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Association between *FTO* polymorphism in exon 3 with carcass and meat quality traits in crossbred ducks

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ABSTRACT. The fat mass and obesity-associated gene (*FTO*) is an excellent candidate gene that affects energy metabolism. Single nucleotide polymorphisms (SNPs) in *FTO* are associated with carcass and meat quality traits in pigs, cattle, and rabbits. The aim of this study was to investigate the association between novel SNPs in the *FTO* coding region and carcass and meat quality traits in 95 crossbred ducks, using DNA sequencing. We found two transitions G/A (SNP 387 and 473) within exon 3. SNP 387 was a synonymous mutation, whereas SNP 473 was a missense mutation. Association analysis suggested that SNP g.387G>A was significantly associated with all of the carcass traits measured, the intramuscular fat content (IMF), cooking yield (CY), pH values 45 min after slaughter (pH45m), drip losses from the breast muscle, and the leg muscle ($P < 0.05$). For SNP g.473G>A, the genotype AA exhibited greater leg muscle weight than the genotypes GG or AG ($P < 0.05$). The D value suggested that the two SNPs exhibited strong linkage disequilibrium. Three haplotypes (G_1G_2, G_1A_2, and A_1A_2) were significantly associated with IMF, CY, the a* value, and all of the carcass traits measured ($P < 0.05$). The results suggest

that *FTO* is a candidate locus that affects carcass and meat quality traits in ducks.

Key words: Crossbred duck; Fat mass and obesity-associated gene; Carcass and meat quality trait; Single nucleotide polymorphism

INTRODUCTION

Carcass and meat quality traits are important in animal breeding programs, and include live weight (LW), carcass weight (CW), pH, semi-eviscerated weight (SEW), eviscerated weight (EW), meat color (MC), drip loss (DL), tenderness, intramuscular fat (IMF) content, and other traits. However, these traits have low heritabilities (Boukha et al., 2011) and are difficult to improve using traditional artificial selection. Moreover, their measurement is both expensive and difficult, and can only be conducted after death. Because of advances in molecular genetics technology, genomic selection strategies and marker-assisted selection programs have been used, and are considered to be the most effective selection approaches for low-heritability traits since they are easily measured (Gao et al., 2007). Furthermore, the identification of gene polymorphisms that are associated with production traits, and linkage analysis, are important and commonly used tools to characterize candidate genes at the DNA level.

Several factors, such as genes, the breed studied, the rearing system, sex, and age could influence carcass and meat quality traits (Santos et al., 2007; Galián et al., 2008; Tůmová et al., 2014). Fat content is a crucial aspect of animal meat quality, and the aim in modern animal breeding is to reduce fat deposits and increase lean growth. DNA markers in several obesity-related genes have been associated with fat deposition and carcass and meat quality traits in different animal populations. For example, the leptin gene, which is also called the obese gene, codes for a circulating protein that regulates dietary intake by binding to leptin receptors (Prokop et al., 2012). Polymorphisms in the leptin gene have been identified, and association analyses have shown that this gene might be an important source of variability in carcass and meat quality traits between different cattle populations (Li et al., 2013; Tian et al., 2013). Therefore, it is important to investigate the association between obesity-related genes and carcass and meat quality traits for duck breeding programs.

The fat mass and obesity-associated gene (*FTO*) was originally cloned in mice (Peters et al., 1999), and energy expenditure is increased in *FTO*-deficient mice (Fischer et al., 2009). *FTO* transcript expression has been detected in all of the tissues tested, and is the highest in the brain (McTaggart et al., 2011; Xing et al., 2013), which plays an important role in regulating feed intake and energy expenditure, commensurate with perceived whole-body energy requirements (Richards and Proszkowiec-Weglarz, 2007). In humans, several single nucleotide polymorphisms (SNPs) of *FTO* that are associated with the body mass index (BMI) have been found, which contribute to obesity and related diseases (Hubacek et al., 2010; Binh et al., 2013). Jia et al. (2012) reported that in chickens, *FTO* is related to glucose metabolism, body weight (BW), and fat content. In pigs, *FTO* mRNA expression increases with increasing BW, and is significantly associated with IMF (Tao et al., 2013). In Italian Duroc pigs, an *FTO* mutation is significantly associated with obesity-related traits (back fat thickness, visible intermuscular fat, and lean cuts) ($P < 0.01$) (Fontanesi et al., 2010). SNP g.167T>G in the 5' flanking region of the pig *FTO* is associated with backfat thickness, abdominal fat weight, and lean meat content in Polish Landrace (Szydlowski et al., 2012). A novel SNP (C1071T) has been

detected in exon 5 of *FTO* using polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis, and DNA sequencing, in five Chinese indigenous cattle breeds, which exhibit significant differences between genotypes (CC,TT, and CT) in backfat thickness and longissimus muscle area (Wei et al., 2011). Fan et al. (2009) found that SNPs c.46-139A>T in intron 1 are significantly associated ($P < 0.01$) with average daily gain (ADG) and the total muscle lipid content. These studies have revealed that *FTO* is important in fatty acid metabolism.

