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Identification and Association of the Single Nucleotide Polymorphisms in Calpastatin (*CAST*) Gene with Carcass Traits in Chicken

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Abstract: Calpastatin (CAST) is a naturally occurring protein that inhibits normal tenderization of meat as it ages postmortem. The aim of this study was to investigate the effect of *CAST* gene polymorphisms on chicken carcass traits. The researchers screened CAST Single Nucleotide Polymorphisms (SNP) in 359 meat type quality chickens from 5 commercial pure lines (S01, S02, S03, S05 and D99; developed from Chinese local breeds), 3 crossbreeds (S01×S05, S01×S10 and S01×D99) and 4 native breeds from Guangdong (Fengkai Xinghua chicken, Huiyang Huxu chicken, Qingyuan Ma chicken) and Guangxi province (Xiayan chicken) in China. Three SNPs (36127T>C, 37752A>T and 37868G>A) were detected by Single Strand Conformation Polymorphism (SSCP) method and DNA sequencing. The linkage disequilibrium analyze found that only 37868G>A SNP in Hardy-Weinberg equilibrium yet one SNP can not compose haplotype, therefore these three SNPs should be analyzed separately rather than as haplotypes. Association analysis showed that the 37752A>T genotypes were significantly associated with Body Weight (BW), Carcass Weight (CW), Breast Muscle Weight (BMW) and Leg Muscle Weight (LMW). The results suggest that CAST SNP is significantly associated with carcass trait in the twelve studied populations and could be useful in selection for changing meat quality in chicken. Further investigations on more chicken populations with larger sample size are needed to confirm this conclusion.

Key words: *CAST* gene, carcass traits, chicken, halplotype, polymorphism, China

INTRODUCTION

The main characteristics in the eating quality of meat are the appearance, color, taste, fat content, texture and tenderness. However, the meat quality is affected by postmortem tenderization (Taylor *et al*., 1995). Some previous researches provided evidence that the calpains had an important role in postmortem tenderization, taking charge of >90% of the tenderization during this period (Taylor *et al*., 1995) while one study has implicated that the calpain proteolytic system was a major cause of muscle protein degradation and then affected postmortem tenderness (Koohmaraie *et al*., 2002). However, calpastatin is regarded as the specific inhibitor of the calpains which could decrease the activity or inactivity during in postmortem muscle (Boehm *et al*., 1998).

Previous studies have defined Restriction Fragment Length Polymorphisms (RFLP) for the bovine calpastatin locus (Bishop *et al*., 1993). To date, several markers have been developed at the *CAST* gene (Barendse, 2002). Although, the lacking relationship of RFLP at the bovine calpastatin locus with calpastatin activity and meat tenderness was studied (Lonergan *et al*., 1995), a SNP at the CAST was significant for tenderness score in two cattle populations with diverse genetic backgrounds and markers at the *CAST* genes are capable to identify animals with the genetic potential to produce meat (Casas *et al*., 2006). The CAST SNP allele C was associated with shear force across days of postmortem aging ($p = 0.005$) was obtained. These studies have shown an association of individual markers at CAST with meat tenderness in beef cattle (Schenkel *et al*., 2006). The Ser66Asn in calpastatin gene are associated with meat quality traits in pigs was reported (Ciobanu *et al*., 2004). A SNP site (35193G>T) in chicken CAST coding region was detected which caused amid acid Tyrosine (Tyr) changed into Aspartic acid (Asp) and the results of association analysis showed that the CAST polymorphism was associated with the muscle fiber density and diameter ($p<0.05$) (Liu *et al*., 2008). However, there were several obvious shortages should be proposed and needed to improve. Firstly, the exon regions of *CAST* gene covered were little when they designed the primer pairs; secondly, population size of the experimental numbers should be expanded in order to screen more

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single nucleotide polymorphisms or obtain a more convictive experimental result; third and foremost, the fixed effect model was used improperly and the family effect had not been taken into consideration. Although, there were adequate investigations of *CAST* gene in beef, few in chickens. Thus, the purpose of this study was to assess the association of Single Nucleotide Polymorphisms (SNP) in the *CAST* gene with carcass quality traits in 359 meat type quality chickens.

