Association of Polymorphisms of Chicken Adipose Differentiation-related Protein Gene with Carcass Traits

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Adipose differentiation-related protein (ADFP) plays an important role in regulating lipid storage and affecting body fat distribution. The objective of the current research was to identify the polymorphisms in chicken *ADFP* gene and their associations with carcass traits. We screened all coding sequence and part of introns (totally 2155 bp) of the *ADFP* gene by using single strand conformation polymorphism method in individuals from Sanhuang chicken, Mountainous Black-bone chicken and a commercial crossbred chicken (totally 427 birds). Three novel single-nucleotide polymorphisms were found in intron 4 (SNP1), intron 5 (SNP2) and exon 8 (SNP3), respectively. The association analyses showed that genotypes of SNP 1 were significantly associated with abdominal fat weight and percentage of abdominal fat ($P < 0.05$), genotypes of SNP2 were significantly associated with breast muscle weight and percentage of abdominal fat ($P < 0.05$), and the main haplotype/haplotypes combination (their frequencies were higher than 5%) were marginally significantly associated with breast muscle weight, abdominal fat weight, and percentage of abdominal fat ($0.05 < P < 0.1$). Haplotype H2 (A-C-A) was an advantageous haplotype for breast muscle weight and H5 (T-C-G) was an advantageous haplotype for weight and percentage of abdominal fat. Conversely, H3H3 (A-T-G/A-T-G) was a disadvantage haplotype combination for accumulation of abdominal fat. These results suggested that polymorphisms of *ADFP* gene were associated with carcass traits, especially with fatness traits. *ADFP* is a potential major gene or in close linkage disequilibrium with the QTL affecting fatness traits in chickens.

Key words: adipose differentiation-related protein, carcass traits, chicken, fat, polymorphism

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Introduction

It is well-known that broilers exhibit an increase in physiological disorders such as over-accumulated abdominal and subcutaneous fat, with increasing growth rate and breast muscle percentage. Redundant fat in the abdomen and under skin leads to the depression of feed efficiency and decrease of meat quality (Zhang *et al.*, 2007). Thus, genetic factors which influence fat deposition have been intensive research topics in poultry (Zerehdaran, *et al.*, 2004; Wang *et al.*, 2006). Previous studies have found over ten candidate genes controlling fat deposition (Qiu *et al.*, 2006; Wang *et al.*, 2006; Wu *et al.*, 2006; Zhang *et al.*, 2006; Lei *et al.*, 2007). Unfortunately, none of them could meet the needs of marker-assisted selection (MAS) of fatness traits in the chicken.

Adipose differentiation-related protein (ADFP) plays a critical role in lipid metabolism and thus affects body fat

distribution and regulation. ADFP, also named adipophilin, is generally upregulated in parallel with stored lipid during lipid droplet formation. It is present on the surface of lipid droplets from the earliest time of their synthesis (Imamura *et al.*, 2002; Wang *et al.*, 2003; Wolins *et al.*, 2005). Exogenous expression of ADFP leads to an increase in neutral lipid mass and over-accumulation of lipid droplets (Magnusson *et al.*, 2006; Prats *et al.*, 2006; Listenberger *et al.*, 2007). ADFP over-expression in cultured hepatocytes simultaneously slows triacylglycerol (TAG) turnover and decreases TAG secretion in very low density lipoprotein (Magnusson *et al.*, 2006). Furthermore, inhibition of *ADFP* expression in mice significantly reduces hepatic TAG accumulation (Chang *et al.*, 2006).

Previous studies have suggested that *ADFP* is a possible candidate gene for human type II diabetes and fat deposition in pigs (Wu *et al.*, 2004; Kim *et al.*, 2005). In this study, we supposed that *ADFP* gene was a candidate gene for MAS of fatness traits in the chicken. The objectives of the current study were to identify the polymorphisms in the exons of *ADFP* gene using the single-strand conformation polymorphism (SSCP) method and to evaluate the

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genetic effects of these polymorphisms on carcass traits, especially fatness traits in the chicken.

