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Association of *HSPB1* and *CRYAB* SNPs with Chicken Meat Quality and Robustness in Five Lines of Korean Native Chicken

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The clinical–chemical traits of blood serum are important biomarkers that can be used to investigate health and metabolic status, both of which can affect meat quality traits. In this study, the SNPs in the genes *HSPB1* (g. 526A > G; 5'UTR) and *CRYAB* (g. 2471T > C; exon 2) were investigated via an association analysis with meat quality and clinical–chemical traits. In total, 597 native Korean chickens were included in the study. There were no significant associations between the SNP in *CRYAB* and either meat quality or serum traits. On the other hand, the SNP in *HSPB1* was significantly associated ($P<0.05$) with water–holding capacity (WHC), lightness (L*), and yellowness (b*) of the thigh muscle. In addition, two clinical–chemical traits, high–density lipoprotein (HDL–c) and amylase (Amy), were significantly associated with this SNP. Of these, the WHC, L*, and b* values of the thigh muscle have the dominant effects only, while Amy trait exhibits both additive and dominant effects. These results can provide useful information that can facilitate the improvement of chicken meat quality, and the understanding of the relationship between blood metabolism and meat quality.

Key words: candidate gene, clinical–chemical trait, Korean native chicken, meat quality

INTRODUCTION

With the rapid globalization and economic growth in recent years, consumer concerns about food selection have changed dramatically. For example, consumers now consider the nutritional value and sensory attributes of the meat and meat products when making purchasing decisions. The sensory characteristics of meat include meat color, flavor, juiciness, and tenderness, all of which can affect a consumer's decision. Meat tenderness is the most important factor in meat selection, because it primarily affects eating quality and is directly evaluated by consumers (Destefanis *et al*., 2008; Kim *et al*., 2009). The tenderness of meat is mainly affected by the contents of the muscle fibers, as well as their structure and protein restriction enzymes (Calkins *et al*., 1981).

In the case of the Korean native chicken (KNC), the National Institute of Animal Science (NIAS) in Korea has launched a project to conserve five lines of KNC. These lines are mainly divided by feather color (gray, black, red, white, and yellow). KNCs are highly favored by Korean consumers, because of their unique taste and quality. However, a large proportion of the demand for chicken meat is fulfilled by a few fast–growing imported chicken breeds. While approximately 10% of chicken meat is that of KNCs, the Korean government is trying to increase the proportion to 30% within the next few years. However, the KNC breed requires improvement, because they are less productive and provide meat of a less desirable texture than broiler chickens. Therefore, the development of desirable characteristics and the improvement of KNC meat tenderness will be an important component of enabling KNCs to compete in the market against commercial broilers.

Livestock animals are severely affected by environmental stress when kept in a small space. In the case of KNCs, their health deteriorates, with significant negative consequences for their productivity. In this respect, maintenance or improvement of their health status would improve their adaptability to limited and dynamic environment conditions: an important part of overcoming the challenges of global warming. With respect to this problem, the KNC is highly adaptable and thus is capable of surviving severe environmental changes. Finding the causal genetic variants for the differences among the clinical–chemical traits of different chicken breeds will be a useful tool for improving meat tenderness. This can be accomplished efficiently using gene markers linked to meat tenderness (Lim *et al*., 2012). Quantitative trait loci (QTL) and candidate gene, proteomics, and genome wide association studies have been performed to elucidate the mechanisms of meat quality and development (Zhang *et al*., 2008; Guillemin *et al*., 2011; D'Alessandro *et al*., 2012; Sun *et al*., 2013).

The expression of Heat Shock Protein Beta 1 (*HSPB1*) and Crystalline Alpha B (*CRYAB*) genes is induced by expression of the heat shock protein (HSP) 27Kda protein, which is affected by either environmental stress or developmental changes. After slaughter, the

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proteins expressed prior to death are responsible for the inhibiting the apoptosis involved in actin rigor. It has been speculated that muscle cell apoptosis could affect meat characteristics. In this context, the main role of the expressed protein (heat shock protein 27Kda) is to facilitate recovery from damage due to the production of stress proteins (Herrera– Mendez *et al*., 2006; Bernard *et al*., 2007). In fact, many candidate gene studies have found that stress has an effect on the tenderness of meat, especially in bovine meat. In proteomics research, the association of protein production with meat tenderness and meat quality has been established.

