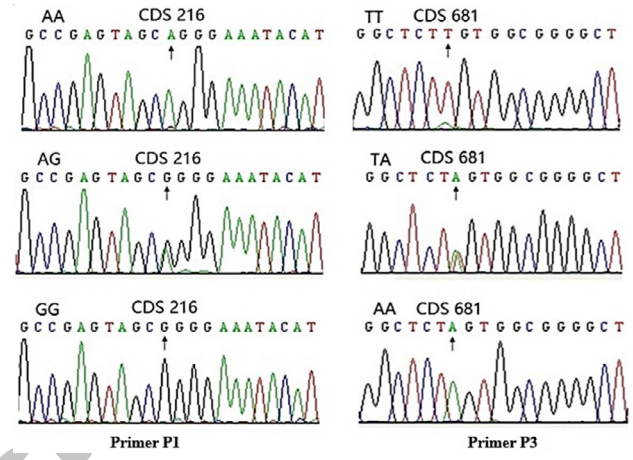

Fig. 1. SNPs detection using PCR production directing sequencing.

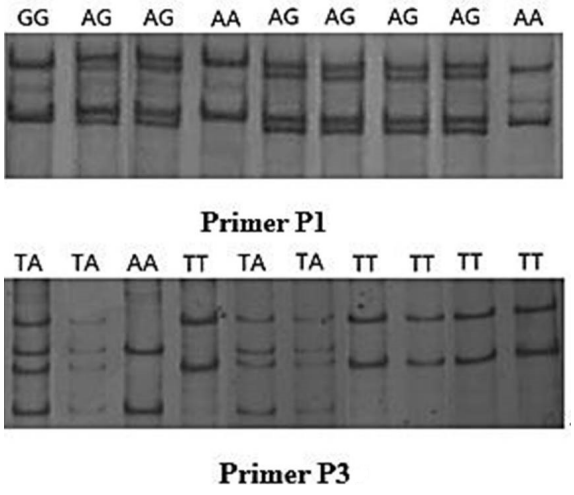

Fig. 2. SNPs cause single-strand conformation polymorphism.

Genotypic frequencies, allelic frequencies, and diversity parameter

Genotypic and allelic frequencies, diversity parameter, and $\chi 2$-value of two SNPs of CYP7A1 gene in Tianzhu Black Muscovy were calculated and summarized in Table II. The A and T allele frequency of CDS 216 A>G

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Table II.- Population genetic information of two SNPs of CYP7A1 gene in Tianzhu Black Muscovy.

SNPs	Genotype frequency			Allele frequency		He	Ne	PIC	χ^2
CDS 216 A>G	AA(60)	AG(96)	GG(39)	A	G	0.494	1.977	0.372	0.003
	0.308	0.492	0.200	0.554	0.446				
CDS 681 T>A	TT(133)	TA(46)	AA(16)	T	A	0.320	1.471	0.269	13.469**
	0.682	0.236	0.082	0.800	0.200				

He, heterozygosity; *Ne*, Effective allele number; *PIC*, polymorphism information content; χ2-test, Hardy-Weinberg equilibrium, $\chi^2_{0.01(2)}$=9.21, $\chi^2_{0.05(2)}$=5.99, **P*<0.05, ***P*<0.01.

Table III.- Haplotypes and diplotypes analysis based on the two SNPs of CYP7A1 gene.

SNPs		CDS 216 A>G	CDS 681 T>A	Frequency
Haplotypes	H1	A	T	0.554
	H2	G	A	0.200
	H3	G	T	0.246
Diplotypes	H1H1(60)	AA	TT	0.308
	H1H2(33)	AG	TA	0.169
	H1H3(63)	AG	TT	0.323
	H2H2(16)	GG	AA	0.082
	H2H3(13)	GG	TA	0.067
	H3H3(10)	GG	TT	0.051

and CDS 681 T>A was 0.554 and 0.800, respectively, which were dominant allele. The AG and TT genotype frequency of CDS 216 A>G and CDS 681 T>A was 0.492 and 0.682, respectively, which are dominant genotype. Chi-square tests (χ2) demonstrated that the genotypic distribution of CDS 216 A>G was in Hardy-Weinberg equilibrium (HWE) (*P*>0.05), however, CDS 681 T>A deviated from HWE (*P*<0.01). The PIC of CDS 216 A>G and CDS 681 T>A was 0.372 and 0.269, respectively, and both belonged to medium levels of genetic diversity according to describe by Banerjee and Chaturvedi (Banerjee and Chaturvedi, 2018).