Based on these observations, *FTO* could be considered an important candidate gene for fat deposition traits, and may affect carcass and meat quality traits. However, no study has yet attempted to detect variation in *FTO* in ducks. Therefore, the objectives of the present study were to estimate allele and genotype frequencies in *FTO* polymorphisms, and to determine the effects of *FTO* polymorphisms on carcass and meat quality traits in crossbred ducks.

MATERIAL AND METHODS

Animals

The study was conducted using 95 F_2 offspring of Cherry Valley ducks x Liancheng white ducks. All of the ducks were reared under the same conditions in the Experimental Farm for Poultry Breeding of the Sichuan Agricultural University, and were slaughtered at14 weeks old. All of the experimental procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University.

Samples collection and phenotypic data

Blood samples (1mL) were collected before slaughter to analyze SNPs in exon 3 of *FTO*. The breast muscle (BM) and leg muscle (LM) were divided into five pieces to evaluate meat quality traits.

The carcass traits included LW, CW, SEW, EW, breast muscle weight (BMW), and leg muscle weight (LMW). CW was measured after the removal of feathers. The SEW was measured after removal of the trachea, esophagus, gastrointestinal tract, spleen, pancreas, and gonads. The EW was measured after the removal of the head, claws, heart, liver, gizzard, glandular stomach, and abdominal fat.

Details of the sample collection and meat quality trait measurements are provided in (Lee et al., 2010, 2012). The IMF was measured using Soxhlet petroleum-ether extraction, and pH values were measured using a pH-Star (RMatthaus, Klausa,Germany) at 45 min (pH_{45min}) and 24h (pH_{24h}) after slaughter. MC parameters (L*, lightness; a*, redness; and b*, yellowness) were measured using a photoelectric spectrocolorimeter (CR-300, Minolta Camera Co., Japan). To determine the cooking yield (CY), a cube of muscle was taken from the BM and the LM, weighed, placed in a bag, and incubated in water bath at 100°C for 30 min to reach a central internal temperature of 71°C. The bag was then cooled at room temperature for 30 min and the solid portion was re-weighed. DL were scored based on size-standardized samples that were weighed, suspended in a plastic bag (ensuring that the samples had not been in contact with the bag) at 4°C for 24 h, removed from the bag, gently blotted dry, and weighed. IMF, CY, and DL were all expressed as a percentage of the initial sample weight. In order to minimize error, the same person was assigned to measure the same trait.

Total DNA extraction and primer design

Genomic DNA was extracted from the blood samples using the phenol/chloroform method following standard procedures (Clements et al., 2008), and stored at -20°C for PCR amplifications. All of the RNA samples obtained were qualitatively and quantitatively assessed by 1.5% agarose gel electrophoresis, and their OD 260/280 ratio was evaluated. The concentration of total DNA was determined spectrophotometrically.

Primers were designed according to duck exon 3 of the *FTO* sequence (NW_004676795.1) from GenBank, for the amplification of a 759-bp product. The 759-bp fragment included exon 3 and parts of introns 2 and 3. The sense and antisense primers were 5'-TACCTCCCATTACTCACC-3' and 5'-TATCCCTGTCCATTCCT-3', respectively. The primers were synthesized by GENEWIZ Inc. (Suzhou, China).

PCR amplifications

The PCR consisted of 2 X 12.5 µL *Taq* PCR Master-Mix (2 X PCR buffer, 0.4 mM of each dNTP, 3 mM $MgCl_2$, and 0.1 U/µL *Taq* DNA polymerase), 1 µL of primer (10 µM of each of the sense and antisense primers), 9.5 µL ddH_2O, and 1 µL genomic DNA template, in a 25-µL final reaction volume. The PCR was performed under the following conditions: one denaturation cycle at 95°C for 5 min, followed by 36 cycles at 95°C for 30 s, 48.1°C for 30 s, and 72°C for 1 min, and an extension cycle at 72°C for 10 min. The PCR products were used for direct sequencing by GENEWIZ Inc. The same primer pairs as listed above were used for the sequencing.

