

Identification and characterization of *SREBF2* expression and its association with chicken carcass traits

F. Ye^{1*}, M.H. Qiu^{2*}, H.Y. Xu^{1*}, X. Lan¹, Q. Zhu¹, X.L. Zhao¹, H.D. Yin¹, Y.P. Liu¹ and Y. Wang¹

¹Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu Campus, Chengdu, Sichuan, China ²China Animal Breeding and Genetics Key Laboratory of Sichuan Province, Sichuan Animal Science Academy, Chengdu, Sichuan, China

*These authors contributed equally to this study. Corresponding author: Y. Wang E-mail: as519723614@163.com

Genet. Mol. Res. 15 (3): gmr.15038514 Received February 1, 2016 Accepted April 8, 2016 Published September 2, 2016 DOI http://dx.doi.org/10.4238/gmr.15038514

Copyright © 2016 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License.

ABSTRACT. The sterol regulatory element-binding transcription factor 2 gene (*SREBF2*) plays an important role in regulating lipid homeostasis. To reveal the genetic factors that underlie carcass fat deposition in chickens, we cloned the coding DNA sequence of chicken *SREBF2*, investigated *SREBF2* mRNA expression levels in various tissues, detected single nucleotide polymorphisms (SNPs) in the exon regions of the gene, and conducted association analyses between single markers/haplotypes and carcass traits. The entire 2859-bp cDNA sequence of chicken *SREBF2* that encoded 952 amino acids was obtained and characterized. *SREBF2* mRNA was highly

Genetics and Molecular Research 15 (3): gmr.15038514

expressed in the uropygial gland, followed by the liver, breast muscle, and leg muscle. Ten SNPs were detected, and four (g.49363077T>A, g.49357503C>T, g.49355533G>A, and g.49354641G>A) were novel. When analyzing the associations between the single mutations and carcass traits, significant differences were found in three SNPs and g.49357915G>A was highly significantly associated with most carcass traits, except for abdominal fat weight and sebum thickness. In addition, haplotype combinations that were constructed using the *SREBF2* SNPs were associated with breast muscle weight. Chickens with the combined genotype H21H21 had the highest live weight, carcass weight, eviscerated weight, and semi-eviscerated weight values. To the best of our knowledge, this is the first study conducted on chicken *SREBF2* polymorphisms, which are predictive of the genetics that underlie the economic performance of chickens.

Key words: Chicken; *SREBF2* gene; Cloning; Expression; Polymorphism

INTRODUCTION

Many factors influence chicken meat quality, including muscle development and tenderness and subcutaneous, abdominal, and intramuscular fat deposition. A suitable fat content can improve the quality of the chicken, but excessive fat deposition has many negative effects. With the continued improvement of living standards in China, the incidences of obesity, diabetes, and cardiovascular system diseases have also increased (Kaidar-Person et al., 2011; Reaven, 2011); therefore, dietary fat content is receiving an increasing amount of attention. Reducing body fat deposition by the regulation of poultry fat metabolism has become an important subject for many researchers.

Sterol regulatory element-binding proteins (SREBPs) belong to the nuclear transcription factors family and are important regulatory factors in animal body fat synthesis. Therefore, SREBP transcription factors are pivotal activators of key enzymes involved in cholesterol synthesis, low-density lipoprotein endocytosis, fatty acid synthesis, and glucose metabolism (Edwards et al., 2000; Zhao and Yang, 2012). There are three SREBP isoforms in mammals and birds: SREBP-1a, SREBP-1c, and SREBP2. SREBP-1a and SREBP-1c are encoded by the *SREBF1* gene, whereas SREBP2 is encoded by the *SREBF2* gene (Le Hellard et al., 2010). The transcriptional activity, tissue distributions, and modes of regulation of the SREBP-1a, SREBP-1c, and SREBP-1c, SREBP-1c, and SREBP-1c, and SREBP-1c, isoforms differ (Shimano et al., 1997; Bommer and MacDougald, 2011). Collectively, SREBPs can activate the transcription of virtually all of the genes involved in the synthesis of cholesterol, fatty acids, and phospholipids (Bommer and MacDougald, 2011).

