Livestock Science 170 (2014) 1–7

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Association of *MC1R* genotypes with shank color traits in Korean native chicken

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ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form
30 September 2014
Accepted 3 October 2014

Keywords:
Korean native chicken
MC1R
Shank color
SNP

ABSTRACT

A nation-wide conservation project for the Korean native chicken (KNC) was launched in 1994 and has been conducted primarily by the Korean government. As a result, five lines of KNC have been developed, classified mainly by plumage color. When the lines were developed, charcoal gray and dark green shank colors were selected to distinguish them from broiler breeds, which have yellow shank colors. After more than 20 generations of selection with the criteria, the shank colors in KNC still display large color variations. From an economic viewpoint, shank color is a very important trait because different consumer preferences are prevalent in different areas of Korea. In this study, 596 F_1 individuals from five KNC lines were used to investigate shank color variation by using a spectrophotometer. Additionally, four SNPs genotyped from the strong candidate gene for pigmentation, *MC1R*, were genotyped using the Fluidigm Dynamic Array. The *L** (lightness), *a** (redness), and *b** (yellowness) values showed normal distributions, and the heritabilities of these traits were estimated as 0.5, 0.37, and 0.63, respectively. The results also indicated strong line effects for *b**, except for the G (gray) and L (black) lines. Two particular SNPs in the *MC1R* gene, c.212C > T and c.427A > G, were significantly associated with the *b** values of the shank colors. The results suggested that the SNP markers in the study could be used for the selection of KNC individuals with desirable shank colors.

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http://dx.doi.org/10.1016/j.livsci.2014.10.001
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1. Introduction

Intensive efforts have recently been conducted to preserve animal genetic resources, especially the activities of the Food and Agriculture Organization (FAO), and documentation of global animal genetic resources may be found on a website of this organization called DAD-IS (http://dad.fao.org). In Korea, the conservation of domestic animal genetic resources has mainly been conducted by a governmental institution known as the National Institute of Animal Science (NIAS). The conservation of native chicken breeds began in 1992 with the collection of chickens from rural areas. The actual conservation project, titled "Restoration of Korean native chickens," was launched by the Korean Poultry Science Association in 1994. The NIAS has since selected birds based on economic traits and plumage colors for more than 15 years. As a result, five lines of Korean native chicken (KNC) have been developed and registered in DAD-IS under the following names: Baeksaek Jaerae-jong (White), Heuksaek Jaerae-jong (Black), Hoegalsaek Jaerae-jong (Gray), Hwanggalsaek Jaerae-jong (Yellow), and Jeokgalsaek Jaerae-jong (Red).

The above five KNC lines are classified mainly based on plumage color. When the lines were developed, charcoal gray or dark green shank colors were selected to distinguish the KNC from broiler breeds, which have yellow shank colors. However, the shank colors in the five lines exhibit great variation, even after at least twenty generations of selection. Shank color is an important economic trait because dark shank colors are preferred for indigenous chicken breeds in Korea. Consumers expect KNC breeds to have dark shank colors, which differentiates them from commercial broilers with yellow shank colors. Therefore, when consumers compare native chickens with broilers, plumage colors are the first indicator for breed recognition, with shank colors a close second.

In chicken, skin pigmentation such as shank color is related to the levels of carotenoids and melanins. The various skin phenotypes are explained by the combination of the two pigmentation substances with specific genes and unidentified modifiers (Smyth, 1990). The carotenoid pigmentation is affected by carotenoid pigments such as xanthophyll and is responsible for the yellow colors of the skin. In contrast, the melanin pigmentation is affected by melanocytes, which are observed in the dermis and epidermis of the skin.

In previous studies, the Mendelian loci that control shank color pigmentation in chicken have been examined. The autosomal dominant *W* locus is known to be responsible for the dominant white (W^+) and recessive yellow (w) shank colors, which are related to carotenoid pigmentation. The sex-linked recessive y mutation also causes yellow shank color. For melanin pigmentation, the sex-linked *Id* locus causes dermal melanization. The inhibitor of dermal melanin (*Id*) is dominant to dermal melanin (id^+). The fibromelanosis (*Fm*) locus has also been investigated as the causative locus for hyperpigmentation in dermal pigment. In addition, the *E* locus has been extensively studied as a major determinant of pigment phenotype. This locus affects epidermal melanization, which is associated with dark shank colors (Smyth, 1990).