Materials and Methods

Resource Populations

Birds (male: female = 1: 1) used in this study were from three chicken breeds: Mountainous Black-bone chicken (MB; $n=66$), Sanhuang chicken (SH; $n=123$) and a commercial crossbred (CC) chicken ($n=238$). The CC breed consisted of five purebreds [S03 ($n=29$), S01 ($n=30$), D99 ($n=30$), S02 ($n=30$) and S05 ($n=29$)] and three crossbreds [S01 $\times$ S10 ($n=30$), S01 $\times$ D99 ($n=30$), S01 $\times$ S05 ($n=30$)] from the Sichuan Daheng Poultry Breeding Company (Zhang *et al.*, 2008). The MB chicken, a native breed in the Sichuan Province, has spotty feathers and black or yellow skin. These chickens have favorable meat quality. The SH chicken is an indigenous breed in the Guangdong Province. It was named because of its yellow plumage, skin and shanks and has a high meat quality. All birds were hatched on the same day, housed on the deep-litter bedding, and moved to the growing pens at 7 wk of age. Birds had access to feed (commercial corn-soybean diets meeting NRC requirements) and water ad libitum.

Phenotyping for Carcass Traits

Prior to sacrificing at 90 days of age, the birds were fasted for 12 h and blood was collected for genomic DNA isolation by phenolic extraction (Salkinoja-Salonen *et al.*, 1989). These DNA samples were used for genotyping the *ADFP* gene. The body weight (BW) and seven carcass traits were then measured. These included: carcass weight (CW), semi-eviscerated weight (SEW), eviscerated weight (EW), breast muscle weight (BMW), leg muscle weight (LMW), abdominal fat weight (AFW) and subcutaneous fat thickness (SFT). The CW was measured on the chilled carcass without feathers. The SEW was measured with the trachea, esophagus, gastrointestinal tract, spleen, pancreas, and gonads removed. The EW was measured on the SEW after the removal of the head, claws, heart, liver, gizzard, glandular stomach, and abdominal fat. The SFT was measured at the caudal spondyle including the skin and fat with a vernier caliper

after dressing. The ratios of AFW to CW were calculated as percentage of abdominal fat (PAF). Intramuscular fat (IMF) was extracted with Soxhlet extraction technique (DeRosa *et al.*, 1983), and expressed as the weight per one hundred gram dry breast muscle.

Amplification and Population Genotyping

Nine primer pairs were designed using OLIGO 6.0 software (Molecular Biology Insights, Inc., Cascade, Co.) to amplify the coding sequence and part of introns of *ADFP* gene according to GenBank database sequences (Accession No: NC_006127, GI: 427237) (Table 1). Primers were synthesized by TaKaRa Biotechnology Co., Ltd (Dalian, China).

PCR amplification was performed in a total volume of 10 μ L containing 0.8 μ L (50 ng/ μ L) template, 5 μ L 2 $\times$ Taq PCR MasterMix (Beijing Tianwei Biology Technique Corporation), and 0.3 μ L of each primer (10 pmol/ μ L). The PCR reactions were carried out at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 50°C (or other annealing temperatures depicted in Table 1) for 30 s, 72°C for 1 min and a final elongation at 72°C for 8 min. PCR products were resolved by 1% agarose gel, and the gel was analyzed on a gel imaging system (Gel Doc™ EQ170-8060). Two μ L of each PCR products was denatured at 98°C for 10 min, cooled on ice for 5 min, and then separated by polyacrylamide gel (12%) electrophoresis at 10 V/cm and 10°C for 10 h. After electrophoresis, the gel was silver stained.