Gene expression studies have revealed that SREBP-1a and -1c preferentially activate the transcription of genes involved in fatty acid synthesis, whereas SREBF2 is involved in cholesterol biosynthesis. At present, SREBF2 research is focused on mice and humans. For example, Yang et al. (2015) found that polymorphisms of *SREBF2* (rs1052717 and rs2267443) contribute to the underlying pathophysiology of metabolic syndrome in patients treated with clozapine. In studying the relationship between *SREBF2* and obesity and serum lipid levels in children and adolescents, Liu et al. (2014) found that carriers of GC/CC genotypes of the

Genetics and Molecular Research 15 (3): gmr.15038514

SREBF2 rs2228314 polymorphism have a higher risk of abnormal high-density lipoprotein cholesterol levels than do individuals carrying the GG genotype. In the chicken, Zhang et al. (2014) found that the *SREBP2* expression level in the liver is highest at the 21days of embryonic stage. Therefore, based on previous research, we speculated that chicken *SREBF2* might play an important role in carcass fat deposition.

To the best of our knowledge, this is the first investigation of chicken *SREBF2* expression and polymorphisms. In this study, we isolated the full coding DNA sequence (CDS) of Erlang Mountain chicken *SREBF2* for the first time, analyzed its nucleotide sequence, investigated its expression levels in different tissues, and detected its sequence variants in 10 chicken populations; subsequently, we investigated the associations between the sequence variants and carcass traits. This study provides useful information on chicken genetics and breeding.

MATERIAL AND METHODS

Sample collection

The study was conducted in strict accordance with the requirements of the Animal Ethics Committee of Sichuan Agricultural University. The chickens that were involved in this study were humanely sacrificed to reduce suffering. Twenty Erlang Mountain chickens (10 hens and 10 cocks, 13 weeks of age) were provided by the poultry breeding farm of Sichuan Agricultural University and used to clone chicken *SREBF2* and for mRNA expression analysis. The chickens were randomly selected and slaughtered at the same time, and six fresh tissue types (liver, breast muscle, thigh muscle, abdominal adipose tissue, sebum cutaneum, and uropygial gland) were collected, immediately placed in liquid nitrogen, and stored at -80°C for RNA extraction.

To screen for single nucleotide polymorphisms (SNPs) and perform an association study, 120 Erlang Mountain chickens (including the SD02, SD03, SD01 x SD02, and SD01 x SD03 lines) and 180 high-quality Sichuan Daheng broilers (including the S01, S02, S03, S05, S06, and D99 lines) were randomly selected; all were 13 weeks old. During their growth period, all of the chickens had access to food and water *ad libitum*, were housed under the same temperature and light conditions, and their nutrition levels were completely consistent. After slaughter on the same day, live weight (LW), carcass weight (CW), eviscerated weight (EW), semi-eviscerated weight (SEW), breast muscle weight (BMW), leg muscle weight (LMW), abdominal fat weight (AW), and sebum thickness (ST) were measured. All of these performance traits were determined as described in "The Poultry Production Performance Terms and Measurement Statistics Method" (NY/T823-2004). Venous blood samples were taken from under the wings and prepared for DNA extraction.

RNA isolation and cDNA synthesis

Total RNA was extracted from the fresh tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer protocol, and was then dissolved in RNase-free water. The integrity of the RNA was evaluated by electrophoresis on 1.0% agarose gels, and the concentration and purity of the RNA were measured using a NanoVue Plus[™] spectrophotometer (Thermo Scientific, USA).

cDNA synthesis was performed in a volume of 10 µL with 1 µg total RNA using

Genetics and Molecular Research 15 (3): gmr.15038514

a PrimeScriptTM RT Reagent Kit (TaKaRa, Dalian, China), according to the manufacturer instructions. The reaction conditions for cDNA synthesis were 37° C for 15 min followed by 85° C for 5 s and storage at 4° C.

Molecular cloning of Erlang Mountain chicken SREBF2 cDNA

The predicted gene sequence of chicken *SREBF2* (XM_416222.2) was downloaded from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi. nlm.nih.gov/). Sequences of all of the primers that were used in this study are listed in Table 1. The polymerase chain reaction (PCR) (total volume of 50 μ L) contained 4.0 μ L first-strand cDNA, 25 μ L buffer, 8.0 μ L dNTP, 0.5 μ L LA *Taq* DNA polymerase (TaKaRa), 2 μ L each primer (10 pmol), and 8.5 μ L RNase-free H₂O. The optimum conditions for amplification were 10 min at 94°C, 35 cycles of denaturation at 94°C for 50 s, and annealing at 58°-63°C. The PCR products were subjected to electrophoresis on 1% agarose gels and purified using an E.Z.N.A.[®] Gel Extraction Kit (Omega, USA). The principal product was cloned into a pMD18-T vector (TaKaRa), and three randomly selected positive clones were sequenced by HuaDa Biotechnology Co. Ltd. (Beijing, China).