Several previous studies have successfully elucidated the molecular mechanisms of skin color phenotypes in chicken. Eriksson et al. (2008) determined that the *BCDO2* gene on GGA24 is the causative gene for yellow skin. The *BCDO2* gene encodes the beta-carotene dioxygenase 2 enzyme, which cleaves apocarotenoids and results in yellow and white skins (Kiefer et al., 2001). Dorshorst et al. (2010, 2011) performed research concerning the *Id* and the *Fm* loci in the black Silkie chicken and identified two loci affecting skin pigmentation. The first locus, *Id*, was located at 72.3 Mb on GGAZ, and the second locus, *Fm*, was located at 10.3–13.1 Mb on GGA20. These authors reported that the duplicated regions containing the *EDN3* gene, which encodes the endothelin 3 protein, cause fibromelanosis in black Silkie chicken.

Many studies have provided compelling evidence that the *E* locus is equivalent to the *MC1R* gene. The *MC1R* gene plays a major role in the regulation of coat and skin color variations in mammals, as well as feather and skin colors in birds, because it influences the synthesis of eumelanin (black/brown) and pheomelanin (red/yellow) (Andersson, 2003; Kerje et al., 2003; Klungland and Våge, 2003; Lin and Fisher, 2007; Ling et al., 2003; Rees, 2003).

In our previous study using KNC, we examined the association of plumage color variation with the *MC1R* gene (Hoque et al., 2013). However, the association of *MC1R* gene polymorphisms with shank color has not yet been investigated using KNC. Therefore, this study aims to investigate the differences in the shank colors among the five KNC lines and to identify the associations between the shank color traits measured quantitatively and objectively using a spectrophotometer and *MC1R* genotypes.

2. Materials and methods

The experimental procedures and chicken care facilities used in this study were performed according to the standards established by the Committee for Accreditation of Laboratory Animal Care at the National Institute of Animal Science in Korea. This study was also performed to meet recommendations described in "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.

2.1. Experimental animals

A within line mating design was applied in this study. Specifically, a total of 88 parents (F_0) of five Korean native chicken lines, consisting 15 sires and 73 dams, respectively, were mated to generate 597 offspring (F_1) samples. These animals were maintained under the standard breeding procedures in NIAS, Korea. Among these, 596F_1 individuals were used for measurement of shank colors, which were divided into 110 gray (G), 90 black (L), 135 red (R), 126 white (W) and 135 yellow (Y) lines. These all individuals were reared under the same environment including feeding conditions.

2.2. Measurement of shank colors

Shank color traits were measured using the spectrophotometer (Minolta CM-3500d, Japan) and expressed as lightness (L^*), redness (a^*), yellowness (b^*). The measurement was conducted by a single personnel.

2.3. DNA extraction and SNP genotyping assay

Blood samples of F_0 and F_1 birds were collected from wing veins by using vacutainer tubes containing EDTA. These blood samples were used for the extraction of DNA according to Miller et al. (1988) methods. The DNA concentration was measured by using Thermo Scientific NanoDrop (NanoDrop Products, USA), and the final DNA concentration was adjusted as 25 ng/μl for each sample. The diluted DNAs were maintained in refrigerator at −20 °C until use.

BioMark HD system was used for SNP genotyping for F_1 animals from the five Korean native chicken lines (Fluidigm® 192.24 SNPtype™, USA). Specific target amplifications (STA) were designed to detect four SNPs in the *MC1R* gene. The STA was performed in 14 cycles using 25 ng/μl of genomic DNA. Furthermore, STA products were diluted for priming in the IFC controller, and then the mixture was loaded into BioMark 192.24 dynamic array according to thermal cycling protocol (SNPtype E 192.24 v1) (Supplementary Table 1). Finally, polymorphisms of the *MC1R* gene in the F_1 birds were analyzed using the SNP genotyping analysis (FC1TM cycler, Fluidigm, USA).

2.4. Statistical analysis

Before the association analysis, we obtained the descriptive statistics and verified normal distribution of phenotype data. When putative outliers were detected, we exclude them, based on the ascertainment of normality, using Minitab version 14.0 program (Minitab, USA). Analysis of variance (ANOVA) using the Minitab was conducted to identify factors influencing phenotypic variation.