The focus of this study was to identify candidate genes or genetic markers that may affect meat quality and animal robustness, and evaluate their potential utility in the improvement of the meat quality.

MATERIALS AND METHODS

Animals and the measurement of phenotypic traits

Eighty–eight parents (G0) of five Korean native chicken lines, consisting of 15 sires and 73 dams, were mated to produce 597 offspring (G1). A within–line mating design was used in this study. The birds were reared under the same feeding and control environment system, and slaughtered using the same procedure in same environment, provided by the National Institute of Animal Science, Republic of Korea. Of these offspring, 593 G1 individuals were used to measure the traits in this study. The G1 offspring were divided into 109 gray (G), 90 black (L), 135 red (R), 125 white (W), and 134 yellow (Y) lines based on their plumage colors. This study was conducted according to the recommendations of "The Guide for the Care and Use of Laboratory Animals," published by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (2012–C–037) in Korea. Genomic DNA was extracted from blood samples collected from the wing vein using the manual extraction method (Miller *et al*., 1988). The extracted genomic DNA concentration was measured using NanoDrop 2000C spectrophotometer (Thermo Scientific, USA), and samples were stored at −20°C until use. The phenotypic traits of meat quality and clinical–chemical traits were measured in a previous study (Jung *et al*., 2015).

Genotype analysis

The heterogeneous single nucleotide polymorphisms (SNPs) in *HSPB1* and *CRYAB* were filtered from the KNC SNP annotation database. Direct sequencing of the candidate genes was performed in order to validate the variations in the genes. A forward and reverse primer pairs were designed to include the target SNP areas of each gene (Table 1).

The PCR mixture contained 10 pmol of primer pair, 50 ng of gDNA, 1× buffer, 1.5 mM of $MgCl_2$ buffer, 0.2 mM of dNTPs, and 2 units of HS Taq polymerase (GenetBio, Korea). The volume was then adjusted by adding 20 μL of distilled water. The size of each amplicon was confirmed via electrophoresis on 2% agarose gels with a 100–bp standard marker. The genotype of each gene was identified using the restriction fragment length polymorphism (RFLP) method.

The reaction was performed in a 20 μL reaction volume composed of 1 unit of restriction enzyme, 15 μL of PCR product, 1× buffer, and 5 μL distilled water. The reaction conditions were as follows: a 20 min incubation at 65°C for *HSBP1* and a 16 h incubation at 37°C for *CRYAB*. The sizes of restricted fragments were confirmed via electrophoresis on a 3% agarose gel with a 100 bp standard marker.

Statistical analysis of phenotype data and association tests

The ascertainment of normality and the descriptive statistical analysis of meat quality and clinical–chemical traits were carried out using MINITAB® Release 14 (MINITAB Inc., USA). The parentage tests of the SNPs were confirmed using the CRI–MAP program.

We applied a linear mixed–effects model to the SNP association analyses of the genes and traits, with simultaneous consideration of the familial structure of the KNC resource population:

$$Y_{ijklm} = \mu + S_i + B_j + L_k + G_l + b1CW_{ijklm} + A_{ijklm} + \varepsilon_{ijklm} \quad \textbf{(MODEL 1)}$$

where, Y_{ijklmn} represents the measured phenotype; μ represents the population mean, S_i is the fixed effect of ith sex, B_j is the fixed effect of jth batch (two levels), Lk is the fixed effect of kth line (five levels), G_l is the fixed effect of lth genotype (three levels) of *HSPB1* or *CRYAB*, b_l is a regression coefficient, CW_{ijklm} is the covariate for the carcass weight, A_{ijklm} is the random additive polygenic animal effect, and ε_{ijklm} is the random residual effect. The mean and variance of the random additive polygenic animal effects can be defined as $A \sim N(0, \mathbf{A}\sigma_a^2)$, where **A** is based on the relationship matrix computed using the nuclear families in this study, and σ_a^2 is the additive poly-

Table 1. Description of the PCR primers and restriction enzymes used to genotype the SNPs in *HSPB1* and *CRYAB* genes

Gene	Primer sequence	Region	Annealing Tm.	Product size	Restriction Enzyme
HSPB1	F: ATTGACCTTGGGATGTTGGA R: TTCTGACCTCTCCCCTCTCC	g.526 (5'UTR)	57	586	*Taq*I
CRYAB	F: AATGCTGTGCATGAATGGAA R: GATGGGCAATCCCTGTTATG	g.2471 (exon 2)	50	840	*Bss*SI

genic variance. The mean and variance of the residual random effect of individuals can be defined as: $\varepsilon \sim N(0, \mathbf{I}\ \sigma_a^2)$, where **I** is the identity matrix and σ_a^2 is the residual variance.