Usage	Name	Sequence (5'-3')	Annealing temperature (°C)	Fragment (bp		
Cloning	1	F: ATGTCCCGTGGAACCAACC	58.0	1453		
U		R:GATGGAGCTTGTGGTAGGC				
	2	F: AAGACGGACGGCAATC	61.5	2663		
		R:TGAGGGCTGGTGAGGTGTTAG				
	3	F: ACCAGTGGAGGGCCAGAGA	63.0	1533		
		R: ATGGAGGAAGTCCGGGCT				
RT-PCR	SREBF2	F: ACTCAATGGGAAGTGGAGCAC	58	161		
RIJER		R: cactatgctgaaacgtgacctc		-		
	B-actin	F: GAGAAATTGTGCGTGACATCA	57.2	180		
	<i>p</i>	R: CCTGAACCTCTCATTGCCA				
	F1	F: GGTCCAGCCTCAGATCATCAA	55	230		
		R: TCCCCACCGTTAGAAA				
	F2	F: GGCTGAATGCTGGTGACACTT	53			
		R: TTACCTTGGCGTCTGT				
SNP	F3	F: AACCCTGGAAGCACGTTGTAC	55	244		
		R: CAATGATAAAGAACCGAAAG				
	F4	F: GAACTGTTGAAGGGCATTGAC	55	230		
		R: TGTGGCCCTTAAGTAACTCTA		- 10		
	F5	F: TITITCATCICCCCCACCAA	60	248		
	F((2	201		
	FO	F: IGUUCAUGUIGAIUUI P: TCCTCCTACGAGACGCATGTG	62	201		
	17		(2	249		
	r/	R: CGCTGGTCTTGGCCTCGTC	65	248		
	F8	F: CCTCCAGCTCCGCTTAC	60	187		
	10	R' TAGCAGAGGACGACACCGTGA	00	107		
	F9	F ⁻ TCCTTTCCCCAGAGCTATTTC	61	236		
	- /	R: GGCTCCCAGGGCAAAGTACA				
	F10	F: CACAAGTTCATCAGGCGTTCT	56	196		
		R: AGACTAACCCGCACATT				
	F11	F: CTTCTTGTTTCAGCGAGTTCT 56				
		R: CATTTCCTGCAGCTAGTGG				
	F12	F: ACCCGTGACTCCGTTCTTG	ACTCCGTTCTTG 60			
		R:TTTAGGAGCAGCACCACGCAC				
	F13	F: CTGTGAAGGCACGGCTCT	60			
		R: ATGGTGTGTAGCCCTTACGTT				
	F14	F: CCTGGTGCTGAGCCGTGTCTG	64	235		
		R: CCAAAGCTCACCTTGCGGTAC				
	F15	F: CCTCCAGGTCTTCCTTCACGA	61	167		
		R: CTACACAGCACCCAATAGCC				
	F16	F: AGGGCAGAGGGAGCGAG	62	217		
		R: AGGGCTGGTGAGGTGTTAGGA	(2)	102		
	F17	F: GCCAGCGTGCCGTCCTG	62	182		
		K: IUCUCAATICUTTIIGCAACA				

RT-PCR = reverse transcription-polymerase chain reaction; SNP = single nucleotide polymorphism.

Genetics and Molecular Research 15 (3): gmr.15038514

Sequence analysis

The assembled cDNA sequence was evaluated by DNAMAN 6.0. Homologs were identified in GenBank using a tBLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search. The open reading frame (ORF) of chicken *SREBF2* was detected using the NCBI ORF-finder tool (http://www.ncbi.nlm.nih.gov/projects/gorf/). The phosphorylation sites of the SREBF2 protein were predicted by the NetPhos 2.0 server (http://www.cbs.dtu.dk/services/NetPhos), and the presence and locations of signal peptides were predicted using the SignalP 4.0 server (http://www.cbs.dtu.dk/services/SignalP). The transmembrane domain of the deduced amino acid (AA) sequence was predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0), and the secondary structures of the deduced AA sequence were predicted by HNN (http://npsa-pbil.ibcp.fr/cgi-bin/npsa automat.pl?page=/NPSA/npsa hnn.html).

Expression analysis of chicken SREBF2

Total mRNA from the six chicken tissues was extracted to investigate the mRNA expression profiles of chicken *SREBF2* using real-time PCR. Each real-time PCR was conducted in triplicate. β -actin (housekeeping gene) was used as an internal control for each sample. The primers were designed according to the predicted mRNA sequence of chicken *SREBF2* (XM_416222.2) and the β -actin sequence (Table 1).

