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Identification of single nucleotide polymorphisms in the *ASB15* gene and their associations with chicken growth and carcass traits

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ABSTRACT. *ASB15* is a member of the ankyrin repeat and suppressor of cytokine signaling box family, and is predominantly expressed in skeletal muscle. In the present study, an F_2 resource population of Gushi chickens crossed with Anka broilers was used to investigate the genetic effects of the chicken *ASB15* gene. Two single nucleotide polymorphisms (SNPs) (rs315759231 A>G and rs312619270 T>C) were identified in exon 7 of the *ASB15* gene using forced polymerase

chain reaction-restriction fragment length polymorphism and DNA sequencing. One was a missense SNP (rs315759231 A>G) and the other was a synonymous SNP (rs312619270 T>C). The rs315759231 A>G polymorphism was significantly associated with body weight at birth, 12-week body slanting length, semi-evisceration weight, evisceration weight, leg muscle weight, and carcass weight ($P < 0.05$). The rs312619270 T>C polymorphism was significantly associated with body weight at birth, 4, 8, and 12-week body weight, 8-week shank length, 12-week breast bone length, 8 and 12-week body slanting length, breast muscle weight, and carcass weight ($P < 0.05$). Our results suggest that the *ASB15* gene profoundly affects chicken growth and carcass traits.

Key words: Chicken; *ASB15* gene; Single nucleotide polymorphism; Growth and carcass traits

INTRODUCTION

The ankyrin repeat and suppressor of cytokine signaling (SOCS) box (*ASB*) family of proteins function as a substrate recognition subunit in a subset of Elongin-Cullin-SOCS (*ECS*) E3 ubiquitin ligases (Andresen et al., 2014). The different SOCS box-containing proteins have variable N-terminal sequences that encode for domains that mediate protein-protein interactions and determine substrate recognition by *ECS* ligases (Kamura et al., 2004). Depending on the nature of such domains, *ECS*s can be divided into subfamilies, such as the SOCS, which exhibit an SH2 domain, and the larger *ASB*, which have 18 members that display ankyrin repeats at their N termini (Kile et al., 2001). *ASB* family members function in multiple biological processes related to cell growth (Guibal et al., 2002), tissue development (Kohroki et al., 2001; Guibal et al., 2002), and insulin signaling (Wilcox et al., 2004).

ASB15 is a member of the *ASB* gene family, and is predominantly expressed in skeletal muscle (Hilton et al., 1998; Kile et al., 2000; McDaneld and Spurlock, 2008). *ASB15* is rapidly downregulated in response to β-adrenergic receptor agonists (McDaneld et al., 2004; Spurlock et al., 2006). In C_2C_{12} myoblasts, *ASB15* causes delayed differentiation and altered protein synthesis, and local overexpression of *ASB15* in skeletal muscle *in vivo* stimulates a significant increase in muscle fiber diameter (McDaneld et al., 2006). *ASB15* plays a role in early myoblast differentiation, and its effects may be mediated in part by the PI3K/Akt signal transduction pathway. As an *ASB* family member, *ASB15* should function in skeletal muscle growth (McDaneld et al., 2006); however, no evidence of such a role has been found in chickens.

An important goal in the production of meat animals is to increase muscle growth. Therefore, the purpose of this study (based on the roles of the *ASB* family as described above) was to examine whether associations exist between single nucleotide polymorphisms (SNPs) in the *ASB15* gene and growth and carcass traits in chickens, in order to identify a possible candidate gene that affects meat production in chickens.

MATERIAL AND METHODS

Resource population

An F_2 resource population as previously described (Han et al., 2010) was used in this study. Gushi (G) chickens (24 hens and two roosters), which is a slow-growing Chinese breed, and Anka (A) broilers (12 hens and four roosters), which is a fast-growing broiler, were hybridized. The F_2 population consisted of four crossed families (A-roosters mated with G-hens) and three reciprocal families (G-roosters mated with A-hens). To build the F_2 population, nine F_1 females were selected from each of seven families (six unrelated rooster families and one half-sib). In total, 63 F_1 females were mated with seven F_1 males from seven families. The resource population was established over two hatches that occurred at two-week intervals, and included 42 grandparents, 70 F_1 parents, and 836 F_2 chickens. All of the chickens were reared under the same conditions, with free access to food and water.

Phenotypic measurements

The growth traits analyzed were body weight (BW) and body size indices, including shank length (SL), breastbone length (BBL), and body slanting length (BSL). BW was recorded every 2 weeks, from birth to slaughter. SL, BBL, and BSL were measured every 4 weeks.