Sequence analysis

Polymorphic sites were detected by sequence comparisons using the DNAMAN software (http://dnaman.software.informer.com/). The duck *FTO* mRNA sequence (XM_005017005.1) was used for confirming the exact location of the two SNPs, and for identifying the mutation type. D' (deviation ratio to reveal the degree of deviation) and r^2 (the coefficient of linkage disequilibrium) were evaluated using Haploview 4.2 (http://haploview.software.informer.com/4.2/). If there was no linkage disequilibrium between the two SNPs, then:

$$f(_{G1G2}) = f_{G1}\ f_{G2} \qquad \text{(Equation 1)}$$

If alleles at the two loci were not randomly associated, then there would be a deviation (D) in the expected frequencies. The D, D', and r^2 were evaluated by the following:

$$D = f(_{G1G2}) - f_{G1}\ f_{G2} \qquad \text{(Equation 2)}$$

$$D' = D/\min(f_{G1}f_{G2},\ f_{a1}f_{a2}) \qquad \text{(Equation 3)}$$

$$r^2 = D/f_{G1}f_{G2}f_{a1}f_{a2} \quad \text{(Equation 4)}$$

F_{G1} and f_{a1} represent the frequency of G and A, respectively, in SNP g.387G>A, and f_{G2} and f_{a2} represent the frequency of G and A, respectively, in SNP g.473G>A.

Statistical analysis

The genotype and allele frequencies were assessed, and the Hardy-Weinberg equilibrium of the SNPs was analyzed, by the χ^2 test using the SPSS software. Association analyses were performed using the Least Significant Difference method with General Linear Models (GLMs) in SAS 8.0, and significant differences ($P < 0.05$) are presented as means ± standard errors (SE). Analyses were conducted for each SNP separately. The linear model used was:

$$Y_{ikm} = \mu + C_i + G_k + S_m + E_{ikm} \quad \text{(Equation 5)}$$

where Y_{iklm} is the observation for the trait, μ is the overall population mean, C_i is the effect of the crossbreed combination (I = 1, 2, 3), G_k is the effect of genotype (k = GG, GA, or AA), S_m is a fixed effect that is associated with sex (m = male or female), and E_{ikm} is the random error.

RESULTS

Identification of SNPs and genotyping

The PCR products were 759-bp long (which was consistent with the target fragment), had good specificity, and could be directly sequenced. Exon 3 was identified by BLAST. By comparing them with *FTO* mRNA sequences, two SNPs were located, called g.387G>A and g.473G>A. Both SNPs were genotyped for three genotypes: GG, AA, and AG (Figure 1). After studying the protein sequence, we found that SNP 387 was a synonymous mutation (GT<u>A</u>/GT<u>G</u> both code ^{129}His), whereas SNP 473 was a non-synonymous mutation (A<u>G</u>G/A<u>A</u>G code ^{158}Ser/^{158}Phe, respectively). Phe is an essential amino acid in humans; therefore, the g.473G>A SNP should be further studied to clarify its expression and the effects of this mutation.

Genotype frequency and population genetic indices

The genotype frequency of AA (0.178) at site g.387G>A was lower than those of genotypes AG (0.411) and GG (0.411); the frequency of alleles G and A were 0.615 and 0.385, respectively (Table 1). The frequency of genotype GG for site g.473G>A was 0.757, and that of genotype AG (0.211) was higher than that of genotype AA (0.032). The frequency of alleles G and A were 0.865 and 0.135, respectively. The χ^2 test showed that the population was not at Hardy-Weinberg equilibrium for the polymorphisms at the sites detected.