MATERIALS AND METHODS

Resource populations: In total, 359 meat type quality chickens (with a male/female ratio as 1:1) were used. These samples included 5 commercial pure lines developed in form of pureline selection by Sichuan Dahen poultry breeding company using local breeds in Sichuan provinces in China (granted the pureline certificate issued by Sichuan province government), 3 crossbreeds (S01×S05, S01×S10 and S01×D99) and 4 Chinese domestic chicken breeds originated from the local breeds in Guangdong (FX, HH, QM) and Guangxi provinces (GX) in China. Two-way cross S01×S05, S01×S10 and S01×D99 are commercial crossbreeds. The appearance of purelines S01, S05 and D99 is yellow partridge plumage with blue shanks and white skin of S02 with yellow partridge plumage with black shanks and black skin and of S03 with yellow plumage with yellow shanks and white skin. The FX, HH, QM and QX chickens are named by their yellow plumage, skin and shank and have a high quality of meat. Different breeds were hatched in different incubators under the same condition. All birds had free access to food and water. Commercial corn-soybean diets that met all NRC requirements were provided in the study. From birth to 3 weeks of age, birds received a starter feed (2.90 Megacalories (Mcal) of Metabolizable Energy (ME) kg^{-1} and 20.5 g kg^{-1} of Crude Protein (CP). Birds were fed a grower diet (3.00 Mcal of ME kg^{-1} and 18.5 g kg^{-1} of CP) from 4-6 weeks of age and were transferred to the growing pens at the age of 7 weeks. Before slaughter, blood was collected and the genomic DNA was isolated by the standard phenol/chloroform method. For each population, 25-36 chickens were randomly sampled for collecting blood and slaughtering.

Phenotypic measurements: Birds were raised in cage according to the conventional program of commercial broilers. Traits were measured at 90 days age including Body Weight (BW) of the live birds after 12 h with no access to feed, Carcass Weight (CW), Semi-Eviscerated Weight (SEW), Eviscerated Weight (EW), Breast Muscle Weight (BMW), Leg Muscle Weight (LMW), Abdominal Fat Weight (AFW). The CW was measured on the chilled carcass removed feathers. Semi-eviscerated weight was measured on the carcass removed trachea, esophagus, gastrointestinal tract, spleen, pancreas and gonad. Eviscerated weight was measured on the semi-eviscerated weight after removal of head, claws, heart, liver, gizzard, glandular stomach and abdominal fat. Subcutaneous fat thickness was measured at the caudal spondyle including the skin and fat width with a vernier caliper after dressing. The ratios of each of the above traits to CW were calculated as Eviscerated Percentage (EP), Semi-Eviscerated Percentage (SEP), Breast Muscle Percentage (BMP) and Leg Muscle Percentage (LMP), respectively.

PCR-SSCP screening for CAST mutations: According to the reported result (Liu *et al*., 2008), the researches found that Liu only found one variation site in *CAST* gene through her scan region, so in this study five primer pairs were designed referred the report of Liu and complementally three other primer pairs designed to

Table 1: Primers used for screening the single nucleotide polymorphisms in the *CAST* gene

Primer no.	Primer (5'-3')	Annealing temperature (°C)	Product length (bp)	Primer location
1	F: AATACAGGGTCACATCG	56	239	36044
	R: AAAGAAACATTCCCTGA			36253
2	F: AAACGAGAAGGTAGCC	55	291	37577
	R: CTGGTATCTTTGGAAGACATA			37867
3	F: CCAAAAGTAGATGAACATTCT	48	249	37743
	R: GCTTCTATTAATTCCTACCT			37992

Table 2: Primers used for screening the single nucleotide polymorphisms in the *CAST* gene used in the study of Liu *et al.* (2008)