Haplotypes and diplotypes analysis of two SNPs of CYP7A1 gene

Linkage disequilibrium coefficient D' and r2 between two SNPs of CYP7A1 gene in Tianzhu Black Muscovy was 1 and 0.310, respectively. The CDS 216 A>G and CDS 681 T>A loci has no strong linkage disequilibrium based on the rule that r^2>0.33 and |D'|>0.8 are thought about strong linkage disequilibrium in different alleles (Slatkin, 2008). Three haplotypes and six diplotypes of both SNPs of CYP7A1 gene were found and showed in Table III. The frequency of haplotypes H1, H2 and H3 was 0.554, 0.200 and 0.246, respectively. H1 was main haplotype. The frequency of diplotypes H1H1, H1H2 and H1H3 were greater than 0.1 (0.308, 0.169 and 0.323, respectively). However, other three diplotypes H2H2, H2H3 and H3H3 frequencies were less than 0.1 (0.082, 0.065 and 0.051, respectively).

Associations between identified SNPs and seven lipid indexes

Association analysis of both SNPs of CYP7A1 gene with lipid traits in Tianzhu Black Muscovy were performed and showed in Table IV. For CDS 216 A>G locus, individuals with genotype AA were higher than those with genotype AG for AFP (*P*<0.01) and TG (*P*<0.05), and individuals with genotype AA were higher EFA (*P*<0.05) and lower UFA (*P*<0.05) than those with genotype GG. For CDS 681 T>A locus, individuals with genotype TT were significantly lower UFA (*P*<0.05) and extremely higher TCH (*P*<0.01) than those with genotype AA, and individuals with genotype TT were extremely higher than those with genotype TA for PUFA and EFA (*P*<0.01), and finally individuals with genotype TA were significantly higher TG (*P*<0.05) and extremely higher TCH (*P*<0.01) than those with genotypes TT and AA.

Associations between diplotypes and seven lipid indexes

Association analysis of combinative diplotypes of CDS 216 A>G and CDS 681 T>A loci with lipid traits in Tianzhu Black Muscovy was done and showed in Table V. Individuals with H1H1 were higher AFP than those with H1H3 (*P*<0.01) and H1H2 (*P*<0.05). Individuals with H1H1 were lower UFA than those with H2H2 and H3H3 (*P*<0.05). For PUFA and EFA, individuals with H1H1 and H1H3 were higher than those with H1H2 and H2H3 (*P*<0.05). For TCH, individuals with H2H3 were extremely higher than other diplotyes except H1H2 (*P*<0.01), individuals with H1H2 were extremely higher than those with H1H1, H1H3, H2H2, and H3H3 (*P*<0.01), individuals with H1H1 were extremely higher than those with H2H2 and H3H3 (*P*<0.01), individuals with H1H3 were higher than those with H2H2 and H3H3 (*P*<0.05), and individuals with H1H2 were lower than those with H2H3 (*P*<0.05).

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Table IV.- Association of two SNPs of CYP7A1 gene with lipid traits in Tianzhu Black Muscovy.

Traits	CDS 216 A>G			CDS 681 T>A		
	AA(60)	AG(96)	GG(39)	TT(133)	TA(46)	AA(16)
IMF / %	5.81±0.13	5.69±0.10	5.49±0.16	5.76±0.09	5.49±0.15	5.61±0.25
AFP / %	1.38±0.05^{A}	1.13±0.04^{B}	1.27±0.07	1.24±0.04	1.21±0.06	1.30±0.11
UFA / %	55.333±0.39^{a}	56.02±0.31	56.85±0.49^{b}	55.76±0.27^{a}	56.03±0.45	57.51±0.77^{b}
PUFA / %	23.05±0.34	22.62±0.27	21.99±0.42	22.97±0.22^{A}	21.70±0.38^{B}	22.50±0.65
EFA / %	22.50±0.32^{a}	22.14±0.25	21.43±0.40^{b}	22.44±0.21^{A}	21.18±0.36^{B}	21.96±0.62
TCH / mmol·L^{-1}	3.51±0.06	3.56±0.05	3.42±0.07	3.46±0.03^{A}	3.84±0.06^{B}	3.08±0.10^{C}
TG / mmol·L^{-1}	1.19±0.02^{a}	1.13±0.02^{b}	1.14±0.03	1.13±0.01^{a}	1.21±0.02^{b}	1.09±0.04^{a}

IMF, intramuscular fat of chest muscle; AFP, abdominal fat percentage; UFA, unsaturated fatty acids; PUFA, Polyunsaturated fatty acids; EFA, essential fatty acid; TCH, serum total cholesterol; TG, serum triglyceride. A, B, and C within the same line with different superscripts indicates $P<0.01$; a, b within the same line with different superscripts indicates $P<0.05$.