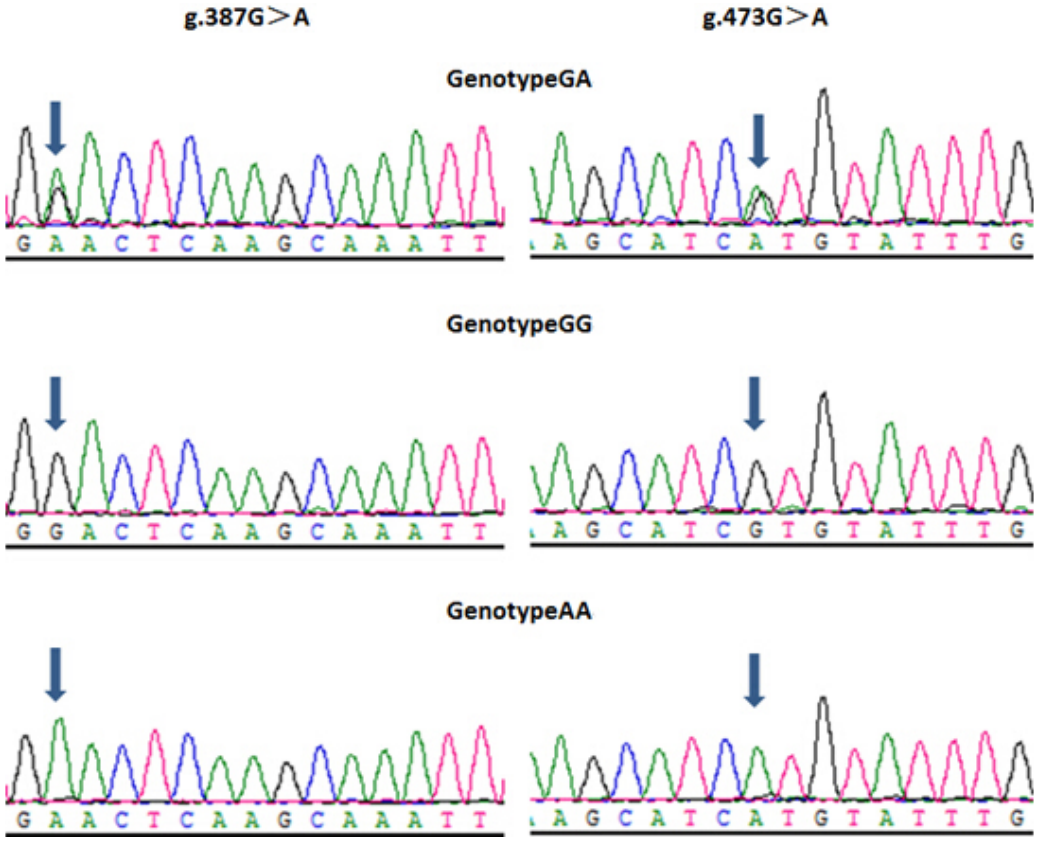

Figure 1. Identification of SNPs and genotyping.

Table 1. Genotype and allele distribution of the *FTO* gene in exon 3 in ducks.

Sequence variant	Genotype frequency			Allele frequency		χ^2
	GG	AA	AG	G	A	
g.387G>A	0.411(39)	0.178 (17)	0.411(39)	0.615	0.385	13.23*
g.473G>A	0.757(72)	0.032 (3)	0.211(20)	0.865	0.135	132.07**

χ^2 (HWE), Hardy-Weinberg equilibrium using the χ^2 test; **significant difference at the P < 0.01 level; *significant difference at the P < 0.05 level.

Haplotype analysis

Three different haplotypes were identified (g.387G>A was the first, followed by g.473G>A) in the duck population: G_1G_2, G_1A_2 and A_1A_2 (Table 2). The frequencies of the three haplotypes were 0.463, 0.305, and 0.232, respectively.

Table 2. Different haplotypes of *FTO* in exon 3 in ducks.

Haplotype	Number	Frequency	D'	r^2
G_1G_2	44	0.463	0.831	0.172
A_1G_2	29	0.305		
A_1A_2	22	0.232		

Association between SNPs in exon 3 of *FTO* and carcass traits

In the present study, *FTO* g.387G>A SNP was significantly associated with all of the carcass traits considered (Table 3). The AA genotype exhibited the highest values for the carcass traits, which were significantly higher than the GG genotype for LW, CW, SEW, EW, and were significantly higher than the GG and GA genotypes for BMW ($P < 0.05$). Therefore, the AA genotype may be positively associated with carcass traits. As with g.387G>A, the AA genotype exhibited the highest carcass trait values in g.473G>A SNP, but was only significantly associated with LMW.

Association between SNPs in exon 3 of *FTO* and meat quality traits

The results of the association analysis of SNP g.387G>A are presented in Table 4. There was a significant association between the *FTO* polymorphisms and IMF, CY, pH_{45min}, and DL in the BM, and IMF and DL in the LM ($P < 0.05$). In the BM, animals with the AA genotype had higher values of IMF, CY, and pH_{45m} than those with the GG genotype ($P < 0.05$), which had a lower DL ($P < 0.05$) than those with the GA genotype. Dominance effects for the DL were significant ($P < 0.05$). In the LM, GG-genotype ducks exhibited a DL of 4.392, which was significantly higher than that of ducks with the AA genotype (3.092; $P < 0.05$). Genotype AA had a significantly higher IMF (12.306) than the genotype GG (9.923) ($P < 0.05$).