The genotypes were confirmed by direct sequencing using BigDye Terminator Chemistry on ABI 3700 (ABI, Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

Single locus analyses

The genetic effects of the polymorphisms were tested using analysis of GLM in the SAS V8.02 package (SAS Institute Inc., Cary, NC). The following model was used: $Y = \mu + B + S + G(S) + bX + e$, where Y = the traits measured; μ = the population mean; B = the fixed breed/line effect; S = the fixed sex effect; $G(S)$ = the nested effect of genotype within sex; e = the residual random effect; b = the regression coefficients of the linear regressions on X (BW). Regression effect was considered because the BW

Table 1. Detailed information of the primers used for the single nucleotide polymorphism

Primers number	Forward Primer (5'-3')	Reverse Primer (5'-3')	Annealed temperature (°C)	Product length (bp)
1	TGAAAGCCTGATGTTTAGTTA	CCACCCTCGACACGAT	49.2	227
2	CGTGTGCGAGGGTGGTA	GTAAGCACATCTGGATACAAA	51.6	260
3	CCAGAACCTTAAATCAACTAT	TCATGGCTTCTAATCTGT	47.9	247
4	TGTAGCTCTCCCTAGCAAAT	CTTCCCAAGACAGTGTTAATG	51.7	247
5	ACTGTCTTGGAAGTCGT	CATGCTCCCTCTCCACTATGA	51.0	211
6	ACCTGTTTCCTTCCAATTAC	CCAACACTGCAATTTTATACC	51.9	253
7	TGGCTGTATTTTCAGATCGAG	GTTTGCCAATATTACCTTGAC	49.6	215
8	AGCACATTGAGTCAAGAACTC	TCATCCAGAGACTCCTTC A	53.5	250
9	CATCCTCCTTCCAGGAATA	CTGTGCAGTTTACTCGCTATT	53.1	245

of individuals was discrepant largely.

Haplotype analyses

Because the *ADFP* gene was on the Z chromosome, there were no heterozygous females and the genetic status of females were haplotype. Haplotypes of male were reconstructed according to all detected SNPs using the program PHASE 2.0 (Stephens *et al.*, 2001) based on the Bayesian algorithm. The genetic status of males was expressed as the combination of two haplotypes (diplotype configuration). Genetic effects of haplotype were performed with the same model mentioned above.

The data of all carcass traits were verified for normal distribution by Shapiro-Wilks test in SAS V8.02. None of them were normally distributed. Thus, the parameters of CW, SEW, EW, BMW, LMW, AFW and SFT were estimated on the square root scale before they were analyzed with GLM. The PAF and IMF traits were shifted and rescaled to give approximate normal distribution and equal variance, with parameters converted by arcsine transformation. Genotype/haplotype effect was considered significant at $P < 0.05$ for F-test of type III sum of squares. Values were presented as least square means (LSM) $\pm$ standard errors (SE) after LSM and SE were converted to their original status.

Results

Base Variants of Amplified Fragments

PCR amplification products of the third primer pairs, the fifth primer pairs and the last primer pairs presented in Table 1 were polymorphic. Three gel profiles were observed for each of them (Fig. 1). Sequencing results confirmed that SNP1 (A $\rightarrow$ T), SNP2 (C $\rightarrow$ T) and SNP3 (A $\rightarrow$ G) were located in intron 4, intron 5 and exon 8 (at the 398th amino acid position) of *ADFP* gene, respectively (Table 2). SNP3 was synonymous mutation based on the analyses results of DNASTAR V7.10 software package (DNASTAR, Inc. WI. USA).

Genotype and Allele Frequencies

Genotype and allele frequencies for three SNPs were summarized in Table 2. The alleles A of SNP1, C of SNP2 and G of SNP3 were predominant in all male populations. They were also predominant in hens for most populations. Breed MB was non-informative in SNP1 and the line S03, S01 $\times$ D99, S01 $\times$ S05 and D99 were non-informative in SNP3. Thus breed MB was not included in the GLM analyses for genetic effect of SNP1 and line S03, S01 $\times$ D99, S01 $\times$ S05 and D99 were excluded in the GLM analyses for genetic effect of SNP3.

Haplotypes and Their Frequencies

Haplotypes constructed based on 3 SNPs and their

Fig. 1. Polyacrylamide gel electrophoresis picture of the polymorphic fragments.