The significance of the fixed effects in **MODEL 1** was computed using the Wald procedure implemented in ASReml–R. P–values <0.05 were considered statistically significant. To estimate the genotypic values and standard error of each SNP genotype, the predict command in ASReml–R was used (Gilmour *et al.*, 2009). In order to estimate the additive and dominance coefficients of each SNP in *HSPB1* or *CRYAB*, Gl in the **MODEL 1** was replaced with the additive variable $X_{additive}$ (coded as 1, –1 for the two homozygotes, 0 for heterozygote), and the dominance variable $X_{dominance}$ (coded as 0 for two homozygotes, 1 for heterozygote). Then, trait values were regressed onto the additive and dominance coefficients at each SNP marker.

RESULTS AND DISCUSSION

Associations with meat quality traits

In this study we selected a functional candidate gene, *HSPB1*, which may affect the meat quality and clinical–chemical traits of chickens. A specific SNP in *HSPB1*, g.526A>G (5'UTR) has significant dominant associations with the water holding capacity (WHC), lightness (L*), and yellowness (b*) values of the thigh muscle. The ultimate pH (pH2), tended to be significantly associated with *HSPB1* g.526A > G (P = 0.086). However, did not detect any significant association with *CRYAB* g.2471T > C (exon 2) in 593 birds of KNC (Fig 1; Table 2). In particular, the WHC, pH2, L*, and b* traits of the thigh meat can directly affect tenderness.

Previous studies have been reported that increasing the pH in the meat tends to decrease the WHC; consequently, decreasing the WHC is likely to reduce the L*

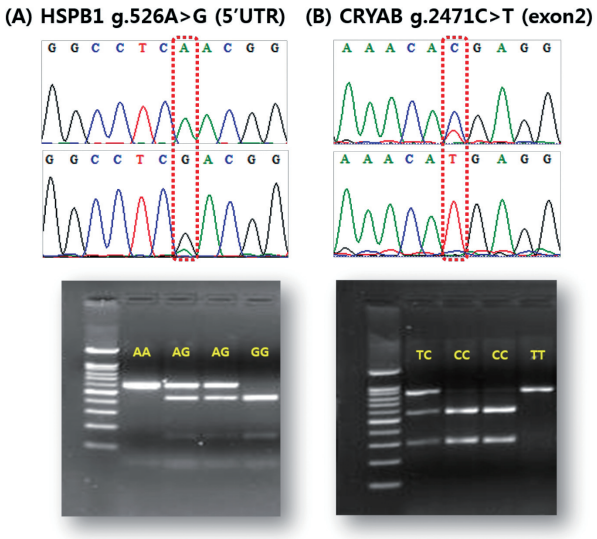

Fig. 1. The evidence of SNPs used to genotype the *HSPB1* (A) and *CRYAB* genes (B).

Table 2. Associations of the SNPs in *HSPB1* and *CRYAB* genes with meat quality traits in Korean native chickens