Meat quality traits were compared between the genotypes in g.473G>A (Table 5). No significant associations were found between g.473G>A and any of the meat quality traits ($P > 0.05$), but additive effects for CY and pH_{45min} in the BM and LM were significant ($P < 0.05$).

Association between different haplotypes and carcass and meat quality traits

The GLM showed that the haplotypes had a significant effect on all of the carcass traits (Table 6). Furthermore, the analysis showed that the A_1A_2 haplotype had the highest values for all of the carcass traits, which were significantly higher than those of the G_1G_2 haplotype for LW, CW, SEW, EW, and BMW ($P < 0.05$). The A_1A_2 and A_1G_2 haplotypes had significantly higher LMW values than the G_1G_2 haplotype ($P < 0.05$).

There were significant associations between the different haplotypes and the different carcass traits (Table 7). Animals with the A_1A_2 haplotype had higher IMF and CL values in the BM than those with the G_1G_2 haplotype ($P < 0.05$). For the LM, haplotype A_1A_2 had a higher IMF than haplotype G_1G_2 ($P < 0.05$), but its a* value was significantly lower than that of haplotype G_1G_2 ($P < 0.05$).

Table 3. Least squares analysis of different genotypes of g.387G>A and g.473G>A with carcass traits in exon3 of the FTO.

SNP	Genotypes	LW	CW	SEW	EW	BMW	LMW
g.387G>A	GG	1775.385 ± 41.373^{B}	1620.897 ± 38.683^{B}	1486.564 ± 37.432^{B}	1352.897 ± 33.854^{B}	90.200 ± 3.152^{B}	74.239 ± 1.682^{C}
	AA	2039.118 ± 62.666^{A}	1872.353 ± 58.590^{A}	1741.176 ± 56.696^{A}	1594.294 ± 51.277^{A}	106.252 ± 4.774^{A}	90.259 ± 2.548^{A}
	GA	1918.077 ± 41.373^{A}	1751.410 ± 38.683^{A}	1672.821 ± 37.432^{A}	1495.923 ± 33.854^{A}	102.583 ± 3.152^{A}	81.433 ± 1.682^{B}
	Additive effect	-71.346 ± 29.255*	-65.256 ± 27.352*	-70.628 ± 26.468**	-71.512 ± 23.939**	-6.191 ± 2.229**	-3.597 ± 1.189**
	Dominance effect	192.387 ± 69.158**	186.199 ± 64.661**	183.984 ± 62.570**	169.884 ± 56.590**	9.861 ± 5.269	12.424 ± 2.812**
g.473G>A	GG	1841.181 ± 31.571	1679.444 ± 29.404	1550.986 ± 28.621	1418.583 ± 26.090	96.279 ± 2.4420	77.872 ± 1.358^{B}
	AA	2038.333 ± 154.666	1880.000 ± 144.048	1765.667 ± 140.215	1621.000 ± 127.813	104.300 ± 11.855	99.007 ± 6.653^{A}
	GA	1977.750 ± 59.902	1817.750 ± 55.790	1683.900 ± 54.305	1541.500 ± 49.502	103.040 ± 4.591	82.288 ± 2.577^{B}
	Additive effect	-68.284 ± 33.856*	-69.152 ± 31.531*	-66.457 ± 30.692*	-61.458 ± 27.978*	-3.380 ± 2.595	-2.208 ± 1.456
	Dominance effect	128.868 ± 158.329	131.403 ± 147.459	148.224 ± 143.535	140.958 ± 130.839	4.641 ± 12.136	18.927 ± 6.811**

Values are reported as means ± SE. Means with different superscript capital letters or *are significantly different ($P < 0.05$). **are significant different at $P < 0.01$. LM = live weight (g); CW = carcass weight (g); SEW = semi-eviscerated weight (g); EW = eviscerated weight (g); BMW = breast muscle weight (g); LMW = leg muscle weight (g).

Table 4. Least squares analysis of different genotypes of g.387G>A with meat quality traits in exon3 of the FTO.