In total, 836 F_2 chickens were slaughtered at the age of 84 days. The carcass traits measured were semi-eviscerated weight (SEW), eviscerated weight (EW), breast muscle weight (BMW), leg muscle weight (LMW), and carcass weight (CW). Blood samples were collected and separated by centrifugation at 3000 *g* for 15 min at 4°C. Serum samples were frozen at -80°C until use.

DNA samples and DNA pool for sequencing

Genomic DNA samples were extracted from the serum using the phenol-chloroform method. The same number of DNA samples from 100 F_2 individuals was pooled and diluted to a working concentration of 50 ng/μL. The pooled DNA was then sent to Taihe Gene Biotechnology Co. Ltd (Beijing) for sequencing, using eight pairs of primers designed according to the *ASB15* gene sequence (Table 1).

Forced polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

In order to amplify 172-bp and 182-bp fragments in exon 7, which contained an rs315759231 A>G mutation and an rs312619270 T>C mutation, two primer pairs were designed according to the sequencing results, and a previously reported gene sequence of the chicken *ASB15* gene (NC_006088). Primer P1 (F1, 5'-GGCTATCTTGGATGATCACATTTCGA-3'; R1, 5'-GTTACCACCTCTCACTGCCAC-3') was designed to amplify the 172-bp fragment that contained rs315759231 A>G. Primer P2 (F2, 5'-GCTGAATCATGGATATAATGTGCAG-3'; R2, 5'-GGCTAACGGAAAGAAGAAAG-3') was designed to amplify the 182-bp fragment that contained rs312619270 T>C. To create *Asu*II (TT/CGAA) and *Pvu*II (CAG/CTG)

restriction sites for genotyping, a point mismatch was introduced into the forward primers of P1 and P2, respectively.

Following optimization, PCRs were performed in a total volume of 25 µL using 50 ng DNA, primers (1 µM each), and 12 µL *Taq* Master Mix (CWBIO, Beijing, China). The PCR profile was as follows: 5 min at 94°C (denaturation), 32 cycles at 94°C for 30 s, 60°C (P1) or 58°C (P2) for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min.

The PCR products were digested by restriction enzymes (*Asu*II for rs315759231 A>G and *Pvu*II for rs312619270 T>C) overnight at 37°C. The digested products were separated by electrophoresis on 2.0% agarose gel that was stained with ethidium bromide. Different genotypes of these two SNPs were confirmed by sequencing.

Statistical analysis

All of the data were analyzed using the SPSS 22.0 software. A linear mixed model procedure was used to analyze the relationship between the two SNPs and the related economic traits of the F_2 resource population. Both additive and dominance effects were also estimated for the traits identified as having significant SNP associations using the REG procedure, where the additive effect was estimated as 1, 0, and -1 for AA (CC), AG (TC), and GG (TT), respectively, and the dominance effect was estimated as 1 and -1 for the homozygote and heterozygote, respectively. The statistical model used was as follows:

$$Y_{ijklm} = \mu + G_i + S_j + H_k + f_l + e \quad \text{(Equation 1)}$$

where Y_{ijklm} represents the measurement of a trait, µ is the overall population mean, G_i is the fixed effect of genotype (i = 3), S_j is the fixed effect of sex (j = 2), H_k is the fixed effect of hatch (k = 2), f_l is the random effect of family (l = 7), and e_{ijklm} is the random error. The least squares method was used to investigate the effects of genotypes of *ASB15* polymorphisms on the target traits. Significance was set at $P < 0.05$, and Bonferroni tests were conducted in order to control for multiple comparisons.

Population genetic indices, including heterozygosity (H_E), effective number of alleles (N_E), and the polymorphism information content (PIC) were calculated according to Nei's methods (Nei and Roychoudhury, 1974) in PopGene (version 1.3.1) (Yeh et al., 1999).

Tests for linkage disequilibrium (LD) were performed using the SHEsis software platform (http://analysis.bio-x.cn/SHEsisMain.htm). D' and r^2 are the most commonly used measures of LD; $r^2 > 0.33$ is indicative of strong linkage disequilibrium (Ardlie et al., 2002). If $r^2 > 0.33$, haplotypes were constructed on the basis of the two SNPs in all of the experimental animals, using the SHEsis program.