(1) The profiles AT, AA and TT represented the heterozygote and two types of homozygote in cocks for SNP1, respectively. (2) The profiles A and T showed the two haplotypes in hens for SNP1. (3) The profiles CT, CC and TT represented the heterozygote and two types of homozygote in SNP2, respectively. (4) The profiles C and T represented the two haplotypes in hens for SNP2. (5) The profiles AG, AA and GG represented the heterozygote and two types of homozygote in SNP3, respectively. (6) The profiles A and G represented the two haplotypes in hens for SNP3.

Table 2. Details of the SNPs in *ADFP* gene

SNPs	Allele	Location	Chromosome Position ²	Amino acid position	Breed ⁵	Allele frequency for cocks ³	Genotype frequency ⁴				
							Cocks			Hens	
							BB	AB	AA	B	A
SNP1	A/T ¹	intron 4	33 394 913	/	S03	0.11	0.79	0.21	0.00	0.80	0.20
					S01	0.13	0.73	0.27	0.00	1.00	0.00
					S01×S10	0.17	0.80	0.07	0.13	0.87	0.13
					S01×D99	0.07	0.87	0.13	0.00	0.60	0.40
					S01×S05	0.07	0.93	0.07	0.00	0.93	0.07
					D99	0.00	1.00	0.00	0.00	0.93	0.07
					S02	0.30	0.40	0.60	0.00	0.93	0.07
					S05	0.00	1.00	0.00	0.00	0.87	0.13
					SH	0.08	0.84	0.16	0.00	0.87	0.13
					MB	0.00	1.00	0.00	0.00	1.00	0.00
SNP2	C/T ¹	intron 5	33 395 676	/	S03	0.04	0.93	0.07	0.00	0.80	0.20
					S01	0.30	0.47	0.47	0.06	0.20	0.80
					S01×S10	0.27	0.60	0.27	0.13	0.80	0.20
					S01×D99	0.33	0.47	0.40	0.13	0.67	0.33
					S01×S05	0.23	0.53	0.47	0.00	0.27	0.63
					D99	0.27	0.47	0.53	0.00	0.87	0.13
					S02	0.17	0.80	0.07	0.13	0.00	1.00
					S05	0.14	0.71	0.29	0.00	0.73	0.67
					SH	0.41	0.52	0.13	0.35	0.58	0.42
					MB	0.20	0.78	0.03	0.19	0.21	0.79
SNP3	G/A ¹	exon 8	33 397 903	398	S03	0.00	1.00	0.00	0.00	1.00	0.00
					S01	0.40	0.47	0.27	0.27	0.27	0.73
					S01×S10	0.00	1.00	0.00	0.00	0.87	0.13
					S01×D99	0.00	1.00	0.00	0.00	1.00	0.00
					S01×S05	0.00	1.00	0.00	0.00	1.00	0.00
					D99	0.00	1.00	0.00	0.00	1.00	0.00
					S02	0.43	0.27	0.60	0.13	0.67	0.33
					S05	0.14	0.71	0.29	0.00	0.67	0.33
					SH	0.21	0.66	0.26	0.08	0.71	0.29
					MB	0.23	0.59	0.32	0.06	0.71	0.29

¹Minor allele is in bold. ²Chromosome position based on NCBI *Gallus gallus*-2.1. ³Minor allele frequency for cocks of each population in single locus. ⁴AA=minor allele homozygote, AB=heterozygote, BB=major allele homozygote. ⁵S03, S01, D99, S02 and S05 were purebreds from the Sichuan Daheng Poultry Breeding Company; S01×S10, S01×D99 and S01×S05 were crossbreds from the Sichuan Daheng Poultry Breeding Company; SH=Sanhuang chicken, MB=Mountainous Black-bone chicken.

frequencies in cocks and hens were shown in Table 3. Eight haplotypes were identified in 427 chickens, in which the five most abundant ones (their frequencies were higher than 5%), designated H1 (A-C-G), H2 (A-C-A), H3 (A-T-G), H4 (A-T-A) and H5 (T-C-G), accounted for 97.97% of the cocks and 94.45% of the hens. The haplotype H1 was predominant in all populations. As the *ADFP* gene was on Z chromosome, all diplotypes were from the male. Eighteen diplotypes were obtained based on these eight haplotypes for cocks. Among them, the main diplotypes (their frequencies were higher than 5%) accounted for 81.51% of the cocks.