Muscle	Genotypes	IMF	CY	pH_{45}	pH_{24}	DL	a*	b*	L*
BM	GG	9.923 ± 0.680^{B}	71.732 ± 0.952^{B}	5.798 ± 0.042^{B}	5.430 ± 0.030	3.745 ± 0.319^{AB}	17.663 ± 0.448	4.234 ± 0.462	33.093 ± 0.680
	AA	12.306 ± 1.003^{A}	75.551 ± 1.422^{A}	5.855 ± 0.061^{A}	5.462 ± 0.044	2.825 ± 0.470^{B}	18.115 ± 0.678	4.641 ± 0.700	35.292 ± 1.030
	GA	10.971 ± 0.690^{AB}	74.025 ± 0.939^{AB}	5.786 ± 0.041^{AB}	5.432 ± 0.029	4.122 ± 0.311^{A}	17.611 ± 0.448	4.567 ± 0.462	34.464 ± 0.680
	Additive effect	-0.523 ± 0.484	-0.147 ± 0.669	-0.039 ± 0.029	-0.001 ± 0.021	-0.188 ± 0.222	0.226 ± 0.406	0.203 ± 0.420	1.099 ± 0.617
	Dominance effect	1.859 ± 1.114	2.673 ± 1.152	0.108 ± 0.068	0.031 ± 0.049	-1.108 ± 0.520*	-0.278 ± 0.605	0.129 ± 0.624	0.272 ± 0.918
LM	GG	7.871 ± 0.643^{B}	68.878 ± 0.927	6.633 ± 0.039	6.043 ± 0.038	4.392 ± 0.294^{A}	21.558 ± 0.453	7.008 ± 0.465	36.864 ± 0.615
	AA	8.739 ± 1.010^{AB}	69.604 ± 8.271	6.368 ± 0.060	6.073 ± 0.059	3.092 ± 0.440^{B}	20.715 ± 0.686	7.636 ± 0.704	38.149 ± 0.931
	GA	10.590 ± 0.635^{A}	70.716 ± 5.269	6.322 ± 0.039	6.034 ± 0.038	3.701 ± 0.302^{AB}	21.088 ± 0.453	7.515 ± 0.465	37.406 ± 0.615
	Additive effect	-1.359 ± 0.452	-0.919 ± 0.656	0.006 ± 0.027	0.005 ± 0.027	0.345 ± 0.211	-0.421 ± 0.411	0.314 ± 0.422	0.642 ± 0.558
	Dominance effect	0.491 ± 1.107	-0.193 ± 1.551	0.049 ± 0.066	0.034 ± 0.065	-0.955 ± 0.488	-0.049 ± 0.611	0.193 ± 0.628	-0.100 ± 0.830

Values are reported as means ± SE. Means with different superscript capital letters or *are significantly different ($P < 0.05$). BM = breast muscle; LM = leg muscle; IMF = intramuscular fat content; CY = cooking yield; pH_{45} = pH values at 45min after slaughter; pH_{24} = pH values at 24h after slaughter; DL = drip losses; Meat colour (MC) parameters (L*-lightness, a* -redness and b*-yellowness).

Table 5. Least squares analysis of different genotypes of g.473G>A with meat quality traits in exon3 of the FTO.

Muscle	Genotypes	IMF	CY	pH_{45}	pH_{24}	DL	a*	b*	L*
BM	GG	10.503 ± 0.483	72.570 ± 0.699	5.737 ± 0.030	5.424 ± 0.021	3.883 ± 0.279	17.731 ± 0.332	4.522 ± 0.342	33.875 ± 0.511
	AA	11.577 ± 2.658	72.953 ± 3.400	5.680 ± 0.145	5.417 ± 0.103	2.677 ± 1.357	17.240 ± 1.617	3.030 ± 1.663	32.593 ± 2.486
	GA	12.116 ± 0.947	75.911 ± 1.317	5.887 ± 0.056	5.479 ± 0.040	3.816 ± 0.526	17.764 ± 0.611	4.378 ± 0.629	34.848 ± 0.940
	Additive effect	-0.831 ± 0.531	-1.670 ± 0.745*	-0.075 ± 0.032*	-0.027 ± 0.023	0.034 ± 0.298	-0.245 ± 0.826	-0.746 ± 0.849	0.641 ± 1.269
	Dominance effect	0.241 ± 2.381	-1.287 ± 3.480	-0.131 ± 0.148	-0.035 ± 0.106	-1.173 ± 1.390	0.279 ± 1.027	0.601 ± 1.057	1.613 ± 1.579
LM	GG	9.193 ± 0.493	69.159 ± 0.662	6.319 ± 0.028	6.036 ± 0.028	4.060 ± 0.292	21.293 ± 0.336	7.072 ± 0.339	37.244 ± 0.456
	AA	8.383 ± 2.702	71.653 ± 3.241	6.473 ± 0.138	6.057 ± 0.138	2.330 ± 1.419	19.557 ± 1.633	6.083 ± 1.649	35.367 ± 2.219
	GA	9.029 ± 0.933	72.527 ± 1.288	6.367 ± 0.055	6.079 ± 0.055	4.773 ± 0.550	21.185 ± 0.617	8.372 ± 0.623	37.841 ± 0.839
	Additive effect	0.081 ± 0.529	-1.684 ± 0.734*	-0.024 ± 0.031	-0.022 ± 0.031	-0.356 ± 0.311	-0.868 ± 0.834	-0.494 ± 0.842	-0.938 ± 1.132
	Dominance effect	-0.727 ± 2.408	0.810 ± 3.321	0.129 ± 0.141	-0.001 ± 0.141	-2.087 ± 1.453	0.760 ± 1.037	1.795 ± 1.047	1.536 ± 1.409