Gene	Trait	*P–value*	AA	AG	GG	Additive	Dominant
HSPB1	Br_WHC	0.800	64.15 ± 0.86	63.52 ± 0.61	63.56 ± 0.69	–0.33 ± 0.7	0.29 ± 0.57
	Br_H2O	0.717	4.29 ± 1.1 E–03	4.29 ± 8.0 E–04	4.29 ± 0.01	0 ± 0	0 ± 0
	Br_cProtein	0.247	24.35 ± 0.05	24.44 ± 0.03	24.43 ± 0.04	0.05 ± 0.04	–0.04 ± 0.03
	Br_cFat	0.062	0.8 ± 0.01	0.82 ± 0.01	0.84 ± 0.01	0 ± 0.01	–0.01 ± 0
	Br_cAsh	0.153	1.16 ± 0.01	1.16 ± 0.01	1.19 ± 0.01	–0.01 ± 0.01	–0.01 ± 0
	Br_Collagen	0.674	1.93 ± 0.04	1.96 ± 0.03	1.99 ± 0.03	0 ± 0.03	–0.02 ± 0.03
	Leg_WHC	**0.010**	**62.34 ± 0.61**	**60.93 ± 0.41**	**62.66 ± 0.46**	**–1.57 ± 0.53**	**–0.16 ± 0.41**
	Leg_H2O	0.225	74.99 ± 0.12	74.93 ± 0.09	75.15 ± 0.11	–0.13 ± 0.1	–0.08 ± 0.08
	Leg_cProtein	0.443	22.01 ± 0.13	21.94 ± 0.09	21.79 ± 0.11	0.04 ± 0.11	0.1 ± 0.09
	Leg_cFat	0.231	1.07 ± 0.02	1.11 ± 0.01	1.12 ± 0.02	0.01 ± 0.02	–0.02 ± 0.01
	Leg_cAsh	0.117	1.11 ± 0.01	1.09 ± 4.9E–03	1.11 ± 0.01	–0.01 ± 0	0 ± 0
	Leg_Collagen	0.875	2.03 ± 0.03	2.05 ± 0.02	2.05 ± 0.03	0.01 ± 0.03	0 ± 0.02
	Br_Cooking loss	0.713	20.87 ± 0.29	20.59 ± 0.19	20.67 ± 0.22	–0.17 ± 0.25	0.09 ± 0.2
	Br_pH1	0.804	1.81 ± 0.01	1.81 ± 0.01	1.81 ± 0.01	0 ± 0.01	0 ± 0.01
	Br_pH2	0.114	1.74 ± 0	1.76 ± 0.01	1.76 ± 0.01	0 ± 0	–0.01 ± 0
	Br_Delta_pH	0.365	0.38 ± 0.04	0.37 ± 0.03	0.32 ± 0.03	0.02 ± 0.03	0.02 ± 0.02
	Br_L*	0.384	59.25 ± 0.48	59.92 ± 0.33	60.08 ± 0.38	0.24 ± 0.4	–0.41 ± 0.32
	Br_a*	0.414	1.95 ± 0.02	1.99 ± 0.02	1.97 ± 0.02	0.02 ± 0.02	0 ± 0.01
	Br_b*	0.986	21.28 ± 0.19	21.30 ± 0.15	21.32 ± 0.16	0 ± 0.13	–0.01 ± 0.11
	Leg_Cooking loss	0.813	29.55 ± 0.54	29.93 ± 0.38	29.86 ± 0.43	0.23 ± 0.44	–0.15 ± 0.36
	Leg_pH1	0.967	6.45 ± 0.04	6.43 ± 0.03	6.44 ± 0.03	–0.01 ± 0.04	0 ± 0.03
	Leg_pH2	0.085	6.03 ± 0.06	6.13 ± 0.04	6.23 ± 0.04	0 ± 0.05	–0.09 ± 0.04