All of the reactions were performed in a CFX96 Real-Time PCR detection System (Bio-Rad, USA). Each reaction (total volume of 15 μ L) contained 7.5 μ L 2X SYBR[®] Premix Ex *Taq*TM, 0.5 μ L each primer, 1 μ L normalized template cDNA from each tissue, and 9.5 μ L sterile water. The reaction was performed as follows: initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s and 58°C for 25 s, and a final temperature increment of 0.5°C/s from 58 to 95°C. Chicken *SREBF2* mRNA expression was calculated relative to the amount of β -actin present.

The real-time PCR data were analyzed using the comparative Ct method (Schmittgen and Livak, 2008); Ct values are the means of the samples, which were tested in triplicate. Gene expression was calculated as $2^{-\Delta ACt}$ ($\Delta \Delta Ct = Ct$ target - Ct internal control), which indicates an n-fold difference relative to the expression of the internal control gene. The differential expression of *SREBF2* among the six tissues was analyzed by analysis of variance in SAS version 6.12 (Statistical Analysis Systems Institute Inc., Cary, NC, USA). Multiple comparison analysis was conducted using the Duncan test. Comparisons were considered significant at P < 0.05.

SNP scanning and genotyping

Seventeen pairs of primers (Table 1) were designed based on the *SREBF2* sequence (ENSGALG00000011916), and were synthesized by Shanghai Yingjun Biotechnology Co. Ltd. (Shanghai, China). The 10- μ L reaction mix contained 5 μ L 2X *Taq* PCR Master Mix (including Mg²⁺, dNTPs, and *Taq* DNA polymerase; Beijing TIAN WEI Biology Technique Corporation, Beijing, China), 0.4 μ L each primer, 0.8 μ L DNA template (50 ng/mL), and 3.4 μ L ddH₂O. The cycling protocol was as follows: 94°C for 4 min, 35 cycles at 94°C for 30 s, 58°C (or another appropriate annealing temperature, as shown in Table 1) for 30 s and 72°C for 1 min, with a final extension at 72°C for 8 min. Genetic variants in the *SREBF2* genomic sequence were analyzed using the PCR-single-strand conformation polymorphism

Genetics and Molecular Research 15 (3): gmr.15038514

(SSCP) method. Briefly, after denaturation at 99°C for 10 min, 3 μ L PCR product was rapidly cooled on wet ice and then loaded onto 16 x 18 cm, 12% acrylamide:bisacrylamide (39:1) gel. Electrophoresis was performed at 200-300 V for 13-15 h in 1X TBE buffer, and the gel was silver-stained. Three DNA samples that exhibited different patterns on the SSCP gel were further amplified and purified, and were then sequenced by the Shanghai Yingjun Biology Technique Corporation.

Statistical analysis

Genic and allelic frequencies were determined for each population by direct counting. The Hardy-Weinberg equilibrium (HWE), heterozygosity, homozygosity, and effective allele number were statistically analyzed according to the previous approaches of Nei and Roychoudhury (1974) and Nei and Li (1979). The polymorphism information content (PIC) was calculated according to Botstein et al. (1980)'s methods.

The linkage disequilibrium (LD) structure, as measured by D' and r^2 , was constructed using the Haploview software version 3.32 (Barrett et al., 2005), and haplotypes were constructed using the PHASE program version 2.0 (Stephens et al., 2001). Association analyses between single SNP-marker genotypes and the carcass traits were performed using the general linear model procedure in SAS 6.12. The model used was as follows:

$$Y = \mu + B_i + S_j + G_k + B_i \times S_j \times G_k + e_{ijk}$$
(Equation 1)

where Y is the trait being measured; μ is the population mean; B_i is the fixed effect of breed; S_j is the fixed effect of sex; G_k is the fixed effect of genotype; $B_i \ge S_j \ge G_k$ is the interaction among breed, sex, and genotype; and *e* is the random error. Values are reported as least square means \pm SEM. Statistical significance was evaluated using the Duncan test, and differences were considered significant at P < 0.05.

RESULTS

Characterization of the chicken SREBF2 sequence

The BLAST result from the NCBI's nucleotide sequence database revealed that Erlang Mountain chicken *SREBF2* was significantly similar to mammal *SREBF2* sequences. The Erlang Mountain chicken *SREBF2* CDS extended from position 1 to 2859 within the cDNA sequence, and encoded a 952-AA protein (Figure 1).

The deduced AA sequence's molecular weight was 103.76 kDa and its isoelectric point was 8.71. The chicken *SREBF2* sequence had 88 negatively charged residues (Asp + Glu) and 104 positively charged residues (Arg + Lys), which indicated that the protein should have an overall positive charge. Hydropathy correlation analysis revealed that the protein was highly hydrophilic. Fifty-eight phosphorylation sites were predicted by the NetPhos 2.0 server (Table 2); no signal peptides were identified by SignalP 4.0. The TMHMM results indicated that the protein had one transmembrane domain that was located between 17 and 33 AA. The secondary structure of the protein was predicted to be 46.95% α -helix, 43.70% random coil, and 9.35% extended strand (Figure 2).