Values are reported as means ± SE. Means with different superscript capital letters or *are significantly different ($P < 0.05$). BM = breast muscle; LM = leg muscle; IMF = intramuscular fat content; CY = cooking yield; pH_{45} = pH values at 45min after slaughter; pH_{24} = pH values at 24h after slaughter; DL = drip losses; Meat colour (MC) parameters (L*-lightness, a*-redness and b*-yellowness).

Table 6. Least squares analysis of different haplotypes with carcass traits.

Haplotype	LW	CW	SEW	EW	BMW	LMW
G1G2	1802.386 ± 39.851^{B}	1646.705 ± 37.205^{B}	1510.250 ± 35.996^{B}	1375.681 ± 32.616^{B}	92.470 ± 3.029^{B}	74.925 ± 1.717^{B}
A1A2	1990.909 ± 56.358^{A}	1830.909 ± 52.615^{A}	1699.318 ± 50.906^{A}	1556.863 ± 46.126^{A}	103.401 ± 4.284^{A}	84.787 ± 2.428^{A}
A1G2	1901.034 ± 49.087^{AB}	1730.344 ± 45.827^{AB}	1614.137 ± 44.339A^{B}	1484.482 ± 40.175A^{B}	102.147 ± 3.731A^{B}	82.329 ± 2.115^{A}

Values are reported as means ± SE. Means with different superscript capital letters are significantly different ($P < 0.05$). LM = live weight (g); CW = carcass weight (g); SEW = semi-eviscerated weight (g); EW = eviscerated weight (g); BMW = breast muscle weight (g); LMW = leg muscle weight (g).

Table 7. Least squares analysis of different haplotypes with meat quality traits.

Muscle	Haplotype	IMF	CY	pH_{45}	pH_{24}	DL	a	b	L
BM	GiG2	9.828 ± 0.620^{B}	72.357 ± 0.903^{b}	5.724 ± 0.039	5.439 ± 0.028	4.144 ± 0.357	17.622 ± 0.348	4.420 ± 0.401	33.723 ± 0.652
	A1A2	12.23 ± 0.338^{A}	75.463 ± 1.262^{a}	5.840 ± 0.054	5.471 ± 0.038	3.615 ± 0.499	17.384 ± 0.493	3.782 ± 0.568	33.884 ± 0.922
	A1G2	11.23 ± 0.899^{AB}	73.034 ± 1.099^{ab}	5.775 ± 0.048	5.415 ± 0.033	3.527 ± 0.434	17.839 ± 0.429	4.764 ± 0.495	34.524 ± 0.803
LM	GiG2	7.990 ± 0.606^{B}	69.183 ± 0.859	6.308 ± 0.037	6.030 ± 0.036	4.575 ± 0.372	21.745 ± 0.395^{a}	7.249 ± 0.416	37.320 ± 0.594
	A1A2	9.385 ± 0.828^{AB}	71.706 ± 1.234	6.382 ± 0.051	6.076 ± 0.051	4.361 ± 0.520	19.907 ± 0.558^{b}	7.640 ± 0.589	37.459 ± 0.840
	A1G2	10.71 ± 0.733^{A}	69.740 ± 1.058	6.336 ± 0.045	6.044 ± 0.045	3.381 ± 0.453	20.518 ± 0.486^{ab}	6.800 ± 0.513	37.261 ± 0.732

Values are reported as means ± SE. Means with different superscript capital letters are significantly different ($P < 0.05$). BM =breast muscle; LM = leg muscle; IMF = intramuscular fat content; CY = cooking yield; pH_{45} = pH values at 45 min after slaughter; pH_{24} = pH values at 24 h after slaughter; DL = drip losses; Meat colour (MC) parameters (L*-lightness, a* -redness and b*-yellowness).