Marker-Trait Association Analyses

The effect of single polymorphisms and haplotype/diplotypes on all carcass traits (CW, SEW, EW, BMW, LMW, AFW, SFT, IMF and PAF) was estimated with GLM. Genotypes of SNP1 had a significant effect on

AFW (type III $F=2.83$, $P=0.0385$) and PAF (type III $F=3.09$, $P=0.0273$). Genotypes of SNP2 had a significant effect on BMW (type III $F=2.76$, $P=0.0421$) and PAF (type III $F=2.76$, $P=0.0421$). And haplotype/diplotypes had a marginally significant effect on BMW (type III $F=1.94$, $P=0.0530$), AFW (type III $F=1.74$, $P=0.0872$) and PAF (type III $F=1.93$, $P=0.0544$). No effect of genotypes at SNP3 on carcass traits approached a statistically significant level according to total type III probability of significance.

Table 4 presented the summary of the association analyses of the *ADFP* polymorphisms and carcass traits. In SNP1, the AFW and PAF of genotype *A* and *T* (45.55 g, 49.71 g, 2.96% and 3.30%) in hens were great significantly higher than those of *AA* and *AT* (21.13 g, 30.48 g, 1.38% and 2.07%) in cocks, respectively ($P<0.01$). And the birds with genotype *AT* had higher AFW and

Table 3. Haplotypes constructed with 3 single nucleotide polymorphisms (SNP) and their frequencies in cocks and hens

Haplotype	SNP			Frequency (%)		Diploptype	Frequency (%)	Diploptype	Frequency (%)
	SNP1	SNP2	SNP3	Cocks (211) ¹	Hens (216) ¹				
H1	A	C	G	56.22	37.96	H1H1	36.97	H2H6	0.47
H2	A	C	A	9.63	6.02	H1H2	10.90	H3H4	3.79
H3	A	T	G	20.42	28.24	H1H3	16.11	H3H5	0.47
H4	A	T	A	5.68	16.67	H1H5	8.53	H3H7	0.95
H5	T	C	G	6.02	5.56	H3H3	9.00	H3H8	0.47
H6	T	C	A	0.88	0.46	H1H4	2.84	H4H4	1.42
H7	T	T	G	0.85	4.63	H2H2	1.42	H4H5	0.47
H8	T	T	A	0.40	0.46	H2H4	2.37	H4H8	0.47
						H2H5	2.37	H5H5	0.95

¹ The number in parentheses is the observed number in cocks or hens.

PAF than those of *AA* ($P = 0.0052$). In SNP2, the *C* and *T* hens had higher BMW (191.11 g and 192.09 g) and PAF (2.91% and 2.69%) than the *CC* and *CT* cocks (166.33 g, 165.44 g, 1.45% and 1.44%) ($P < 0.01$) and the BMW and PAF of the *CC* and *CT* birds were significantly higher than those of *TT* birds (152.57 g and 0.96%), too ($P < 0.01$). The main diplotypes were marginally significantly associated with BMW, AFW and PAF ($0.05 < P < 0.1$). The H2 haplotype had more BMW than other haplotypes (H1, H3, H4 and H5) and haplotype combinations (H1H1, H1H2, H1H3, H1H5 and H3H3). And haplotype H5 had highest AFW and PAF, compared with other haplotypes (H1, H2, H3 and H4) and haplotype combinations (H1H1, H1H2, H1H3, H1H5 and H3H3). It suggested that haplotype H2 was an advantageous haplotype for BMW and H5 was an advantageous haplotype for weight and percentage of abdominal fat. Conversely, diploptype H3H3 was a disadvantage haplotype combination for accumulation of abdominal fat as its AFW and PAF were lowest compared with other diplotypes and haplotypes.