RESULTS

SNP identification and genotyping

The chicken *ASB15* gene is composed of nine exons, which code for a protein with 588 amino acids. Firstly, we cloned and sequenced a 9389-bp fragment, which included the entire coding region and the intron sequences of the *ASB15* gene. Eight pairs of prim-

ers were then designed to detect SNPs by DNA pool sequencing in the nine exons and introns (Table 1). Six SNPs in the introns and two SNPs in the seventh exon were identified (Figure 1). Finally, we selected the two SNPs in the seventh exon for the next step. The SNPs rs315759231 A>G and rs312619270 T>C were confirmed using the forced PCR-RFLP method. The former was a missense mutation from Asn to Ser, and the latter was a synonymous mutation.

Table 1. Primer pair sequences and characteristics of the chicken *ASB15* gene.

Position	Name	Primer (5'-3')	Size (bp)	Tm (°C)	Notes
1-484	E1	F: ATGACGGATGAAAGCGAAG R: TGAGTGATGCCAGGGAGGT	484	58.0	Exon1, intron1
1432-2452	E2-3	F: ACTTTTGTTGTGGGGGCTG R: GTAGTGAGGTGGGGGTCGG	1021	62.0	Exon2-3, intron2-3
2194-3472	E4	F: ACTCAGTCCAACCATTCACCC R: TCCCAAAGAAATCATCCAAAA	1279	60.0	Exon4, intron4
4793-5622	E5	F: TGTTTTTATTGTTGCTTTTCC R: TATGTAGGTCCCTCCTTGATT	829	57.5	Exon5, intron5
5544-6504	E6	F: TTCCTACACTTCCATAATCCA R: AAGAAAATGACCATCACACAG	961	59.0	Intron5, exon6, intron6
6428-7167	E7	F: GGTGCTTCTGTGTTAGGATTTT R: GGCTAACGGAAAGAAGAAAGTG	740	56.0	Exon7
7475-8010	E8	F: TCAAACCTGTCAAGAGGAAT 536 R: CAGTGGCAACAGGTAAAGA	57		Exon8, intron8
8680-9389	E9	F: TTAGTGTTAGTGTCATGGGC R: CTATTCTAAATGTATCTCTTTTCCA	709	56.0	Exon9, intron8

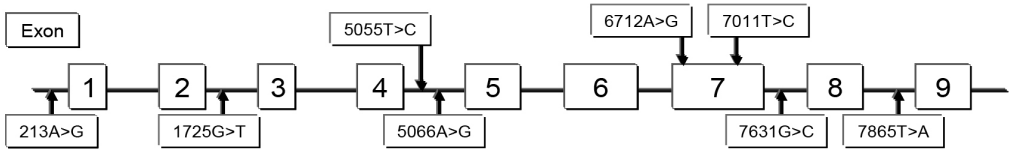

Figure 1. Single nucleotide polymorphism positions in the chicken *ASB15* gene.

Three genotypes were detected from each of the two SNPs. The 172-bp PCR products that were digested into two fragments (146 and 26 bp) were marked AA, undigested (172 bp) fragments were marked GG, and those that were digested into three fragments (172, 146, and 26 bp) were marked AG (Figure 2). The 182-bp PCR products were marked CC (157 and 25 bp), TT (182 bp), and CT (182, 157, and 25 bp) (Figure 3). Although the 26-bp and 25-bp fragments were too short to be visible on 2.0% agarose gel stained with ethidium bromide, three genotypes were confirmed by sequencing the corresponding PCR products (Figures 4 and 5). For the rs315759231 A>G site, the frequencies of alleles A and G were 0.491 and 0.509, respectively. The genotype frequencies of AA, AG, and GG were 0.221, 0.576, and 0.203, respectively (Table 2). The frequencies of alleles A and G were almost same, and the AA genotype frequency was approximately the same as the GG genotype frequency. For rs312619270 T >C, the allele frequencies of T and C were 0.747 and 0.253, respectively, and the genotype frequencies of TT, TC, and CC were 0.581, 0.332, and 0.087, respectively. Allele T was dominant in the F_2 population, and the TT genotype was more frequent than the other genotypes.