Table V.- Association of diplotypes with lipid traits in Tianzhu Black Muscovy.

Diplotypes	H1H1(60)	H1H2(33)	H1H3(63)	H2H2(16)	H2H3(13)	H3H3(10)
IMF / %	5.81±0.13	5.54±0.18	5.77±0.13	5.61±0.25	5.36±0.28	5.46±0.32
AFP / %	1.38±0.05^{Aa}	1.19±0.07^{b}	1.10±0.05^{Bb}	1.30±0.10^{ab}	1.24±0.11^{ab}	1.25±0.13^{ab}
UFA / %	55.32±0.39^{b}	56.28±0.53^{ab}	55.88±0.38^{ab}	57.50±0.76^{a}	55.42±0.84^{ab}	57.65±0.96^{a}
PUFA / %	23.05±0.34^{a}	21.79±0.45^{b}	23.06±0.33^{a}	22.49±0.65^{ab}	21.44±0.72^{b}	21.93±0.82^{ab}
EFA / %	22.50±0.32^{a}	21.33±0.43^{b}	22.56±0.31^{a}	21.96±0.62^{ab}	20.82±0.69^{b}	21.36±0.78^{ab}
TCH / mmol·L^{-1}	3.51±0.05^{C}	3.76±0.07^{ABb}	3.46±0.05^{CDc}	3.08±0.09^{Dd}	4.04±0.11^{Aa}	3.14±0.12^{Dd}
TG / mmol·L^{-1}	1.19±0.02^{A}	1.18±0.03^{a}	1.09±0.02B^{b}	1.09±0.04B^{b}	1.27±0.04^{A}	1.04±0.05^{Bb}

IMF, intramuscular fat of chest muscle; AFP, abdominal fat percentage; UFA, unsaturated fatty acids; PUFA, Polyunsaturated fatty acids; EFA, essential fatty acid; TCH, serum total cholesterol; TG, serum triglyceride. A, B, C, D within the same line with different superscripts indicates $P<0.01$; a, b, c, d within the same line with different superscripts indicates $P<0.05$.

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Furthermore, individuals with H3H3 were extremely lower TG than those with H1H1 and H2H3 ($P<0.01$), and individuals with H1H2 were higher TG than those with H1H3, H2H2 and H3H3 ($P<0.05$). In addition, there were no significant difference with the lipid indexes between other diplotypes ($P>0.05$).

DISCUSSION

CYP7A1 is an important factor in hepatic lipid metabolism through effects on cholesterol or fatty acid biosynthesis depending on Acetyl-coenzyme A (AcCoA) metabolic pathways (Hubacek and Bobkova, 2006). Previous studies have shown that single-nucleotide polymorphisms (SNPs) of CYP7A1 gene are related with total cholesterol, triglyceride and low-density lipoprotein (LDL) levels, risk of lipid metabolism-related diseases, and other phenotypes. The combination of SNP rs3808607 and SNP rs9297994 in human CYP7A1 gene was associated with hepatic CYP7A1 mRNA expression, total cholesterol and LDL levels (Wang *et al*., 2018). The combination of CYP7A1 rs3808607-TT with ABCG5 rs6720173-CC and DHCR7 rs760241-GG genotypes was related with serum cholesterol responses to dairy consumption (Abdullah *et al*., 2018). The CYP7A1 gene rs3808607 variant was associated with nonalcoholic fatty liver disease (Zhaldak *et al*., 2017). The rs38088607 mutation of CYP7A1 gene was highly associated with serum TCH and LDL levels in different cohort characteristics (Teslovich *et al*., 2010). However, in avian species, the function of the SNPs of CYP7A1 gene is less known except for the fact that its expression was involved in cholesterol and fatty metabolism, and bile acid synthesis (Zhao *et al*., 2011; Sato *et al*., 2008; Chen *et al*., 2012; Huang *et al*., 2015; Hu *et al*., 2015).