Table 2. Continued

Gene	Trait	*P–value*	AA	AG	GG	Additive	Dominant
	Leg_Delta_pH	0.935	0.26 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0 ± 0.02	0 ± 0.02
	Leg_L*	**0.004**	**48.53 ± 0.41**	**49.32 ± 0.28**	**47.96 ± 0.32**	**–1.07 ± 0.35**	**0.28 ± 0.28**
	Leg_a*	0.746	13.69 ± 0.20	13.72 ± 0.14	13.57 ± 0.16	0.09 ± 0.16	0.06 ± 0.13
	Leg_b*	**0.018**	**20.42 ± 0.16**	**20.53 ± 0.11**	**20.03 ± 0.12**	**–0.3 ± 0.14**	**0.19 ± 0.11**
CRYAB	Br_WHC	0.998	63.67 ± 0.91	63.72 ± 0.64	63.74 ± 0.87	0.01 ± 0.79	–0.03± 0.69
	Br_H2O	0.371	4.29 ± 1.0 E–03	4.29 ± 0.01	4.29 ± 1.0 E–03	0	0
	Br_cProtein	0.761	24.40 ± 0.05	24.42 ± 0.03	24.38 ± 0.05	0.03 ± 0.04	0 ± 0.04
	Br_cFat	0.950	0.82 ± 0.01	0.82 ± 0.01	0.82 ± 0.01	0 ± 0.01	0 ± 0.01
	Br_cAsh	0.059	1.15 ± 0.01	1.17 ± 0.01	1.20 ± 0.01	0 ± 0.01	–0.02 ± 0
	Br_Collagen	0.725	1.98 ± 0.04	1.95 ± 0.03	1.99 ± 0.04	–0.03 ± 0.04	0 ± 0.03
	Leg_WHC	0.354	60.92 ± 0.64	62.03 ± 0.45	62.21 ± 0.62	0.47 ± 0.59	–0.63 ± 0.5
	Leg_H2O	0.126	75.12 ± 0.13	74.93 ± 0.09	75.18 ± 0.13	–0.22 ± 0.11	–0.02 ± 0.09
	Leg_cProtein	0.117	21.95 ± 0.14	21.94 ± 0.11	21.61 ± 0.14	0.15 ± 0.12	0.16 ± 0.11
	Leg_cFat	0.401	1.09 ± 0.02	1.11 ± 0.01	1.14 ± 0.02	–0.01 ± 0.02	–0.02 ± 0.02
	Leg_cAsh	0.340	1.09 ± 7.8E–03	1.11 ± 0.01	1.11 ± 0.01	0 ± 0	0 ± 0
	Leg_Collagen	0.749	2.05 ± 0.03	2.07 ± 0.02	2.04 ± 0.03	0.02 ± 0.03	0 ± 0.02
	Br_Cooking loss	0.646	20.92 ± 0.30	20.67 ± 0.21	20.95 ± 0.29	–0.26 ± 0.28	–0.01 ± 0.24
	Br_pH1	0.249	1.79 ± 0.01	1.82 ± 0.01	1.81 ± 0.01	0.02 ± 0.01	0 ± 0.01
	Br_pH2	0.691	1.76 ± 0.01	1.76 ± 0.01	1.75 ± 0.01	0 ± 0	0 ± 0
	Br_Delta_pH	0.203	0.29 ± 0.04	0.37 ± 0.03	0.39 ± 0.04	0.02 ± 0.03	–0.05 ± 0.03
	Br_L*	0.150	60.65 ± 0.53	59.74 ± 0.37	59.05 ± 0.51	–0.11 ± 0.47	0.79 ± 0.4
	Br_a*	0.395	1.97 ± 0.03	1.98 ± 0.02	1.94 ± 0.03	0.02 ± 0.02	0.01 ± 0.02
	Br_b*	0.429	21.31 ± 0.20	21.28 ± 0.15	21.05 ± 0.19	0.09 ± 0.14	0.12 ± 0.12
	Leg_Cooking loss	0.292	29.37 ± 0.61	29.59 ± 0.43	30.54 ± 0.58	–0.36 ± 0.51	–0.58 ± 0.45
	Leg_pH1	0.740	6.47 ± 0.04	6.47 ± 0.02	6.44 ± 0.03	0.02 ± 0.03	0.01 ± 0.03
	Leg_pH2	0.137	6.23 ± 0.06	6.17 ± 0.04	6.04 ± 0.06	0.03 ± 0.06	0.09 ± 0.05
	Leg_Delta_pH	0.505	0.25 ± 0.02	0.26 ± 0.01	0.30 ± 0.02	–0.01 ± 0.02	–0.02 ± 0.02
	Leg_L*	0.780	48.56 ± 0.36	48.57 ± 0.25	48.27 ± 0.35	0.15 ± 0.33	0.14 ± 0.28
	Leg_a*	0.190	13.42 ± 0.21	13.81 ± 0.15	13.58 ± 0.20	0.31 ± 0.17	–0.08 ± 0.15
	Leg_b*	0.554	20.29 ± 0.14	20.32 ± 0.10	20.14 ± 0.14	0.1 ± 0.13	0.07 ± 0.11