Genetics and Molecular Research 15 (3): gmr.15038514

1 atgtcccgtggaaccaaccggccccaaacgttccctttcctcctt	811 gatgcaaaggttaaggatgaacctgactctcctcctgtggctctt
MSRGTNRPQTFPFLL	
A E M L Q F V S N Q A G D F P	GMVDRSRILLCALTF
91 gacctgttttcggaccctctgtgcggcaccttccagggtggcggt	901 ctgtgcctctccttcaaccctttgacatcccttctggatgcccga
D L F S D P L C G T F Q G G G 136 ggcggtggtggtggtagtggcacgggcgttctggtccagcctcagatc	L C L S F N P L T S L L D A R 946 gggagtccggagtccgacagcctcacgcgccacggctctggcagg
G G G G S G T G V L V Q P Q I 181 atcaagacggactccctcgtgctgaccacgctgaagacggacg	G S P E S D S L T R H G S G R 991 aacaigetgaccattgagtecgacacaggtggatggtttggetgg
IKTDSLVLTTLKTDG	N M L T I E S D T G G W F G W
226 aatcctgtcatggctgcagtgcagaacccagcgctcaccgcgctc	1036 atgatgcccacgctgatcctgtggctcgtgaatggtgtgatcgtc
271 accactcccatccagaccacagcactgcagacccttgttgggagc	1081 ctcagcgtgtttgtgaagctcctcgtgcacggagagccagtgacc
316 aatggaaccattctgaccacatgccagtgatgatgggggggg	1126 cggctgcactcgaggtcgtcggtcacgttctggaggcaccgcaag
361 aaggtgcctatcaacaagttcctggaggtgtcaagcaaccagac	1171 caggcagacctggacctggcacggggggattttgctgcggcagca
406 ccacctaaggaaggagaaggagaacaactcacaacatcattgaa	1216 tcaaacctgcagacctgcctgcccgggccgagcactgccg
PPKEGERRTTHNIIE	SNLQTCLSVLGRALP
451 aagcgttaccgatcctctataaatgacaagatcattgagctgaag K R Y R S S I N D K I I E L K	1261 gcctcccgcctggacctggcctgcagcctctcctggaacgtcatc A S R L D L A C S L S W N V I
496 gacctcatcatggggacagacgccaagatgcacaagtctggagtc D L I M G T D A K M H K S G V	1306 cgctacagoctgcagaagctagcgctggtggggggggggg
541 ctgagaaaagccattgattacatcaaatacctgcagcaggccaac	1351 aggaceteteaceagtggagggeeagagaggeeactgegggetet
L R R A I D Y I K Y L Q Q A N	N T S H Q W R A R E A T A G S
H K L R Q E N H V L K L A N Q	E D E A K T S A R D A A L A Y
631 aaaaacaaactgttgaagggcattgacctaagcagtctggttgac K N K L L K G I D L S S L V D	1441 cacaagetecatcagetecacatcacaggtaaacteceetecage
676 aacgatgcggacctgaagatagatgacttcaaccagaatgttctt N D A D L K I D D F N O N V L	1486 tccgcttactccggtttgcacatggccctgtgtgccgtcaatctg
721 ctgatgtctcctcccgcctctgattcgggatcacaggctggct	1531 gccgagtgtgccgaagagaagatcccgcccagcacaatggctgaa
L M S P P A S D S G S Q A G F	AECAEEKIPPSTMAE
S P Y S I D S E P G S P L L D	1576 atccacctgacggctgcagttggcctgaagacccgctgcggaggc
	I I I I A A T O I A I A C O O
1621 aagctgggcttcctggcgagctatttcctaagccaggcgcagagc	2251 agctcactgagtcactgcgagagggccagcagccacctgtggaac
KLGFLASYFLSOAOS	SSLSHCERASSHLWN
1666 statacagetcagagegeagegeatcoccaactcoctagettag	2296 agcetcaacatgagcagcggtgcetccagcactecectcagcaac
	S L N M S S G A S S T P L S N
	2341 gtgatccagctgctggcctgtgacttgctgctgtccctccgcacc
	VIOLLACDLLLSLRT
LCHPLGQKFFVLKSW	
1/56 acggtgaagtetgetgecaaggagageetgtaetgeacceagagg	2386 adoct at accade a a cad contrat a a cad contrat a a
	2386 agcctgtggcagaagcagcggcgagcagcagccaggcggtgag
TVKSAAKESLYCTQR	2386 agcctgtggcagaagcagcgagcagcagcagcggggtgag S L W Q K Q A S S S Q A L G E
T V K S A A K E S L Y C T Q R 1801 aaccccgcggatcccatagcacaagttcatcaggcgttctgtgag	2386 agoctgtggcagaagcagcagcagcagcagcagggtggg S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcaccccccgagctcactggcttccaggtgac
T V K S A A K E S L Y C T Q R 1801 aaccccgcggatcccatagcacaagttcatcaggcgttctgtgag N P A D P I A Q V H Q A F C E	2386 agcctgtgcagaagcaggcagcagcagccagccggtggtaga S L W Q K Q A S S S Q A L G E 2431 acctaccatgctcacccccgagctactggctcaggtgac T Y H A S P P E L T G F Q R D
T V K S A A K E S L Y C T Q R 1801 aaccccgcgatccatagcacaagtcatcagcggtctgtag N P A D P I A Q V H Q A F C E 1846 aacctgctggagagagcagtggatcccttgtgaagcctaggac	2386 agcctgtgcagaagcaggcgagcagcagccagctggtgag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccgagctcactggcttccaggctga T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcagctggccccaggctcaggccccggtac
T V K S Å Å K E S L Y C T Q R 1801 aaccegeggatcceatagcacaagttcatcaggggttctgtag N P Å D P I Å Q V H Q Å F C E 1846 aacctgetggagagegeggtggattcettggeaggecteagae N L L E R Å V D S L V K P Q T	2386 agcctgtgcagaagcaggcagcagcagccagccggtggtag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccgagctcactggcttccagcgtgac T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgccacgcgccacggctcacggccccacgtac L G S L R K L A H G F R P A Y
T V K S A A K E S L Y C T Q R 1801 aaccccgcgatccatagcacaagtcatcagcgcttctgtag N P A D P I A Q V H Q A F C E 1846 aacctgctggagagagcagtggatcccttgtgaagcctcagac N L L E R A V D S L V K P Q T 1891 aqgaaagagtgtgtggacaagaggaggagcagcagatcc	2386 agcctgtgcagaagcaggcagcagcagccagccggtggtgag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccgagctcactggcttccaggtgag T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcaagctggcccacggcttcaggcccgcgtac L G S L R K L A H G F R P A Y 2521 cgcaaggtcttcctcactgagccacgtgcqccgctgcqcggggggg
T V K S Å Å K É S L Y C T Q R 1801 aaccecgeggatcecatagcacaagtteateateagegttetgtgag N P Å D P I Å Q V H Q Å F C E 1846 aactgetggagagagagtggatteettggaggetggagt N L E R Å V D S L V K P Q T 1891 aggaaagagtggtggtggacaaggaggacgaacgetgegagtte R K E V V G Q E E D E P C E F	<pre>2386 agcctgtgcagaagcaggcagcagcagccagctggtgag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccgagctcactggcttcaggcggg T Y H A S P P E L T G F Q R D 2476 ctcggcagctgcqcaagtggcgccacgggctcaggccccgctac L G S L R K L A H G F R P A Y 2521 cgcaaggtcttcctcagccgcggggg R K V F L H E A T V R L M A G</pre>
T V K S A A K E S L Y C T Q R 1801 aacccqcqqatcccataqcacaagttcatcaqqcgtctcqtaq N P A D P I A Q V H Q A F C E 1846 aacctqctqqaqaqaqcqqtqqattcccttgqaaqcctaqacc N L E R A V D S L V K P Q T 1891 aqqaaaqaqgtqqtqqqacaaqaqqqqqcqaaccqtqcqagttc R K E V V G Q E E D E P C E F 1936 tccaqqcqcqstqqaatacctqaactqcttdqac	2386 agcctgtgcagaagcaggcagcagcagcagccagctggtgag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccaggctcactggctccaggtgac T Y H A S P P E L T G F Q R D 2476 ctcggcagctgcgcaagctggcccacggcttcaggccgcgtac L G S L R K L A H G F R P A Y 2521 cgcaaggtttccttcagaggccaccgtgcgcctgatggcggg R K V F L H E A T V R L M A G 2566 gccagcctaccgcacccacgctgtcggagacagttcagg
T V K S A A K E S L Y C T Q R 1801 aaccccgcggatcccatagcacaagtcatcagcaggctccgtagg N P A D P I A Q V H Q A F C E 1846 aacctgctggagagcagtggatcccttgtgaagcctcagacc N L L E R A V D S L V K P Q T 1891 aggaaagagtgtgggacaaggaggaggagacggggtc R K E V V G Q E E D E P C E F 1936 tccagcggatggatccctgaactgtccaactcttcctggac S A M E Y L K L L N S F L D	2386 agcctgtgcagaagcaggcagcagcagccagctggtgtag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccaggctcactggcttcaggctgca T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcaagctggcccagggctcaggcccgcgtac L G S L R K L A H G F R P A Y 2521 cgcaaggtcttcctcaggccccgcgcggcg R K V F L H E A T V R L M A G 2566 gccagcctacccgccaccaccaccgcgcggcggaga A S P T R T H Q L L E H S L R