Discussion

The approach of using a candidate gene, selected on the basis of known relationships between physiology and production traits, is a useful method to investigate associations of gene polymorphisms directly, with certain traits of interest in farm animals (Rothschild *et al.*, 1997; Jiang *et al.*, 2000). Because the role of adipose differentiation-related protein (ADFP) is controlling lipid droplet homeostasis (Brasaemle *et al.*, 1997; Gross *et al.*, 2006), research efforts have been increasingly directed toward the association of SNPs in *ADFP* gene and obesity-related diseases in humans, and fatness traits in livestock. In the East Chinese population, 23 SNPs of *ADFP* gene were detected. The allelic and genotypic frequencies of the SNP located in *ADFP* gene promoter in population with type II diabetes were significantly different with those of the controls. Two missense mutations were typically transferred in two families with type II diabetes (Wu *et al.*, 2004). Moreover, several sequence variations in por-

cine *ADFP* gene were observed from nine Korean native pig breeds (Kim *et al.*, 2005). The biological role and the mapping localization of this gene indicate that the porcine *ADFP* is a possible candidate gene for fat deposition and marbling traits. The chicken *ADFP* gene, located in chromosome Z, contains 7775 bp including 8 exons and 7 introns. Two SNPs were detected in exon 1 (the first amino acid) and exon 8 (the 409th amino acid) (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=427237). But, no association analysis of these two SNPs and carcass traits was reported.

In the current study, three novel SNPs of chicken *ADFP* gene were identified. Although none of them leads to amino acid changes, the efficiency of transcription or translation of the *ADFP* gene might be affected by these mutations (Nielsen *et al.*, 2007). And the degree that mutations affected the efficiency of transcription or translation of the *ADFP* gene might be different. So, the differences of the results of marker-trait association among the three SNPs were the differences of genetic effect brought on by the three SNPs. Interestingly, the results of single locus analyses and haplotype analyses showed that genotypes of SNP1 had significant effect on AFW and PAF, SNP2 had significant effect on BMW and PAF, and haplotype/diploptype had marginally significant effect on AFW, PAF and BMW ($0.05 < P < 0.1$). These results were in accordance with adipose differentiation-related protein's function controlling metabolism of triglyceride and participating in the formation of lipid droplets, which supported our hypothesis that these mutations were associated with chicken carcass traits, especially fatness traits. The phenotype of carcass traits of each breed is not described in this manuscript, for all the three populations in this study were local chicken breeds for meat type and we initially failed to find the significant differences of the average of traits between breeds before we performed the marker-trait association analyses. If there are the differences of the average of traits between breeds, the linkage disequilibrium not always means the true linkage. Thus we only considered the nested effect of genotype within sex in the model used in this study.