Heritabilities of lightness (L^*), redness (a^*) and yellowness (b^*) were estimated with the following animal model using univariate analyses:

$$Y_{ijkl} = \mu + S_i + B_j + L_k + a_{ijkl} + e_{ijkl} \quad (\textbf{Model 1})$$

where, S_i is the fixed effect of sex, B_j is the fixed effect of batch, L_l is the fixed effect of line, a_{ijkl} is the random additive polygenic effect of each animal, and e_{ijkl} is a random residual effect. The genetic and residual variance components were estimated using the restricted maximum likelihood method in the ASReml-R program (Gilmour et al., 1995).

We also established an additional linear mixed-effects model to perform association analysis between the polymorphisms of the *MC1R* gene and shank color traits with simultaneous consideration of familial relationship in the F_1 pedigree:

$$Y_{ijkl} = \mu + S_i + B_j + L_k + G_l + a_{ijklm} + e_{ijklm} \quad (\textbf{Model 2})$$

where, S_i is the fixed effect of sex, B_j is the fixed effect of batch, L_l is the fixed effect of line, G_l is the fixed effect of the *MC1R* polymorphism, a_{ijklm} is the random additive polygenic effect of each animal, and e_{ijklm} is a random residual effect.

To estimate the additive and dominance coefficients of each SNP, G_l in the **Model 2** was replaced by additive variable X_{add} (coded as 1, −1 for the two homozygotes, 0 for heterozygote), and dominance variable X_{dom} (coded as 0 for two homozygotes, 1 for heterozygote). Then, phenotypic values were regressed onto the additive and dominance coefficients at each marker.

For the two models, the mean and variance for the random additive polygenic term was assumed to be $a \sim N(0, A\sigma_a^2)$, where A is the additive genetic relationship matrix computed from the F_1 pedigree in this study and σ_a^2 is the additive polygenic variance. The mean and variance for the residual random effect of individuals were assumed to be $\varepsilon \sim N(0, I\sigma_e^2)$, where I is the identity matrix and σ_e^2 is the residual variance. The covariance between a and e was assumed as zero.

To assess the significance of *MC1R* effect on the shank color traits, we used likelihood ratio tests (LR), defined as:

$$\text{LR} = 2 \times (\text{Log Likelihood of } \textbf{Model 2} - \text{ Log Likelihood of } \textbf{Model 1})$$

The LR is assumed to be asymptotically Chi-squared distribution with one degree of freedom. In addition, F_0 parents and F_1 individuals were used to construct haplotype. The PedPhase program based on the pedigree information was applied to construct the haplotypes in the KNC population (Li and Jiang, 2005). The haplotype data were then used to conduct haplotype-based association analyses. The effects of haplotypes on the shank color traits were evaluated by restricted maximum likelihood based mixed effects model analyses (SAS institute Inc., USA).

The percentage of additive variance ($\%Var_{add}$) explained by the inclusion of the *MC1R* genotype or haplotype in the analysis model was calculated using the following equation:

$$\%Var_{add} = \left[(Var_{\textbf{add_Model1}} - Var_{\textbf{add_Model2}})/Var_{\textbf{add_Model1}}\right] \times 100$$

where $Var_{\textbf{add_Model1}}$ and $Var_{\textbf{add_Model2}}$ were additive genetic variance of **Model 1** and additive genetic variance of **Model 2** respectively.

The percentage of total variance ($\%Var_{total}$; sum of additive genetic variance and residual variance) explained by the inclusion of the *MC1R* genotype or haplotype in the analysis model was calculated using the following

equation:

$$\%Var_{\text{total}} = \left[(Var_{\textbf{total_Model1}} - Var_{\textbf{total_Model2}})/Var_{\textbf{total_Model1}}\right] \times 100$$

where $Var_{\textbf{add_Model1}}$ and $Var_{\textbf{add_Model2}}$ were total variance of the **Model 1** and total variance of the **Model 2** respectively.

To address multiple testing issues in this study, Bonferroni's correction was applied to nominal *P*-values in single marker association analysis: threshold for significant association was 0.05/4=0.0125 for four SNP markers in the *MC1R* gene. Results with Nominal *P*-values ranging from 0.0125 to 0.05 were regarded as suggestive associations.

3. Results and discussion

Since the conservation project began to establish the five KNC lines in 1994, the plumage colors have been virtually fixed within each line. Therefore, the names of the lines represent their plumage colors. As indicated, during the establishment of the five lines, charcoal gray and dark green shank colors were selected to distinguish between KNC and broiler breeds. However, after almost 20 years of selection, shank color variation remains within the lines (Fig. 1).