DISCUSSION

As a transcriptional coactivator (Wu et al., 2010), *FTO* has been identified as an obesity-related gene (Gerken et al., 2007). Wang et al. (2012) reported that the hypothalamus and cerebellum exhibit relatively high *FTO* mRNA expression levels in male leghorn layers. By sequencing 2.0 kb of the 5' flanking region of porcine *FTO*, the SNP g.1191A >T has been detected, and was significantly associated with the IMF content in a Jinhua x Pietrain F_2 reference population ($P < 0.05$) (Zhang et al., 2009). The results of these studies indicate that *FTO* variants are associated with fatness traits in breeds selected for a low fat content, and can be used as important candidate genes for molecular markers of carcass and meat quality traits.

The several *FTO* SNPs that have been detected are significantly associated with production performance. In commercial pig populations, allele C of the *FTO* SNP g.400C>G in exon 3 is significantly associated with backfat depth, and allele G is significantly associated with muscle traits and has the greatest effect on thoracic tissues (Dvořáková et al., 2012). The *FTO* SNPs c.499G>A and c.453C>A in exon 3 are significantly associated with BW at 35, 70, and 84 days of age in New Zealand rabbits ($P < 0.01$), and the synonymous SNP c.660T>C is significantly associated with BW at 84 days of age, ADG, and the IMF content of the longissimus lumborum in Ira rabbits ($P < 0.01$) (Zhang et al., 2013). Fontanesi et al. (2009) found that the *FTO* SNP g.276T>G in intron 4 is associated with IMF deposition in Italian Duroc pigs, and the feed: gain ratio in Italian Large White pigs. The *FTO* SNP c.594C>G in exon 3 is significantly associated with the ADG and the muscle total lipid content in ISU Berkshire x Yorkshire pigs ($P < 0.01$). In addition, *FTO* polymorphisms are associated with BW and ADG, as well as hot carcass weight (HCW), in crossbred beef cattle ($P < 0.05$) (Rempel et al., 2012), LW at slaughter, CW, and lean weight in paternal half-sib families of Slovenian Simmental cattle (Jevšinek Skok et al., 2011). Our study showed that *FTO* polymorphisms in exon 3 had significant effects on carcass and meat quality traits. To the best of our knowledge, this is the first study that has demonstrated an association between *FTO* coding regions and carcass and meat quality traits in ducks. It is also important to determine whether *FTO* plays a role in the development of other traits in ducks (such as growth traits).

All of the ducks studied deviated from the Hardy-Weinberg equilibrium for the two SNPs sites ($P < 0.05$), which may indicate that artificial selection has been more important than natural selection at these gene sites. The three haplotypes that were found to support the results of a previous study by Lee et al. (2012), which also reported strong linkage disequilibrium (Zhao et al., 2013). Analysis at the protein level has revealed that the substitution of serine by a phenylalanine in SNP 473 would not affect its secondary and tertiary structures, confirming the lack of a significant association with meat quality traits. Carcass and meat quality could be affected by LW (Galián et al., 2009; Choi et al., 2013), which is what we found in SNP 387. The AA genotype of SNP 387 did not differ significantly from the GA genotype for most of the traits, indicating a dominant effect of the A allele.

Joo et al. (2013) showed that the quality of fresh meat can be controlled by the manipulation of muscle fiber characteristics, and that the IMF content is positively correlated with the amount of red muscle fiber but is negatively correlated with the amount of white muscle fiber. It should be noted that there were differences between the BM and LM results, which may indicate that muscle fiber characteristics are also a crucial aspect of duck meat quality. Estimated aerobic capacity data have suggested that the BM and LM are mainly composed of fast and slow muscle fibers, respectively, in poultry (Turner and Butler, 1988). Meat tenderness,

IMF, and cooking loss could be affected by the degradation of cytoskeletal proteins in chickens, which is slower in the LM than the BM (Tomaszewska-Gras et al., 2011). In embryonic stages of the Peking duck, the development of the BM always lags behind that of the LM (Li et al., 2010). Tang et al (2013) reported that the level of myostatin mRNA expression in the LM is higher than that in the BM at 70 days of age in the Wanxi White goose. Therefore, the different effects of *FTO* polymorphisms on meat quality (Tables 4 and 5) between the BM and the LM could be associated with their different physiological characteristic and anatomical positions.

In contrast to previous reports, the synonymous mutation g.387G>A was significantly associated with carcass and meat quality traits; however, there was no significant association between the missense mutation SNP g.473G>A and meat quality traits. It is important to understand the mechanism of action of these polymorphisms on the traits in question, particularly those that do not cause amino acid changes but that may be linked to other, as yet unknown, causative mutations. Based on the significant association we found between SNP g.387G>A of *FTO* and carcass and meat quality traits, we suggest that *FTO* may be an important candidate gene that affects carcass and meat quality traits in ducks.

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