Table 4. Associations of *ADFP* polymorphisms and carcass traits in chickens

Marker	Genotype	Number	Traits ¹				
			CW (g)	SEW (g)	EW (g)	BMW (g)	LMW (g)
SNP1	AA	147	1552.24± 12.13	1422.73± 3.55	1175.20± 3.90	171.92± 2.26	264.85± 8.68
	AT	30	1527.85± 25.47	1414.19± 7.48	1156.16± 8.16	166.20± 4.70	260.36± 18.32
	TT	12	1598.37± 100.41	1478.57± 29.25	1208.88± 31.96	176.56± 18.82	258.85± 72.92
	A	148	1528.74± 11.21	1414.00± 3.29	1182.21± 3.63	197.79± 2.25	264.89± 8.06
	T	24	1531.86± 28.09	1418.84± 8.25	1176.61± 9.06	196.81± 5.63	256.62± 20.05
SNP2	CC	130	1521.35± 12.30	1384.93± 3.82	1159.75± 4.09	166.33± 2.23 ^{B2}	255.43± 8.28
	CT	47	1499.32± 19.23	1388.75± 6.01	1155.99± 6.43	165.44± 3.51 ^B	258.71± 13.16
	TT	34	1484.20± 23.58	1367.41± 7.35	1139.06± 7.87	152.57± 4.16 ^C	253.94± 16.12
	C	108	1487.25± 13.09	1374.38± 4.09	1157.01± 4.40	191.11± 2.58 ^A	260.03± 8.99
	T	108	1488.09± 13.45	1380.83± 4.21	1163.61± 4.52	192.09± 2.65 ^A	253.11± 9.11
SNP3	AA	13	1438.52± 41.58	1312.66± 11.90	1098.84± 12.39	159.35± 7.04	243.64± 28.50
	AG	44	1437.22± 23.30	1329.93± 6.77	1115.97± 7.06	162.53± 4.00	243.25± 15.92
	GG	95	1466.64± 16.57	1323.09± 4.74	1116.19± 4.95	156.04± 2.75	244.83± 11.17
	A	51	1423.30± 21.13	1313.87± 6.09	1121.35± 6.40	185.32± 3.87	241.85± 14.35
	G	105	1425.10± 15.41	1323.22± 4.46	1116.51± 4.66	186.65± 2.83	252.20± 10.64
Diplotype	H1H1	78	1555.19± 16.95³	1395.29± 4.76	1172.09± 4.69	168.10± 2.89	258.24± 11.41
	H1H2	23	1499.21± 31.07	1396.39± 9.00	1186.03± 8.92³	175.37± 5.59	250.60± 21.43
	H1H3	34	1508.29± 25.02	1398.30± 7.12³	1165.84± 6.99	164.26± 4.27	266.42± 17.39
	H1H5	18	1492.36± 33.22	1386.82± 9.45	<u>1150.83± 9.26⁴</u>	160.88± 5.64	253.06± 22.71
	H3H3	19	<u>1489.97± 33.35⁴</u>	1380.02± 9.47	1157.69± 9.34	<u>151.15± 5.50⁴</u>	253.79± 22.87
	H1	75	1501.75± 17.25	1387.28± 4.90	1170.79± 4.84	192.18± 3.19	268.93± 12.02³
	H2	13	1492.73± 39.56	<u>1369.54± 11.17⁴</u>	1173.48± 11.12	202.85± 7.54³	<u>245.95± 26.75⁴</u>
	H3	61	1498.24± 19.01	1393.32± 5.42	1170.87± 5.34	196.33± 3.55	254.95± 12.93
	H4	36	1492.11± 25.11	1390.04± 7.16	1177.06± 7.08	192.40± 4.66	255.60± 17.18
	H5	12	1508.38± 41.24	1391.80± 11.67	1161.31± 11.47	185.76± 7.49	253.35± 28.16

¹ CW = carcass weight, SEW = semi-eviscerated weight, EW = eviscerated weight, BMW = breast muscle weight, LMW = leg muscle weight, AFW = abdominal fat weight, PAF = percentage of abdominal fat, SFT = subcutaneous fat thickness, IMF = intramuscular fat content.

² The least square means within a line lacking a common uppercase superscript differ great significantly ($P < 0.01$).

³ The bold numbers represent the advantageous diplotypes.

⁴ Underlined numbers represent the negative diplotypes.

However, usually the problem arises as to whether it is necessary to consider the variation in candidate genes as phased haplotypes or whether a single locus at-a-time approach might perform as well. Quite a few people (Morris and Kaplan, 2002; Horne and Camp, 2004) considered haplotype method has greater statistical power than tests based on the underlying single marker in view of the interaction between non-alleles and linkage disequilibrium between the SNPs. But Clark (2004) reported that the haplotype-based approach may perform worse than the single locus approach if the causal connection between SNP and a phenotype is truly driven by just a single SNP. The association results of single SNP/haplotypes with carcass traits in the current study confirmed the conclusion of Clark (2004).