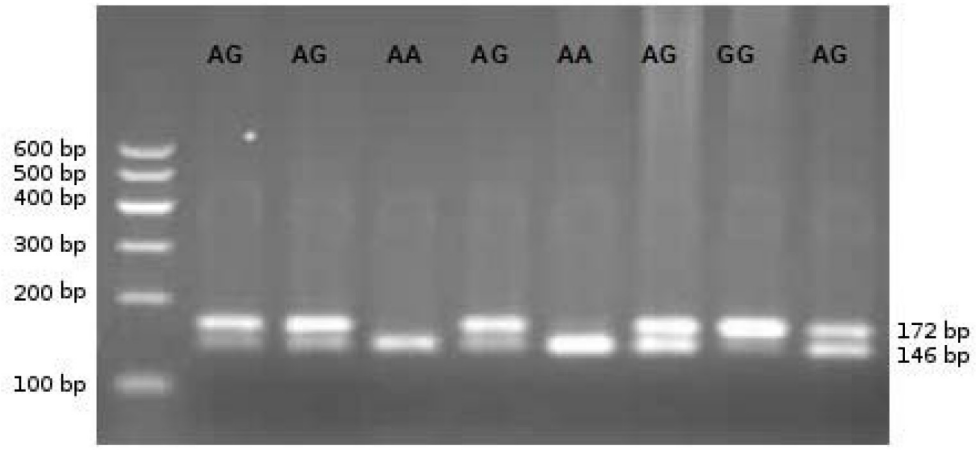

Figure 2. Agarose gel electrophoresis (2.0%) showing *Asu*II-restriction fragment length polymorphism fragments of the *ASB15* gene polymerase chain reaction amplification products.

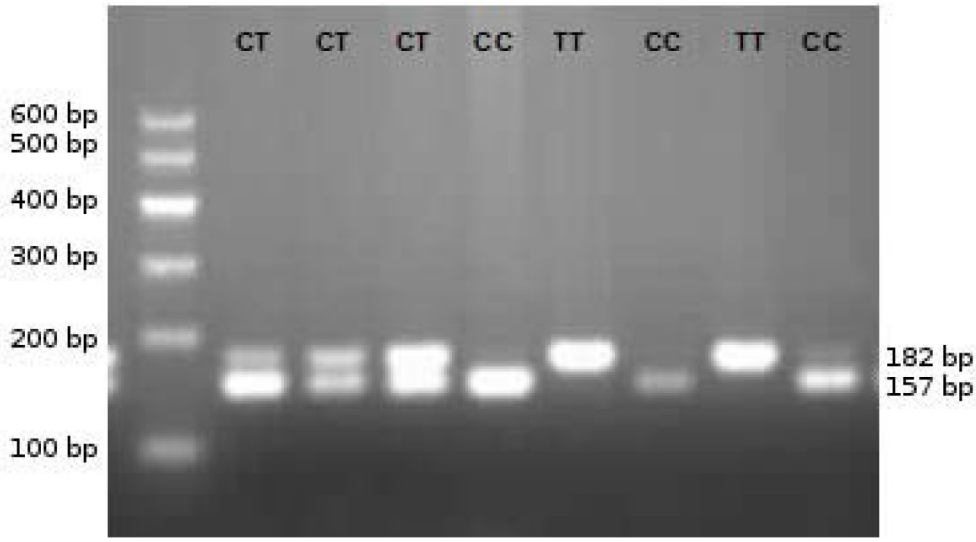

Figure 3. Agarose gel electrophoresis (2.0%) showing the *Pvu*II-restriction fragment length polymorphism fragments of the *ASB15* gene polymerase chain reaction amplification products.

Table 2. Genotypic and allelic frequencies (%), and related genetic parameters.

Locus	Genotypic and allelic frequencies					H_E	N_E	PIC
rs315759231	GG	AG	AA	A	G			
	0.203	0.576	0.221	0.491	0.509	0.499	1.999	0.375
rs312619270	TT	CT	CC	T	C			
	0.581	0.332	0.087	0.747	0.253	0.378	1.608	0.307

H_E = gene heterozygosity; N_E = number of allele; PIC = polymorphism information content.

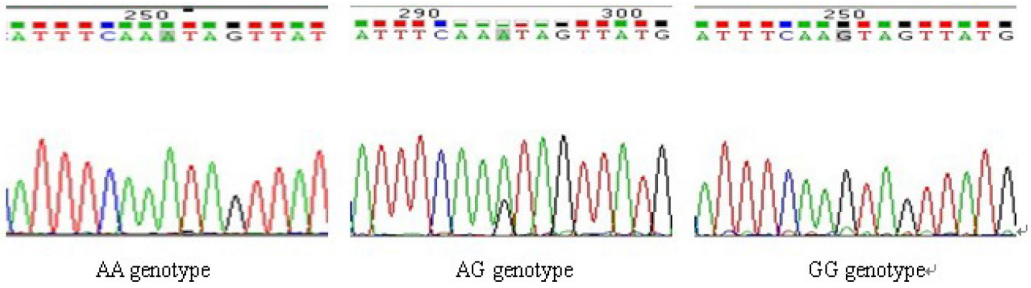

Figure 4. DNA sequencing files of AA, AG, and GG genotypes.