Primer no.	Primer (5'-3')	Annealing temperature (°C)	Product length (bp)	Primer location
1	F: ATGAAAAAATAAGGAGAGGTA	46.9	247	35669
	R: AGTAAAAGATTGCGTG	-	-	35900
2	F: CAAATGTAGCCAAGTCGAA	49.0	254	36550
	R: TTTATTTTTTAATGGAGCAAG			36783
3	F: AGCAGTGTTGAATGTATGATA	46.4	217	38233
	R: GACCAGAAAAAGTTTTATAG			38430
4	F: GGGAATGTCTGTGACTATGTC	49.4	254	39230
	R: ATGGCACAGAGAGAAGTGA			39465
5	F: TGAGTCACCCATACCACGTA	51.8	216	41881
	R: TTGAATGAACAGCAGCACTAT			42076

amplify CAST exons containing variant regions according to the sequence of Gallus gallus CAST (accession No. NC_006127.2). The primer pairs for target fragment were shown (Table 1 and 2) and synthesis was completed by the TaKaRa Biotechnology Co., Ltd (Dalian, China). The PCR reaction was performed with the following condition; one cycle of denaturation at 94°C for 6 min followed by 35 cycles at 94°C for 45 sec, 56°C for 45 sec, 72°C for 1 min and ended with an extension cycle at 72°C for 8 min. Total 10 μL reaction volume included 0.8 μL DNA template (50 ng μL^{-1}), 5 μL 2×Taq PCR MasterMix (Beijing TIAN WEI Biology Technique Co., Beijing, China), 3.6 μL ddH_2O and 0.3 μL of each primer (10 pmol μL^{-1}). PCR products were separated on 1% agarose gel and were visualized on gel imaging system (Gel DocTMEQ170-8060) and photographed. The 3 μL PCR products was denatured at 98°C for 10 min then were placed on ice for 5 min. Electrophoresis was run at 100 V cm^{-1} on a 12% polyacrylamide gel electrophoresis for 9-0 h at 20°C. Gels were stained using the silver staining. Individual single strand conformation polymorphism banding pattern was determined under visible light. Samples showing different bands in the gel were further amplified and purified and were sequenced by a commercial sequencing company (Shanghai Yingjun Biology Technique Co., Shanghai, China).

Statistical analyses: All analyses were performed using the Statistical software SAS8.0 (SAS Institute, Inc., Cary, NC). The genotype frequencies of each polymorphism were calculated for deviations from Hardy-Weinberg equilibrium by Chi-square (χ^2) test (significance based on $p<0.05$). Estimate of Linkage Disequilibrium (LD) between SNPs were calculated using D' by Haploview. Association of the genotypes for the SNP in the *CAST* gene with some carcass traits including BW, CW, SEP, EP, BMW, BMP, LMW, LMP, AFW and AFP were evaluated using the GLM (General Linear Models procedures), fitting the following model which included the known SNP genotypes as fixed effects:

$$Y = \mu + G + S + B + f + d(f) + G \times S + G \times B + BW_{12} + e$$

Where:

Y = The dependent variable
μ = The overall mean for the trait
G = The effect of the genotype for the SNP in the *CAST* gene
S = The fixed effect of the sex (hen or cock)
B = The fixed effect of breed

But the family (f) and dam nested within the family (d(f)) as random effect. G×S, G×B as interaction of G×S and G×B and BW at 12 week (BW_{12}) as a linear covariate (except for the percentage traits and the BW traits), μ was population mean and e was the random error. The other interactions of each 2 terms in the model were not significant for any of trait which was not included in the final model. Significant differences between least squares means of the different genotypes were calculated using Duncan's multiple range test ($p<0.01$).

RESULTS AND DISCUSSION

SNPs of the chicken *CAST* gene: The PCR-SSCP method was developed for screening the nucleotide substitutions. Eight target gene fragments were amplified, denatured and then subjected to polyacrylamide gel electrophoresis to find SNPs. No SNPs site was detected using the five primer pairs (Table 1) referred to the results of Liu *et al.* (2008). Three profiles were observed in each gel picture (Fig. 1). Three SNPs, a T/C variant at position nt36127 (located in exon 8), an A/T variant at nt37752 (located in exon 11) and a G/A variant at nt37868 (located in intron 11) were identified in chicken using the other three primer pairs (Table 1).