Br: breast muscle; Leg: thigh muscle; cFat: crude fat content (%); cAsh: crude ash content (%); H2O: crude moisture (%); cProtein: crude Protein; L*: CIE lightness value; a*: CIE redness value; b*: CIE yellowness; WHC; water holding capacity (%); pH1: after slaughter 15 min pH; pH2: ultimate pH

and b* values of the meat's color (Qiao *et al*., 2001). Appropriate moisture levels in meat can impart a soft texture and enrich the flavor of the gravy. In addition, the water content of the meat can affect its color, as too much water will cause it to look pale. In addition, the lower moisture content of the dark meat can make it less desirable to consumers. It has also been reported that pH changes the water holding capacity of the meat, and thus alters the color of the meat. In light of these results, the tenderness of meat from cattle is considered to be affected by *HSPB1*, and changes in pH were confirmed to be significantly associated with the expression of *HSPB1*. This study confirmed that high pH in animals of the GG genotype conferred a high WHC value. This may be attributed to the fact that KNC has darker meat than that of broilers, an observation supported by a previous study of KNC meat quality (Jung *et al*., 2011).

The chicken QTLdb (http://www.animalgenome.org/cgi–bin/QTLdb/GG/index) includes QTLs for 10 different traits, including meat color and drip loss traits. The QTLs related to the color of thigh meat were found in GGA1, 2, 11, 26 (Jia *et al*., 2012; Yoshida *et al*., 2013). In these QTL results, the b* value–affected QTLs were found in GGA1 and GGA2; meanwhile the other QTLs in GGA11 and GGA26 were not associated with thigh meat coloration. In addition, similar WHC traits, such as drip loss trait QTLs, were reported in GGA1, 11, and 14 (Nadaf *et al*., 2007; Jia *et al*., 2012). These QTL regions (not including GGA19) mostly affected breast meat coloration, because these studies did not measure traits separately. Therefore, more research into thigh meat color and WHC traits are needed, because *HSPB1* is located on the

Table 3. Associations of the SNPs in *HSPB1* and *CRYAB* genes with the clinical-chemical traits in Korean native chickens

Gene	Trait	*P–value*	AA	AG	GG	Additive	Dominant
HSPB1	Glu	0.448	254.57 ± 2.73	258.23 ± 1.89	256.22 ± 2.14	2.83 ± 2.32	–0.82 ± 1.86
	T_Pro	0.995	4.21 ± 0.07	4.21 ± 0.05	4.2 ± 0.05	0 ± 0.06	0 ± 0.05
	GPT	0.741	2.91 ± 0.04	2.94 ± 0.03	2.91 ± 0.03	0.02 ± 0.03	0 ± 0.02
	Cre	0.589	–0.18 ± 0.03	–0.22 ± 0.02	–0.23 ± 0.02	–0.01 ± 0.02	0.02 ± 0.02
	HDL_c	**0.014**	**100.57 ± 3.80**	**101.51 ± 3.02**	**92.47 ± 3.27**	**–4.98 ± 2.48**	**4.05 ± 2.12**
	T_Cho	0.295	135.77 ± 4.23	138.41 ± 3.15	132.41 ± 3.51	4.32 ± 3.14	1.67 ± 2.65
	GOT	0.736	5.36 ± 0.04	5.37 ± 0.02	5.4 ± 0.03	–0.01 ± 0.03	–0.01 ± 0.02
	Amy	**0.010**	**5.35 ± 0.07**	**5.38 ± 0.05**	**5.13 ± 0.05**	**–0.13 ± 0.06**	**0.1 ± 0.05**
Gene	Trait	*P–value*	CC	CT	TT	Additive	Dominant
CRYAB	Glu	0.734	259.39 ± 3.12	256.43 ± 2.23	256.77 ± 3.01	–1.64 ± 2.67	1.3 ± 2.35
	T_Pro	0.968	4.22 ± 0.08	4.24 ± 0.05	4.23 ± 0.07	0.01 ± 0.06	0 ± 0.05
	GPT	0.830	2.93 ± 0.05	2.95 ± 0.03	2.92 ± 0.05	0.02 ± 0.04	0 ± 0.03
	Cre	0.573	–0.22 ± 0.03	–0.18 ± 0.02	–0.2 ± 0.03	0.03 ± 0.03	–0.01 ± 0.02
	HDL_c	0.900	98.87 ± 4.11	100.76 ± 3.29	100.73 ± 3.95	0.95 ± 2.83	–0.92 ± 2.56
	T_Cho	0.515	135.91 ± 4.44	136.47 ± 3.32	141.38 ± 4.26	–2.17 ± 3.5	–2.73 ± 3.13
	GOT	0.504	5.33 ± 0.05	5.38 ± 0.03	5.42 ± 0.04	0 ± 0.04	–0.04 ± 0.03
	Amy	0.975	5.28 ± 0.07	5.31 ± 0.05	5.31 ± 0.07	0.01 ± 0.06	0 ± 0.05