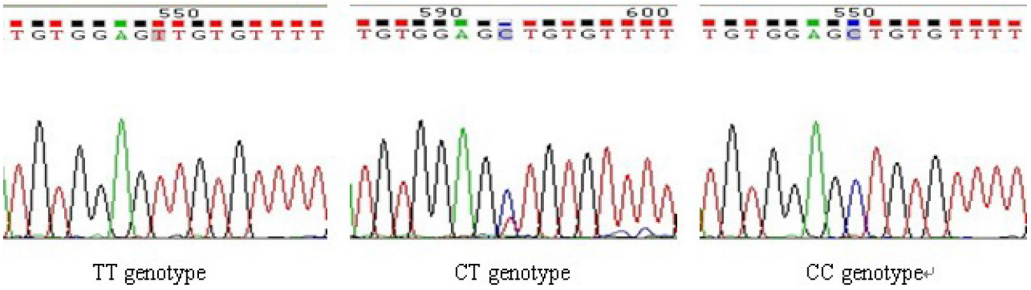

Figure 5. DNA sequencing files of TT, CT, and CC genotypes.

Diversity analysis and linkage disequilibrium

The genetic characteristics (H_E, N_E, and PIC) of the F_2 population are presented in Table 2. The value of H_E was approximately 0.5 for rs315759231 and rs312619270, and the N_E approached 2; the PIC values were 0.375 and 0.306, respectively. PIC values greater than 0.50 were indicative of high levels of polymorphism, between 0.50 and 0.25 of intermediate levels of polymorphism, and lower than 0.25 of low levels of polymorphism. The F_2 population exhibited intermediate levels of genetic diversity at these two SNPs. These results reflect a low overall level of genetic diversity of the chicken gene in the analyzed population. Genetic diversity is essential for species conservation, and the potential improvement of production traits in selected breeds.

The values of D' and r^2 of the two SNPs were 0.115 and 0.004, respectively; as $r^2 < 0.33$, the SNPs were not in linkage disequilibrium.

SNP associations with growth and carcass traits

A total of 17 growth traits and five carcass traits were used in an analysis of association. Regarding the growth traits, the rs315759231 A>G polymorphism was only significantly associated with BW0 and BSL12 (Table 3). A significant dominant effect on BW0 was found, while the additive effect was significant on BSL12 (Table 5). The rs312619270 T>C polymor-

phism was significantly associated with BW0, 4, 8, 12, and SL8, BBL12, BSL8, and BSL12 (Table 4); the CT individuals had significantly higher values of these traits than those with CC and TT genotypes.

Table 3. Relationships between rs315759231 A>G single nucleotide polymorphisms (SNPs) and chicken growth and carcass traits.