Linkage disequilibria of the SNP in the chicken *CAST* gene: To further define the haplotype structures of the *CAST* gene, haplotype blocks were analyzed using the Haploview program. According to the four-gamete

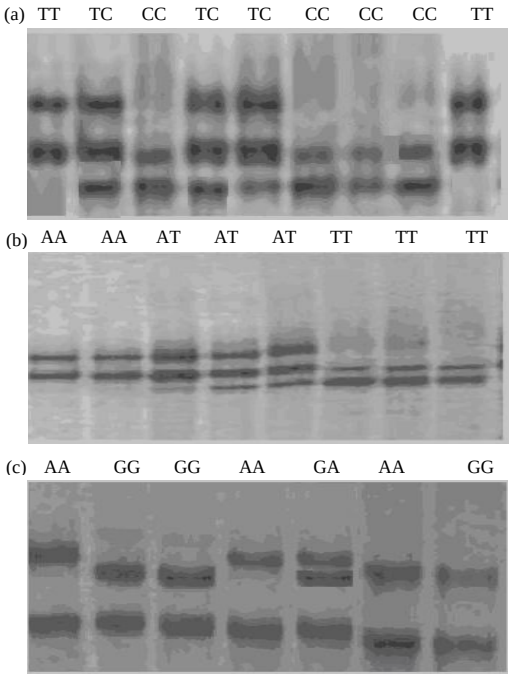

Fig. 1: Genotypes of the three single nucleotide polymorphisms in the *CAST* gene; a) Genotypes of T36127C; b) Genotypes of A37752T and c) Genotypes of G37868A

Table 3: Genotype and allele frequency of SNPs in the *CAST* gene

		T36127C						A37752T						G37868A					
Lines	N	CC	CT	TT	C	T	p[a]	AA	AT	TT	A	T	p[a]	GG	GA	AA	G	A	p[a]
S03	27	0.70	0.11	0.19	0.76	0.24	<0.01	0.56	0.11	0.33	0.61	0.39	<0.01	0.30	0.52	0.18	0.56	0.44	0.790
S01	28	0.43	0.43	0.14	0.64	0.36	0.72	0.25	0.25	0.50	0.38	0.62	0.01	0.25	0.46	0.29	0.48	0.52	0.710
D99	27	0.52	0.37	0.11	0.70	0.30	0.56	0.33	0.56	0.11	0.61	0.39	0.38	0.41	0.44	0.15	0.63	0.37	0.810
S02	28	0.36	0.57	0.07	0.64	0.36	0.15	0.07	0.57	0.36	0.36	0.64	0.19	0.43	0.39	0.18	0.62	0.38	0.390
S05	27	0.48	0.37	0.15	0.67	0.33	0.38	0.41	0.26	0.33	0.54	0.46	0.01	0.26	0.48	0.26	0.50	0.50	0.850
S01×S10	29	0.45	0.38	0.17	0.64	0.36	0.33	0.41	0.21	0.38	0.52	0.48	<0.01	0.34	0.24	0.42	0.47	0.53	0.005
S01×S05	28	0.39	0.46	0.15	0.62	0.38	0.96	0.29	0.25	0.46	0.41	0.59	0.01	0.32	0.25	0.43	0.45	0.55	0.008
S01×D99	25	0.48	0.44	0.08	0.70	0.30	0.81	0.36	0.56	0.08	0.64	0.36	0.28	0.40	0.20	0.40	0.50	0.50	0.003
XH	34	0.65	0.29	0.06	0.79	0.21	0.55	0.29	0.53	0.18	0.56	0.44	0.67	0.41	0.18	0.41	0.50	0.50	<0.010
QYM	34	0.53	0.18	0.29	0.62	0.38	<0.01	0.30	0.35	0.35	0.47	0.53	0.09	0.35	0.41	0.24	0.56	0.44	0.340
HX	36	0.50	0.44	0.06	0.72	0.28	0.52	0.17	0.44	0.39	0.39	0.61	0.70	0.33	0.50	0.17	0.58	0.42	0.860
XY	36	0.44	0.44	0.12	0.67	0.33	1.00	0.33	0.39	0.28	0.53	0.47	0.19	0.28	0.44	0.28	0.50	0.50	0.510