T V K S A A K E S L Y C T Q R 1801 aacccqcqqatcccataqcccaagttcatcaqqqqttqq N P A D P I A Q V H Q A F C E 1846 aacctqctqqaqaqqqtqqattccttgtqaqqctcaqac N L E R A V D S L V K P Q T 1891 aqqaaaqaqgtqgtqgqcaaqaqqqqqacqaccqtqcqaqttc R K E V V G Q E E D E P C E F 1936 tccaqcqcqtqtqqatacctqaattqctaacttcttcctqqcc S S A M E Y L K L L N S F L D 1981 tcascqaqqatcqtqqacctttgccaqttcta	2386 agcctgtgcagaagcaggcagcagcagcagccggtgtggtag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccgagctcactggctccaggtgac T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcaagctggcccacggtcacggccgcgtac L G S L R K L A H G F R P A Y 2521 cgcaaggtcttccttcacgaggccaccgtgcgcctgatgggggg R K V F L H E A T V R L M A G 2566 gccagcctaccgcaccacgacgtgggagcaggatctagg A S P T R T H Q L L E H S L R 2611 cgtcgacaaccgtagagcacagatggaccggtggacggtggacggatggacggtggacggtggacggtggacggtggacggatggacggtggacggatggacggtggacggatggacggtggacggtggacggtggacggatggacggtggacggatggacggtggacggatggacggtggacggatggat
T V K S A A K E S L Y C T Q R 1801 aaccccgcgatccatagcacaagtcatcaggcgttctgtag N P A D P I A Q V H Q A F C E 1846 aactgctggagagagcgtggttccttgtgaagcctcagacc N L L E R A V D S L V K P Q T 1891 aggaaagagtggtggtgggacaagaggaggagcgaaccgtgggttc R K E V V G Q E E D D P C E F 1936 tccaggcgatggtggactcaattgctcaattcttctggac S S A M E Y L K L L N S F L D 1981 tcatgggaagtggagcaccgcctttgccagcagttttatgctg M G S G A P F A S S M L	2386 agcctgtgcagaagcaggcagcagcagcagccagctggtgtag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccaggctcactggcttccaggctgca T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcaagctggcccacggctcaggcccgcgtac L G S L R K L A H G F R P A Y 521 cgcaaggtctcctcacgagcagcagcgcgcgaggg R K V F L H E A T V R L M A G 2566 gccagcctcaccgcaccaccagctgctggagcacagtccagg A S P T R T H Q L L E H S L R 2611 cgccgcacacccgaagcagcaaggaggagcggagaccggaacagg R K V F L H Z A T V R L M A G 2566 gccagccctacccgcagctgctggagcacagtccagg A S P T R T H Q L L E H S L R 2611 cgccgcacacccgaacagcaagcaaggaggagctggacacagctg
T V K S A A K E S L Y C T Q R 1801 aacccqcqqqacccatqqcacatctcatcaqqqqtctqtqq N P A D P I A Q V H Q A F C E 1846 aacctqctqqaqaqqqcqtqqtcctctqtqaqqctcaqac N L E R A V D S L V K P Q T 1891 aqqaaqaqqtqqtqqqcacaqqqqqacaaccqtqcqaqttc R K E V V G Q E E D E P C E F 1936 tccaqcqcqtqqaatacctqaattqctaactctttcctqqac S S A M E Y L K L L N S F L D 1981 tccatqqqaqtqqqqaccccttqccaqttcttqtq S M G S G A P P F A S S S M L 2026 aattcaqcqcatqq	2386 agcctgtgcagaagcaggcagcagcagcagccagctggtgag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccaggctcactggcttcaggctgag T H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcaagctggccccaggctcaggcgcgcg L G S L R K L A H G F R P A Y 2521 cgcaaggtcttccttcacgaggccaccgtgcgcctgatggggg R K V F L H E A T V R L H A G 2566 gccagcctaccgcacccacgacgctggagcacagtctcagg A S P T R T H Q L L E H S L R 2611 cgtcgcacaaccgagacagctggagccggaccgattcag R R T T Q N S K Q G E L D T L 2656 cacaggagaaggaggtggactgaccgt