Trait		Genotype			P value
		GG (149)	AG (422)	AA (162)	
Growth traits	**BW0 (g)**	**30.925 ± 0.454[b]**	**30.304 ± 0.419[ab]**	**31.240 ± 0.449[a]**	**0.000**
	BW2 (g)	120.765 ± 2.812	122.525 ± 2.522	122.965 ± 2.787	0.515
	BW4 (g)	317.135 ± 7.913	321.102 ± 7.323	325.386 ± 7.846	0.247
	BW6 (g)	558.694 ± 14.736	559.896 ± 13.579	571.554 ± 14.599	0.278
	BW8 (g)	804.623 ± 20.589	812.688 ± 18.739	835.888 ± 20.354	0.062
	BW10 (g)	1100.295 ± 26.620	1110.925 ± 24.474	1131.061 ± 26.423	0.196
	BW12 (g)	1335.878 ± 30.202	1350.352 ± 27.357	1383.184 ± 29.896	0.067
	SL0 (cm)	2.578 ± 0.012	2.574 ± 0.009	2.593 ± 0.011	0.127
	SL4 (cm)	5.469 ± 0.075	5.497 ± 0.054	5.543 ± 0.073	0.685
	SL8 (cm)	7.876 ± 0.061	7.912 ± 0.038	7.988 ± 0.059	0.372
	SL12 (cm)	9.345 ± 0.082	9.395 ± 0.067	9.455 ± 0.081	0.373
	BBL4 (cm)	6.181 ± 0.065	6.210 ± 0.055	6.248 ± 0.064	0.505
	BBL8 (cm)	8.905 ± 0.081	8.880 ± 0.066	8.985 ± 0.079	0.281
	BBL12 (cm)	10.937 ± 0.049	10.996 ± 0.080	11.004 ± 0.092	0.652
	BSL4 (cm)	11.311 ± 0.099	11.385 ± 0.083	11.488 ± 0.098	0.138
	BSL8 (cm)	1 6.132 ± 0.108	16.257 ± 0.077	16.295 ± 0.105	0.400
	BSL12 (cm)	**19.643 ± 0.117[b]**	**19.776 ± 0.089[ab]**	**19.955 ± 0.114[a]**	**0.046**
Carcass traits	**SEW (g)**	**1086.655 ± 26.317[b]**	**1 098.473 ± 24.017[ab]**	**1128.924 ± 25.991[a]**	**0.041**
	EW (g)	**903.108 ± 23.999[b]**	**919.915 ± 22.176[ab]**	**943.985 ± 23.740[a]**	**0.024**
	BMW (g)	68.507 ± 2.585	70.238 ± 2.386	71.956 ± 2.555	0.110
	LMW (g)	**97.158 ± 3.014[b]**	**99.308 ± 2.785[ab]**	**101.826 ± 2.986[a]**	**0.049**
	CW (g)	**1195.165 ± 29.04[b]**	**1211.406 ± 26.722[ab]**	**1247.964 ± 28.747[a]**	**0.031**

[a,b]Means within a row of each SNP site with no common superscript were significantly different ($P < 0.05$); bold numbers indicate significant differences. BW0, BW2, BW4, BW6, BW8, BW10, and BW12 represent body weight at 0, 4, 8, and 12 weeks of age; SL4, SL8, and SL12 represent shank length at 4, 8, and 12 weeks of age; BBL4, BBL8, and BBL12 represent breast bone length at 4, 8, and 12 weeks of age; BSL4, BSL8, and BSL12 represent body slanting length at 4, 8, and 12 weeks of age; SEW, semi-evisceration weight; EW, evisceration weight; BMW, breast muscle weight; LMW, leg muscle weight; CW, carcass weight.

Regarding the carcass traits, the rs315759231 A>G polymorphism was significantly associated with SEW, EW, LMW, and CW. The values of these traits in the AA genotype were significantly higher than those in the GG and AG genotypes (Table 3). Values of SEW, EW, LMW, and CW were higher in the AA genotype than in the GG and AG genotypes. The rs312619270 T>C polymorphism was only significantly associated with BMW and CW; trait values in the CT genotype were higher than those in the TT and CC genotypes. Both the dominant and additive effects were significant for weight (except BW0), SL8, BBL12, BSL8, BSL12, and BMW (Table 6).

DISCUSSION

Tremendous progress has been achieved in the fields of genetics and genomics over the last two decades (Gheyas and Burt, 2013). SNPs are by far the most commonly used tools for detecting DNA sequence variations. Although the vast majority of SNPs are found in noncoding regions of the genome, and most that are found in coding regions do not change gene products in deleterious ways, they are thought to be the basis for much of the genetic variation found in

humans and animals (Kwok, 2003). The function of SNPs is a hot topic of debate for geneticists (Rao and Zhang, 2007). Among farm animals, the chicken has emerged as an important model organism, in fields as diverse as embryological development, immunology, oncology, virology, evolution, genetics, and genomics, and particularly as a model for other avian species. Studies of chicken SNPs have mainly focused on important economic characteristics, such as growth (Amills et al., 2003; Wang et al., 2011; Liu et al., 2012; Xie et al., 2012; Ahsan et al., 2013; Jin et al., 2013), carcass characteristics (Fang et al., 2010; Uemoto et al., 2011; Lu et al., 2012; Boschiero et al., 2013; Sun et al., 2013), egg quality (Bennett et al., 2006; Fatemi et al., 2012), fatness (Nie et al., 2010; Han et al., 2012), reproduction (Tang et al., 2010; Moonen and Villamor, 2011; Liu et al., 2012), and disease resistance (Legarra et al., 2011; Meydan et al., 2011).

Table 4. Relationships between rs312619270 T>C single nucleotide polymorphisms (SNPs) and chicken growth and carcass traits.