In avian species, increasing the percentage of chest muscles and the content of intramuscular fat (IMF) is an important breeding goals in modern poulty production. In this study, we firstly identified two novel silent mutation loci: CDS 216 A>G and CDS 681 T>A in

Tianzhu Black Muscovy CYP7A1 gene, and they have no strong linkage disequilibrium. Both SNPs changed the amplified fragment DNA single-stranded conformation. The genotypic distribution of CDS 216 A>G locus was in HWE and indicated that it has not been affected by effect factors including selection, mating system, migration, and random genetic drift or regained balance due to long-term artificial selection breeding (Chen *et al*., 2017). However, the genotypic distribution of CDS 681 T>A locus might be influenced by effect factors, especially selection and genetic drift resulting in deviation from HWE. In addition, for a selected breed, the loci related to measured traits should be expected to deviate from HWE and PIC>0.25 (Puig *et al*., 2017; Saqlain *et al*., 2018).

Association analysis demonstrated that two SNPs were significant relation with at least four of seven lipid indexes. Genotype AA of CDS 216 A>G mutation and genotype TT of CDS 681 T>A mutation was the highest for IMF, PUFA and EFA, respectively, and suggested that they were advantageous to the improvement of meat quality. Furthermore, three haplotypes and six diplotypes were identified by the combination of two SNPs. Diplotypes were dominant affected on tested lipid indexes except for IMF. Diplotype H1H1 was the most beneficial for improving meat quality, which showed that haplotype H1 may play a positive role on meat quality. Based on our results, we considered that two SNPs might be the valid candidate markers to improve meat quality. However, it also increased the content of abdominal fat when the meat quality was improved, and led to the conflict between improving meat quality and reducing body fat deposition. But we can resolve the contradiction through formulating the timing of breeding objectives. For TG and TCH, we suggested that it has dominance/over-dominance effect between homozygous diplotypes, and obtained proper the levels of TG and TCH through the heterosis between different homozygous diplotypes (Xu *et al*., 2013; Dekkers *et al*., 2004). Furthermore, the previous many studies verified that single-strand DNA conformation change caused the difference of gene copy number, promoter activity, transcription and translation levels, and consequently influenced on animal performance (Stefl *et al*., 2013). CYP7A1 as a target gene of LXRs, CYP7A1 signaling has been mentioned to participate in lipid metabolism, and many molecules including FXR, FAS, SREBP-1c, LXRα, CD36, lecithin cholesterol acyltransferase (LCAT), and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) were involved, and then modulated the dynamic balance of serum TG and TC levels through fat deposition (body fat and IMF) and oxidative decomposition (Meng *et al*., 2018; Zhang *et al*., 2018; van Solingen *et al*., 2018; Chiang, 2003). Therefore, two SNPs of CYP7A1 gene may cause the expressive difference of genes involved in lipid metabolism, and achieved to regulate fat deposition. Certainly, further work is necessary implement, such as the effects of SNPs and its haplotypes/diplotypes on expression of CYP7A1 shall be evaluated in other Muscovy, and association of CYP7A1 gene with other lipid metabolism-related genes should be confirmed in Tianzhu Black Muscovy.

CONCLUSIONS

In this study, two novel silent mutation loci in exon 2 (CDS 216 A>G) and exon3 (CDS 681 T>A) of CYP7A1 gene were identified in Tianzhu Black Muscovy, respectively. Association analysis revealed that two SNPs were significant association with the content of UFA, EFA and TG. Allele A of CDS 216 A>G locus and allele T of CDS 681 T>A locus were favorable to improving meat quality, respectively. Three haplotypes and six diplotypes were identified by the combination of two SNPs. Diplotypes had dominantly affected on tested lipid indexes except for IMF. Diplotype H1H1 was advantageous for the improvement of meat quality. Based on our results, we considered that two SNPs of CYP7A1 gene were potential candidate markers for improving meat quality. It also provides reference data for the further studies of poultry CYP7A1 gene.

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ACKNOWLEDGMENTS

The funding for this work was supported by the National Natural Science Foundation of China (31760663) and science and technology project of Guizhou province (QKHPTRC[2017]5788).

Statement of conflict of interest

Authors have declared no conflict of interest.

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