In the population of all five KNC lines, the average lightness (L^*), redness (a^*), and yellowness (b^*) values were 54.01, 0.14, and 8.38, respectively (Table 1). The descriptive statistics for the lightness (L^*), redness (a^*), and yellowness (b^*) of the shank colors were also obtained for each line (Table 1). The range of lightness (L^*) was the widest in the G line (46.02–68.56), while the ranges of redness (a^*) and yellowness (b^*) were the widest in the R line (−2.37–4.48 and 0.82–24.11, respectively). The overall measurement values were distributed widely and showed normal distributions, but compared with broiler breeds (Sirri et al., 2010), the range of yellowness (b^*) was narrow.

When the measurements of lightness (L^*), redness (a^*), and yellowness (b^*) were compared among the five lines, the lowest lightness (L^*, 46.50) value was observed in the L line, while three lines (R, W, Y) were not statistically different (Table 1). The mean value of lightness (L^*) in KNC was relatively very low compared with that of the broiler breeds (79.96) (Sirri et al., 2010). In case of yellowness (b), the lowest value (2.66) was observed in the G line, and the highest value (11.20) was observed in the R line. Previous studies have shown that the yellowness (b^*) of broilers was 53.99 (Sirri et al., 2010), indicating that KNC have relatively lower values of yellowness (b^*) compared to that of broiler breeds. As for redness (a^*), the least square means were significantly different among the five lines. The results indicated that the five KNC lines exhibit shank color variation. The heritability estimates for lightness (L^*), redness (a^*), and yellowness (b^*) were all in the moderate to high range, with estimates of 0.5, 0.37, and 0.63, respectively (Table 1).

To examine the association between *MC1R* gene polymorphisms and shank color traits in KNC, we used the publicly reported SNPs in the *MC1R* gene. We chose six SNPs that had previously been used to discriminate the KNC lines (Hoque et al., 2013). Among these six SNPs, five SNPs

Table 1
Least square means and standard errors for the shank color traits in the five lines of Korean native chicken.

Line	L^*	a^*	b^*
G	53.80 ± 0.32^{b}	-0.77 ± 0.08^{e}	2.66 ± 0.48^{d}
L	46.50 ± 0.35^{c}	0.03 ± 0.09^{c}	3.77 ± 0.52^{d}
R	56.10 ± 0.29^{a}	0.90 ± 0.07^{a}	11.20 ± 0.43^{b}
W	55.22 ± 0.30^{ab}	-0.29 ± 0.08^{d}	8.06 ± 0.44^{c}
Y	56.47 ± 0.29^{a}	0.52 ± 0.08^{b}	13.82 ± 0.43^{a}
Mean[1]	54.01 ± 0.20	0.14 ± 0.04	8.38 ± 0.27
Heritability	0.5	0.37	0.63

(a)–(e) Different letters within same column differ significantly ($P < 0.05$).

Table 2
The SNP information for Fluidigm genotyping assay in *MC1R* gene.

SNP No.	Nucleotide position	Amino acid position	Effect on protein due to amino acid change
1	c.212C > T	p.M71T	Nonpolar to polar
2	c.274A > G	p.E92K	Polar to polar
3	c.376A > G	p.A126I	Nonpolar to nonpolar
4	c.427A > G	p.T143A	Polar to nonpolar

Animal	G062	L054	R074	R020	W268	Y057	Y210
L*	52.32	46.07	56.49	55.528	54.283	52.64	56.156
a*	-1.052	0.467	1.392	2.49	-0.773	0.373	0.360
b*	-0.326	11.896	7.878	12.53	8.100	1.468	16.784

Fig. 1. The shank color variations in Korean native chickens. The L^*, a^* and b^* values are also provided. Note that the shank colors in the same line are different (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