SNP	Trait		Genotype			P value
			TT (441)	CT (252)	CC (66)	
rs312619270 T>C	Growth traits	BW0 (g)	**30.560 ± 0.566^{ab}**	**30.942 ± 0.486^{a}**	**29.983 ± 0.47^{b}**	**0.027**
		BW2 (g)	121.475 ± 2.432	124.301 ± 2.570	121.229 ± 3.280	0.169
		BW4 (g)	**317.188 ± 6.336^{b}**	**327.011 ± 6.68^{a}**	**325.779 ± 8.187^{ab}**	**0.033**
		BW6 (g)	556.124 ± 12.300	568.690 ± 12.956	565.124 ± 15.874	0.229
		BW8 (g)	**804.461 ± 16.014^{b}**	**832.264 ± 17.043^{a}**	**820.168 ± 22.182^{ab}**	**0.042**
		BW10 (g)	1100.614 ± 20.902	1131.654 ± 22.209	1114.304 ± 28.241	0.086
		BW12 (g)	**1338.955 ± 23.176^{b}**	**1379.751 ± 24.845^{a}**	**1351.119 ± 32.806^{ab}**	**0.048**
		SL0 (cm)	2.576 ± 0.011	2.584 ± 0.012	2.598 ± 0.016	0.211
		SL4 (cm)	5.497 ± 0.063	5.494 ± 0.071	5.407 ± 0.105	0.643
		SL8 (cm)	**7.852 ± 0.043^{b}**	**8.002 ± 0.051^{a}**	**7.981 ± 0.088^{ab}**	**0.016**
		SL12 (cm)	9.355 ± 0.053	9.468 ± 0.060	9.435 ± 0.092	0.091
		BBL4 (cm)	6.193 ± 0.053	6.249 ± 0.058	6.257 ± 0.080	0.375
		BBL8 (cm)	8.896 ± 0.064	8.948 ± 0.071	8.868 ± 0.104	0.570
		BBL12 (cm)	**10.918 ± 0.064^{b}**	**11.128 ± 0.105^{a}**	**11.080 ± 0.072^{ab}**	**0.008**
		BSL4 (cm)	11.325 ± 0.083	11.550 ± 0.124	11.490 ± 0.091	0.057
		BSL8 (cm)	**16.123 ± 0.061^{b}**	**16.403 ± 0.077^{a}**	**16.385 ± 0.144^{ab}**	**0.006**
		BSL12 (cm)	**19.707 ± 0.067^{b}**	**19.908 ± 0.081^{a}**	**19.821 ± 0.14^{ab}**	**0.048**
	Carcass traits	SEW (g)	1090.872 ± 20.696	1122.278 ± 22.016	1097.838 ± 28.316	0.076
		EW (g)	911.268 ± 19.188	939.342 ± 20.266	918.281 ± 25.384	0.063
		BMW (g)	**69.114 ± 2.076^{b}**	**72.688 ± 2.184^{a}**	**69.700 ± 2.733^{ab}**	**0.018**
		LMW (g)	98.669 ± 2.584	100.972 ± 2.704	99.233 ± 3.313	0.299
		CW (g)	**1199.710 ± 22.337^{b}**	**1242.634 ± 23.713^{a}**	**1213.363 ± 30.354^{ab}**	**0.015**

^{a,b}Means within a row of each SNP site with no common superscript were significantly different ($P < 0.05$); bold numbers indicate significant differences. BW0, BW2, BW4, BW6, BW8, BW10, and BW12 represent body weight at 0, 4, 8, and 12 weeks of age; SL4, SL8, and SL12 represent shank length at 4, 8, and 12 weeks of age; BBL4, BBL8, and BBL12 represent breast bone length at 4, 8, and 12 weeks of age; BSL4, BSL8, and BSL12 represent body slanting length at 4, 8, and 12 weeks of age; SEW, semi-evisceration weight; EW, evisceration weight; BMW, breast muscle weight; LMW, leg muscle weight; CW, carcass weight.

Table 5. Estimated additive and dominance effects for the rs315759231 A>G.

Traits	Additive effective		Dominance effective	
	a ± SE	P value	d ± SE	P value
BW0	0.237 ± 0.150	0.114	0.401 ± 0.098	0.000
BSL12	0.124 ± 0.063	0.047	0.010 ± 0.042	0.801
SEW	14.790 ± 9.024	0.102	0.000 ± 0.075	0.993
EW	14.726 ± 7.762	0.058	-0.117 ± 0.074	0.113
LMW	1.665 ± 0.969	0.086	-0.606 ± 0.643	0.346
CW	0.223 ± 0.112	0.048	-4.201 ± 6.409	0.512

Table 6. Estimated additive and dominance effects for the rs312619270 T>C.