Commercial pure lines-S01, S02, S03, S05 and D99; Commercial crossbreds-S01×S10, S01×S05 and S01×D99; XH-Fengkai Xinghua chicken; QYM-Qingyuan Ma chicken; HX-Huiyang Huxu chicken; XY-Guangxi Xiayan chicken; [a]p-value is the probability of the Chi-square (χ^2) test for the Hardy-Weinberg equilibrium; [b]p-value is the probability of the Chi-square (χ^2) test for genotype frequency; T36127C ([b]p-value: $\chi^2 = 24.2$, p = 0.338); A37752T ([b]p-value: $\chi^2 = 48.44$ p = 0.0009); G37868A ([b]p-value: $\chi^2 = 24.93$ p = 0.3005)

Table 4: Effects of breed, sex and haplotypes on carcass traits

Traits	Breed	Sex
BW	<0.0001	<0.0001
CW	<0.0001	<0.0001
SEP	<0.0001	0.0440
EP	<0.0001	0.3540
BMW	0.0300	<0.0500
BMP	0.0530	0.5280
LMW	0.0450	0.1150
LMP	0.2370	0.0930
AFW	0.0260	0.2160
AFP	<0.0001	0.0001

[a]Overall significance value for an effect of the breed, sex and genotype

testing, the researchers found that there only exist 37868G>A SNP thus one SNP can not compose haplotype, therefore these three SNPs should be analyzed separately rather than as haplotypes.

Genotype and allele frequencies in different chicken populations: Table 3 shows the allele and genotype frequencies of the three SNPs in nine chicken populations. For SNP 36127T>C, the allele C was the major allele and presented the highest allele frequencies among all chicken populations (average = 68.08%). For SNP 37752A>T, the allele frequency of A was higher than that of T in populations S03, D99, S05, S01×S10, S01×D99, XH and XY.

For SNP 37868G>A, the allele frequency of G was lower than that of A in populations S01, S01×S10 and S01×S05. The Chi-square (χ^2) test for the genotype frequency showed that there were statistically significant differences among the twelve chicken populations for 37752A>T (p<0.01) whereas the difference for 36127T>C and 37868G>A was not significant (p>0.05). The Chi-square (χ^2) test was performed to examine the Hardy-Weinberg Equilibrium (HWE). Variant 36127T>C was significantly deviated from HWE in samples S03 and QYM, variant 37752A>T in samples S03, S01, S05, S01×S10 and S01×37868G>A in samples S01×S10, S01×S05, S01×D99 and XH.

Association of breed and sex with carcass traits: The effects of breed and sex on carcass traits in chickens were analyzed. The significance of main effects of breed and sex on chicken carcass traits is shown in Table 4, respectively. The breeds differed in BW, CW, SEP, EP and AFP (p<0.01) and in BMW, LMW and AFW (p<0.05) while not for BMP and LMP (p>0.05). The sex differed in BW, CW and AFP (p<0.01) and in SEP and BMW (p<0.05) while not for EP, BMP, LMW, LMP and AFW (p>0.05).

Associations of CAST SNP with carcass traits: The results of association analysis by using the GLM between the *CAST* gene polymorphisms and carcass traits were shown in Table 5. For 36127T>C, LMW of chickens with the CC genotype were significantly higher than those chicken with the TT genotypes (p<0.05). No significant difference was detected for other carcass traits.