Glu: Glucose (mg/dl); T_Pro: Total protein (g/dl); Cre: Creatinine (mg/dl); HDL-C: High-density lipoprotein cholesterol (mg/dl); T-Cho: Total cholesterol (mg/dl); GOT: Glutamic oxaloacetic transaminase (IU/L); Amy: Amylase (IU/L)

GGA19, and most previous studies have focused on the breast muscles. This further research is necessary because the thigh and breast muscles can be constructed via different mechanisms.

Moreover, *HSPB1* was not reported to be associated with meat coloration, and the WHC–related QTL and association results only indicated that abdominal fat weight and content were affected by genes in the GGA19 region. In our previous QTL results, we could not identify any QTLs for either meat color or WHC traits in the GGA19 region. QTLs related to thigh meat coloration, WHC, and pH traits were found to be located in GGA8 (thigh L*), GGA10 (thigh Cooking Loss), GGA8, 24 (thigh WHC), and GGA5, 7, 17 (thigh pH). It is possible that the QTL results were affected by the additive effect estimation model, but these association results have dominant effects. Therefore, *HSPB1* requires further study, in order to validate these findings in other breeds or populations. This candidate gene is significantly associated with thigh meat color and WHC traits.

Associations with clinical–chemical traits

The traits for meat tenderness can be affected by environmental stress, a fact that has been established by previous research (King *et al*., 2006). Investigations of the relationships between meat quality and changes in environmental stress have employed the quantification of clinical–chemical traits to determine an animal's health status; these studies will be useful in the development of genetic markers for stress resistance in animals, and for the development of meat quality evaluation methods. Interestingly, in this study, we identified significant associations between the additive and dominance effects of high–density lipoprotein cholesterol (HDL–c) and amylase (Amy) traits, via the SNP in *HSPB1* (Table 3). High levels of HDL–c in the blood serum are positive indicators of animal and human health. Moreover, a high amylase content is indicative of good health, specifically in relation to metabolism (Ueha *et al*., 1971). The birds of the GG genotype had lower HDL–c and Amy values than birds of the AA and AG genotypes. This may indicate that the AA and AG genotypes confer good digestibility and health statuses. Interestingly, the birds of the GG genotype have significantly darker meat and higher pH values than the birds of the AA and AG genotypes.

The clinical–chemical components are factors that affect the likelihood of change in the direct meat quality, and are considered important factors to evaluate using functional studies. However, of these traits the serum can be seen as a particular indicator of nutritional status, and in the case of these traits, factors that can make a difference to the smooth supply of nutrients to the muscles affect the smoothness of the metabolism. In addition, we cannot exclude the possibility that *HSPB1* acts pleiotropically on meat quality and clinical–chemical traits at the same time, as a factor of stress resistance. This is because it acts during stress to restore damaged proteins. Thus, the expression of the gene may affect the generation and modification of the protein, and these changes must be considered in order to fully understand the mechanism, because they provide a variety factors that may drive changes in phenotypic characteristics. However, further research is required to understand the relationship between the characteristics.

Clinical–chemical components have been used as useful biomarkers that can measure the current health

status of animals (diseased or immune). However, chicken serum clinical–chemical traits cannot be used as indicator for health status because it was not established standard yet. However, if change in the various characteristics of the serum can affect the quality of meat and the health of the chickens relative to manage the health of the production side, it is considered to make use for animal breeding as genetic marker. In addition, clinical–chemical traits are a matter of developing breeds with a robustness that can adapt to global warming and the harsh environment of rapid climate change; these concerns are believed to be very important to preparations for future challenges in the livestock industry.

In this study, tests for the association between meat quality and animal clinical–chemical traits were performed using an SNP in *HSPB1*. The results indicate that birds of the GG genotype have dark meat, a tendency to have high pH values, and less HDL–c with Amy than animals of the AA or AG genotypes. With further verifications, these results can provide useful information with which to improve chicken meat quality and our understanding of the relationship between blood metabolism and meat quality.

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