Traits	Additive effective		Dominance effective	
	a ± SE	P value	d ± SE	P value
BW0	0.296 ± 0.160	0.640	0.020 ± 0.106	0.851
BW4	5.983 ± 2.629	0.023	-5.681 ± 1.753	0.001
BW8	16.347 ± 7.582	0.031	-18.806 ± 4.984	0.000
BW12	24.062 ± 11.497	0.037	-33.814 ± 7.477	0.000
SL8	0.082 ± 0.038	0.033	-0.063 ± 0.025	0.013
BBL12	0.153 ± 0.040	0.000	-0.088 ± 0.026	0.001
BSL8	0.142 ± 0.066	0.030	-0.098 ± 0.043	0.023
BSL12	0.146 ± 0.062	0.019	-0.093 ± 0.041	0.023
BMW	2.253 ± 0.889	0.011	-3.140 ± 0.575	0.000
CW	23.104 ± 10.267	0.025	-0.065 ± 0.078	0.408

In this study, two SNPs were found in the chicken *ASB15* gene in exon 7. One was a missense SNP (rs315759231 A>G) and the other was a synonymous SNP (rs312619270 T>C). They were detected and genotyped by DNA pool sequencing and the forced PCR-RFLP method. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP), PCR-RFLP, and direct DNA sequencing are three popular methods for the detection of DNA mutations. DNA pool sequencing and forced PCR-RFLP were used in this study in preference to the numerous steps of the SSCP method, its inaccuracies, complicated technical demands, slow speed, and poor reproducibility. Interestingly, in this study the forced PCR-RFLP method was able to accurately detect polymorphisms in the chicken *ASB15* gene, and the positions of the mutations were found by DNA pool sequencing; the birds were genotyped using forced PCR-RFLP.

In view of the function of *ASB15* in growth and carcass traits, we firstly studied its SNPs. Our results revealed that two SNPs (rs315759231 A>G and rs312619270 T>C) of the *ASB15* gene are located in exon 7 and belong to different mutations. The rs315759231 A>G polymorphism results in an amino acid change, from Asn to Ser. This missense SNP polymorphism was mainly associated with traits related to carcass traits, including SEW, EW, LMW, and CW. Therefore, rs315759231 A>G may affect chicken carcass traits, particularly of the leg muscle. Its AA genotype had significantly higher trait values than the GG and AG genotypes. McDaneld and Spurlock (2008) reported that *ASB15* is an *ASB* gene family member that is predominantly expressed in skeletal muscle. *ASB15* participates in the regulation of protein turnover and muscle cell development, by stimulating protein synthesis and regulating the differentiation of muscle cells. The synonymous SNP (rs312619270 T>C) was associated with traits related to growth and carcass traits, including BW4, 8, 12, SL8, BBL12, BSL8, BSL12, BMW, and CW. These traits have significant additive effects, which would inhibit their use in breeding. Therefore, rs312619270 T>C may affect chicken growth. The trait values of the CT genotype were higher than those of the TT and CC genotypes. Dong et al. (2013) reported that *ASB15* is only expressed in heart and skeletal muscle, and has different expression levels in skeletal muscle at different stages. The expression level of *ASB15* is increasing with its growth at different stages (Dong et al., 2013).

Polymorphisms in the *ASB15* gene probably affect transcription factor binding and mRNA expression levels. These associations can be explained as follows: firstly, the missense mutation (rs315759231 A>G) could affect mRNA expression; secondly, the synonymous SNP (rs312619270 T>C) is a silent mutation; however, some synonymous changes can affect splicing, mRNA stability, or translational efficiency (Chamary et al., 2006).

In conclusion, the SNPs rs315759231A>G and rs312619270 T>C were identified in the seventh exon of the chicken *ASB15* gene. The rs315759231 polymorphism was significantly related to SEW, EW, LMW, and CW, and the rs312619270 polymorphism was significantly related to BW and body measurements. Therefore, the *ASB15* gene probably has a profound effect on growth and carcass traits in the chicken. These SNPs can assist researchers in selecting optimal strains and phenotypes for further experiments, because growth and carcass traits are important characteristics in the poultry industry. Our analyses also provide a basis for the further functional annotation of *ASB15*. *ASB15* may be a good candidate gene to improve growth and carcass traits in the chicken, but further research is required to confirm this.

Conflicts of interest

The authors declare no conflict of interest.

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