For 37752A>T, BW, CW and LMW of chicken with the AA and TT genotype were significantly higher than those with the AT genotypes, respectively (p<0.05). The BMW of chicken with the AA genotype was significantly higher than those with the AT and TT genotypes (p<0.05), respectively. No significant difference was detected for other carcass traits.

For 37868G>A, LMW of chickens with the AA and GG genotype were significantly higher than those chicken with the GA genotypes (p<0.05), respectively. No significant difference was detected for other carcass traits. The researchers used the conservative bonferroni procedure, one of the multiple testing corrections, to adjust p-values for the genotypes of each SNP (Bender and Lange, 2001) and found that only part of the carcass traits including BW (p = 0.0014), CW (p = 0.0015), LMW (p = 0.0421) and BMW (p = 0.0041) for 37752A>T site reached a significant level. However after the bonferroni testing, no result for the other two SNPs was significant with a large false positive rate >p-value cutoff (p<0.05).

Table 5: Least square mean carcass traits by genotype of chicken *CAST* gene for each locus

		Traits									
Sites	Genotypes	BW	CW	SEP	EP	BMW	BMP	LMW	LMP	AFW	AFP
T36127C	CC	1597±26	1442±29	88.53±0.21	73.21±0.21	172±3	13.51±0.12	246±5^{a}	19.05±0.15	30.65±1.60	2.40±0.11
	CT	1583±30	1425±34	88.44±0.25	73.33±0.25	167±4	13.31±0.14	246±6^{a}	19.23±0.17	34.85±1.86	2.60±0.13
	TT	1580±51	1388±57	89.01±0.42	72.99±0.42	169±7	13.54±0.24	234±11^{b}	18.49±0.29	34.37±3.13	2.60±0.23
A37752T	AA	1674±35^{a}	1519±39^{a}	88.84±0.29	73.22±0.29	180±5^{a}	13.54±0.16	253±7^{a}	18.80±0.20	35.04±2.15	2.56±0.15
	AT	1507±33^{b}	1338±36^{b}	88.18±0.27	72.71±0.27	161±4^{b}	13.49±0.15	229±7^{b}	18.94±0.19	33.37±2.00	2.70±0.14
	TT	1579±35^{a}	1399±39^{b}	88.96±0.29	73.60±0.29	166±5^{b}	13.33±0.16	243±7^{a}	19.03±0.20	31.47±2.14	2.34±0.16
G37868A	AA	1612±37	1473±40	88.79±0.30	72.92±0.30	170±5	13.29±0.17	247±8^{a}	19.05±0.21	34.91±2.26	2.65±0.16
	GA	1558±33	1373±36	88.77±0.27	73.22±0.27	167±4	13.64±0.15	236±7^{b}	18.77±0.19	31.00±2.00	2.37±0.14
	GG	1590±32	1410±36	88.42±0.26	73.38±0.26	169±4	13.44±0.15	243±7^{a}	18.95±0.18	33.96±1.98	2.57±0.14

The superscripts lacking a common lowercase differ significantly ($p<0.05$). The superscripts lacking a common uppercase differ great significantly ($p<0.01$). BW = Body Weight (g); CW = Carcass Weight (g); EW = Eviscerated Weight (g); SEW = Semi-Eviscerated Weight (g); BMW = Breast Muscle Weight (g); LMW = Leg Muscle Weight (g); AFW = Abdominal Fat Weight (g); % indicates these traits relative to CW; values are shown by the least squares means±standard error

What problem of the chicken industry always faces up to is inconsistency in meat tenderness at the consumer level. Determination of genetic polymorphisms which may relate to chicken tenderness could benefit the industry in two ways. Firstly, identification of a particular genotype could be used as a predictor of chicken tenderness, allowing breeding decisions that would enhance the trait. Secondly, such a marker could be used to predict meat tenderness before slaughter in groups of unrelated animals if the marker detects sequence differences responsible for genetic variation in tenderness. The calpain system, a Ca^{2+} activated protease family, plays an important role in postmortem tenderization of skeletal muscle due to its involvement in the degradation of important myofibrillar and associated proteins (Koohmaraie, 1992) and also is involved in cytoskeletal remodeling, myofibrillar turnover and regulation of muscle growth (Goll *et al*., 2003; Wendt *et al*., 2004). Several isoforms of calpastatin exist due to alternative promoter usage and differential splicing (Parr *et al*., 2001; Raynaud *et al*., 2005).