There was no report showed the QTLs for chicken carcass traits localized at the *ADFP* gene area. However, in chromosome Z, there were 16 QTLs closely associated with growth traits. Especially, two of them affected abdominal fat weight as reported (Abasht *et al.*, 2006). The data from the current study also showed that polymorphisms in chicken *ADFP* gene were significantly

associated with BMW, AFW and PAF. *ADFP* is a potential major gene or in close linkage disequilibrium with QTL affecting fatness traits in chickens.

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Table 4. (Continuation) Associations of *ADFP* polymorphisms and carcass traits in chickens

Marker	Genotype	Number	Traits ¹			
			AFW (g)	SFT (mm)	IMF (%)	PAF (%)
SNP1	AA	147	21.13± 1.36 ^{C2}	3.17±0.08	4.03±0.23	1.38±0.10 ^{C2}
	AT	30	30.48± 3.49 ^B	3.23±0.17	4.44±0.48	2.07±0.25 ^B
	TT	12	27.87±13.85 ^{ABC}	3.41±0.53	4.26±1.99	1.79±0.97 ^{ABC}
	A	148	45.55± 1.85 ^A	4.33±0.10	4.96±0.24	2.96±0.31 ^A
	T	24	49.71± 4.89 ^A	4.56±0.21	5.33±0.60	3.30±0.35 ^A
SNP2	CC	130	21.31± 1.41	3.21±0.09	4.03±0.23	1.45±0.10 ^{B2}
	CT	47	21.26± 2.24	3.12±0.12	3.84±0.30	1.44±0.16 ^B
	TT	34	14.45± 2.30	3.10±0.21	4.21±0.33	0.96±0.17 ^C
	C	108	43.58± 2.16	4.34±0.11	4.68±0.24	2.91±0.16 ^A
	T	108	41.04± 2.15	4.28±0.11	4.11±0.23	2.69±0.15 ^A
SNP3	AA	13	14.88± 3.97	3.21±0.09	4.03±0.23	1.45±0.10
	AG	44	17.62± 2.39	3.12±0.12	3.84±0.30	1.44±0.16
	GG	95	20.19± 1.77	3.10±0.21	4.21±0.33	0.96±0.17
	A	51	38.01± 3.12	4.34±0.11	4.68±0.24	2.91±0.16
	G	105	41.87± 2.37	4.28±0.11	4.11±0.23	2.69±0.15
Diplotype	H1H1	78	19.33± 1.69	3.20±0.11	3.99±0.29	1.30±0.12
	H1H2	23	19.36± 3.31	3.36±0.21	3.81±0.42⁴	1.30±0.24
	H1H3	34	22.43± 2.73	3.19±0.15	4.00±0.36	1.56±0.20
	H1H5	18	31.97± 4.36	3.09±0.23	4.02±0.49	2.24±0.32
	H3H3	19	18.63± 3.38⁴	3.05±0.26⁴	3.92±0.42	1.18±0.24⁴
	H1	75	43.70± 2.60	4.24±0.14	4.68±0.29	2.89±0.19
	H2	13	35.86± 5.51	4.36±0.36	5.53±0.61	2.35±0.39
	H3	61	42.65± 2.83	4.23±0.14	3.97±0.28	2.65±0.20
	H4	36	39.20± 3.62	4.26±0.19	4.18±0.35	2.59±0.26
	H5	12	50.30± 6.73³	4.56±0.30³	5.57±0.71³	3.42±0.49³

¹ CW=carcass weight, SEW=semi-eviscerated weight, EW=eviscerated weight, BMW=breast muscle weight, LMW=leg muscle weight, AFW=abdominal fat weight, PAF=percentage of abdominal fat, SFT=subcutaneous fat thickness, IMF=Intramuscular fat content.

² The least square means within a line lacking a common uppercase superscript differ great significantly ($P<0.01$).

³ The bold numbers represent the advantageous diplotypes.

⁴ Underlined numbers represent the negative diplotypes.

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