associated with plumage colors were initially investigated. After quality control screening of the primers using the Fluidigm genotyping assay, four SNPs were finally selected. The information for these four SNPs, including their amino acid changes, is presented in Table 2. As shown in Table 3, three SNPs (i.e., c.212C > T, c.274A > G and c.427A > G) in the *MC1R* gene were associated with shank color traits in KNC. In the case of c.427A > G, a significant association (nominal $P=0.001$) was identified with yellowness (b^*), while a suggestive association was found with lightness (L^*) (nominal $P=0.022$). For c.274A > G, a suggestive association (nominal $P=0.020$) was detected with yellowness (b^*). In addition, a significant association of yellowness (b^*) with c.212C > T (nominal $P=0.007$) was detected. The significant and suggestive SNPs showed additive effect, as no significant dominant effect was observed (data not shown). Regarding the SNP c.427A > G, the G427 allele was associated with higher values of yellowness (b^*) and lightness (L^*). For the SNP c.274A > G associated with yellowness (b^*), G274 allele showed higher value than A274 allele. Finally, the T212 allele was associated with higher values of yellowness (b^*). For L^* and b^* shank traits, the effect of each *MC1R* genotype explained a small proportion of the additive genetic variance (2.18–6.34%) and total variance (1.26–1.78%) in the F_1 generation (Table 3). Furthermore, genotypic distributions of the four SNPs in the *MC1R* gene from each line of KNC are shown in Supplementary Table 1. Since each line of KNC has been established based on its plumage color and we have selected the *MC1R* SNPs associated with plumage colors, some lines showed strong tendency of allelic fixation in specific SNPs. In this within line association analyses, we could detect several positive associations between the *MC1R* SNPs and shank color traits. These results suggest that there is some variation for the *MC1R* SNPs co-segregated with shank color traits within each line of KNC (Supplementary Tables 2–5). In addition, we constructed haplotypes of the *MC1R* gene to evaluate the effects of the *MC1R* haplotype on the variation of shank colors in the KNC population. Haplotype and haplogenotype frequencies of the *MC1R* gene in the F_1 birds are listed in Table 4. The results of the haplotype-based association analyses indicated that there were significant association between the haplogenotype and L^* trait in F_1 progeny (Table 5). The F_1 birds with ht1/ht1 haplogenotype had significantly ($P < 0.01$) higher L^* value than those with ht1/ht2. The F_1 birds with ht2/ht2 haplogenotype had significantly ($P < 0.05$) lower L^* value than those with ht2/ht4. The effect of *MC1R* haplotype on L^* trait accounted for a moderate proportion of the additive genetic variance (15.29%) and a small proportion of total variances (4.49%) (Table 5). No significant association of the haplogenotypes with a^* and b^* values was detected in F_1 birds. The *MC1R* gene encodes the melanocortin receptor 1 and controls the eumelanin (black/brown) and pheomelanin (red/yellow) pigmentation in the melanocyte. Therefore, the *MC1R* gene affects body color variation in mammals and birds (Andersson, 2003; Kerje et al., 2003; Klungland and Våge, 2003; Lin and Fisher, 2007; Ling et al., 2003; Rees, 2003). Several nucleotide substitutions in the chicken *MC1R* gene

Table 3
Association between four SNPs in *MC1R* gene with color values for shank color in five lines of Korean native chicken.

Trait	c.212C > T			Additive effect	LR	*P*-value	$\%Var_{add}$	$\%Var_{Total}$
	CC (162)	CT (76)	TT (354)					
L^*	52.43 ± 0.66	53.32 ± 0.55	54.06 ± 0.50	0.80 ± 0.42	1.80	0.180	–	–
a^*	−0.14 ± 0.17	−1.00 ± 0.14	0.30 ± 0.12	0.26 ± 0.11	0.19	0.664	–	–
b^*	6.69 ± 1.02	6.78 ± 0.86	9.00 ± 0.78	1.41 ± 0.62	7.16	**0.007***	**2.18**	**1.26**
Trait	c.274A > G			Additive effect	LR	*P*-value	$\%Var_{add}$	$\%Var_{Total}$
	AA (176)	AG(68)	GG(348)					
L^*	53.11 ± 0.67	52.95 ± 0.57	53.76 ± 0.52	0.47 ± 0.42	0.42	0.518	–	–
a^*	−0.14 ± 0.17	−0.04 ± 0.14	0.29 ± 0.13	0.25 ± 0.11	1.37	0.242	–	–
b^*	6.70 ± 1.02	7.13 ± 0.87	8.96 ± 0.80	1.34 ± 0.62	5.39	**0.020†**	**3.30**	**1.33**
Trait	c.376A > G			Additive effect	LR	*P-value*	$\%Var_{add}$	$\%Var_{Total}$
	AA (0)	AG (16)	GG (576)					
L^*	–	54.40 ± 1.17	53.41 ± 0.39	0.99 ± 1.14	1.01	0.314	–	–
a^*	–	0.05 ± 0.30	0.11 ± 0.09	0.06 ± 0.29	2.40	0.121	–	–
b^*	–	6.71 ± 1.78	8.01 ± 0.63	1.30 ± 1.71	1.65	0.199	–	–
Trait	c.427A > G			Additive effect	LR	*P-value*	$\%Var_{add}$	$\%Var_{Total}$
	AA (202)	AG (39)	GG (351)					
L^*	53.44 ± 1.13	52.22 ± 0.93	53.57 ± 0.82	0.99 ± 0.52	5.26	**0.022†**	**6.34**	**1.78**
a^*	−0.32 ± 0.30	0.03 ± 0.25	0.43 ± 0.21	0.39 ± 0.14	2.73	0.098	–	–
b^*	5.34 ± 1.66	7.51 ± 1.38	9.97 ± 1.22	2.42 ± 0.76	11.67	**0.001***	**2.45**	**1.66**