Previous studies have suggested that genetic variation at the CAST locus contributes to variation in meat tenderness traits (Barendse, 2002) but the data presented here represent the 1st report in the scientific literature of the association of the CAST SNP with meat tenderness.

The purpose of this study was to find functional SNPs by linkage disequilibrium and associational analyses of the SNPs with the economically important trait. There were some successful examples in chicken. Lei *et al*. (2009) identified A17299834G SNP of the *IGF1R* gene associated with carcass traits based on genetic diversity and linkage disequilibrium. Cao *et al*. (2007) also reported the polymorphisms in *Spot14α* gene and identified a significant association between the Spot14α polymorphism and growth traits by linkage disequilibrium. So, some important SNPs or QTL would be found by linkage disequilibrium analysis. In addition, Daly *et al*. (2001) demonstrated haplotype or haplotype block provided a practical solution to resolve some problems such as noisy, unsatisfied and obscured important localization information. The haplotypes generally provided more information content (heterozygosity) than one SNP did (Stephens *et al*., 2001). Thus, both haplotype diversity and the method of SNP selection based on maximizing haplotype diversity were preferred using single SNPs (Huang *et al*., 2003; Zhang *et al*., 2004). However in the study, the researchers did not found two of these SNPs had high linkage disequilibrium through this method there only exist 37868G>A SNP thus one SNP can not compose haplotype. Therefore, three SNPs should be analyzed separately rather than as haplotypes. Researchers analyzed the association of CAST genotypes with carcass traits and found three SNPs (36127T>C, 37752A>T and 37868G>A) which showing associations ($p<0.05$) with partial carcass traits. Carcass traits were affected by genetic, nutritional and environmental factors. In this study, researchers analyzed the association among breed and sex for carcass traits and the results showed that some traits (BW, CW and BMW) were affected not only by genetic but also affected by breed and sex. The BW, CW and AFP were found to be mainly associated with breed and sex, the SEP and EP with breed. Strong interactions between the *CAST* gene and genetic background were detected for associating the *CAST* gene polymorphism with some carcass traits in populations which illustrates the importance of defining gene effects in specific populations before future applications using marker-assisted selection programs.

It should be mentioned that the native chicken breeds (XH, QYM, HX and XY), the five commercial pure lines and three crossbreeds have been subjected to intensive artificial selection on commercial traits such as carcass weight, eviscerated weight and breast muscle weight. Moreover, it is hard to include a term in the statistical

analysis to adjust for relatedness among the individual chickens from the population. Therefore, different chicken populations with various domestication backgrounds and selection histories are needed to verify the genetic effects of the *CAST* gene.

However, the particular concern is that the variation in allele frequency across subgroups of a population to a certain extent reflects a form of confounding known in the genetics literature as population stratification in the case-control studies (Thomas and Witte, 2002). It may cause a certain number of false-positive results and the inflation of the type I error rate. Researchers valued this problem and solved it by establishing the appropriate simulating model in this study and recognized the importance of marker assisted selection programs to define gene effects in specific populations when the strong interactions between the *CAST* gene and genetic background. In the further studies, it may not be a bad idea to use the simple formulas suggested by Lee and Wang (2008) for measuring the potential impacts of population stratification bias to avoid the suspicious of false positive.

CONCLUSION

In this study, three SNPs of *CAST* gene were found in a large cohort of chicken samples and there was an association between *CAST* gene and carcass traits. It is suggestive that CAST may be a potential major gene for carcass traits in chicken. Future study is necessary to discern the regulatory effect of CAST on carcass and meat quality traits in chickens.

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