Numbers in the parenthesis represents the number of birds in each genotype
† Suggestive association ($0.0125 < P < 0.05$)
* Significant associated with trait ($P < 0.0125$)

Table 4
Haplotype and haplogenotype frequencies of the *MC1R* gene in the F_1 birds.

Haplotype	Number	Frequency[a]	Haplogenotype	Number	Frequency
TGGG (ht1)	738	0.62	ht1/ht1	354	0.61
CAGA (ht2)	418	0.35	ht1/ht2	30	0.05
TGGA (ht3)	22	0.02	ht2/ht2	161	0.28
TAAA (ht4)	14	0.01	ht2/ht3	22	0.04
			ht2/ht4	14	0.02

[a] Haplotypes with frequency less than 0.005 are dropped in this table

Table 5
Effects of haplogenotype of *MC1R* on shank color traits for the F_1 birds.

Haplogenotype	*L**	*P*-value	*a**	*P*-value	*b**	*P*-value
ht1/ht1	53.72 ± 1.26^{a}		0.6029 ± 0.34		11.17 ± 1.86	
ht1/ht2	51.81 ± 1.37^{b}		0.3318 ± 0.37		9.61 ± 2.02	
ht2/ht2	53.10 ± 1.84^{abc}	**0.005**	-0.6534 ± 0.50	0.379	3.44 ± 2.71	0.261
ht2/ht3	54.88 ± 2.04^{abcd}		-0.6560 ± 0.55		3.95 ± 3.02	
ht2/ht4	55.73 ± 2.19^{abd}		-0.7607 ± 0.59		2.52 ± 3.26	

[a,b,c] least squares means with different superscripts within same column are significantly different ($P < 0.05$). For *L**, $\%Var_{add}$ of *MC1R* haplogenotype is 15.29 and $\%Var_{total}$ of *MC1R* haplogenotype is 4.49.

are associated with different plumage colors (Guo et al., 2010; Kerje et al., 2003; Ling et al., 2003; Takeuchi et al., 1996). Previous studies have shown that six nonsynonymous SNPs (p.M71T, p.E92K, p.A126I, p.T143A, p.C213R, and p.H215P) are strongly associated with plumage colors in chicken. In this study, two SNPs (c.212C > T causing p.M71T and c.427A > G causing p.T143A) were associated with shank color traits (Table 3).

Ling et al. (2003) argued that amino acid polarity could affect the signal transduction of the *MC1R* gene and ultimately lead to color variations. In this study, two SNPs (c.212C > T and c.427A > G) did change the amino acid polarity (Table 2), indicating that polarity may be a key factor in shank color variation. Previously, Knox (1935) suggested that the extension of black pigment, which is currently known as the main allele of the *MC1R* gene, had an effect on both plumage and shank colors. Therefore, our results confirm the early findings of Knox (1935).

4. Conclusion

In this study, the shank color variations in five KNC lines were quantitatively and objectively measured using a spectrophotometer. The associations between SNPs in the *MC1R* gene and shank colors were investigated, and two SNPs (c.212C > T and c.427A > G) were found to significantly affect the yellowness (*b**) values in KNC. After verifying the positive associations in other populations, we believe that the results presented here can be used for the selection of individuals with desirable shank colors in KNC populations.

Conflict of interest statement

None.

Acknowledgments

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0081330), Rural Development Administration, Republic of Korea.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.livsci.2014.10.001.

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