T V K S A A K E S L Y C T Q R 1801 aaccccgqgatcccatagcacaagtcatcaggcgttctgtgag N P A D P I A Q V H Q A F C E 1846 aacctgctggagagagcgtggattcccttggaagcctcagac N L L E R A V D S L V K P Q T 1891 aggaaagagtggtgtgggaacagagagagagagacgaaccgcgagttc R K E V V G Q E E D E P C E F 1936 tccaggcgsattggagaattgctaattgctaactctttctcgac S S A M E Y L K L L N S F L D 1981 tcaatgggaagtggagcaccgcctttgccaggtgttcttgctg S M G S G A P P F A S S S M L 2026 aaatacacctgggccggatggtgtgtgtggtggtggtgggt	<pre>2386 agcctgtgcagaagcaggcagcagcagcagctggtgtgg S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccgagctcactggcttccaggctg T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcaagctggcccacggctcactggcccgcgtac L G S L R K L A H G F R P A Y 2521 cgccaaggtctctctcacgagcgccgccgtggcggg R K V F L H E A T V R L M A G 2566 gccagcctacccgcaccactgctggggcacagtctcagg A S P T R T H Q L L E H S L R 2611 cgccgcacacccgaagcagcaaggagggggggggggg</pre>
T V K S A A K E S L Y C T Q R 1801 aacccqcqqqacccataqcacaaqttcatcaqqqqtctqqq N P A D P I A Q V H Q A F C E 1846 aacctqctqqaqaqqcqqtqqtccctqqqaqacctcaqacc N L E R A V D S L V K P Q T 1891 aqqaaqaqqtqqtqqqacaaqaqqaqacaacqqtqqqqtc R K E V V G Q E D E P C E F 1936 tccaqcqqqqqqaatacctqaaattqctcaactctttcctqqac S S A K E Y L K L L N S F L D 1951 tccatqqqaqqtqqqqcacqcctttgccaqcaqttctatqctq S M G S G A P P F A S S S M L 2026 aactacaqcccqqcqcqqtqqtqtqqqtqqqtqqqtqqqt	2386 agcctgtgcagaagcaggcagcagcagcagccagctggtgag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccaggctcactggcttcaggctgac T Y H A S P P E L T G F Q R D 2476 ctcggcagcatgctggccacaggtctcaggccccggtac L G S L R K L A H G F R P A Y 2521 cgcaaggtctcctcagccgcagtg R K V F L H E A T V R L M A G 2566 gccagccctacccgcaccaccagctggtggagcacagtctcagg A S P T R T H Q L L E H S L R 2611 cgtcgcacaacccagacagcaggaggaggtggacacgtg R R T T Q N S K Q G E L D T L 2656 ccagggagaggaggactgaccatgcctgctggcg R R T T Q N S K Q G E L D T L 2656 ccaggcacaggaggagctggacatgcctgcgqc P G Q R E R A T A I L L A C R
T V K S A A K E S L Y C T Q R 1801 aacccgcggatccatagcacaagttcatcaggggttcggtag N P A D P I A Q V H Q A F C E 1846 aacctgctggagagagcgtggtggagtccatggaggcctaagac N L E R A V D S L V K P Q T 1891 aggaaagaggtgggggacaagaggaggacgaaccgtgcgagttc R K E V V G Q E E D E P C E F 1936 tccagcggatggatacctgaattgctcatctttcctgac S S A M E Y L K L L N S F L D 1981 tcaatgggagaggaggacgacgtggtggtcggc S K G S G A P P F A S S S M L 2026 aaatcagccgatgggtgggtggtggtggtgggtggcgga K S A L G P D V V C R W M S A 2071 gcagtcgatatggcataggtagtaggaggaggacaagg	<pre>2386 agcctgtgcagaagcaggcagcagcagcagctggtgtgg S L W Q K Q A S S S Q A L G E 2431 acctaccactgcctcacccccgagctcactggcttcaggctggtgag T Y H A S P P L L T G F Q R D 2476 ctcggcagcetgcgcaagctggcccacggctcacggccgcgtac L G S L R K L A H G F R P A Y 2521 cgcaaggtcttcctacggccacgtgcgcqcgg R K V F L H E A T V R L M A G 2566 gccagcctaccgcaccacacgctgtggagcaaggtccaag A S P T R T H Q L L E H S L R 2611 cgccgcacacaccagacgacgaggagcaggtgcacacgt R R T T Q N S K Q G E L D T L 2656 ccagggagaggagcaggcacctggcccgcggcgcg R R T T A N A C R 2701 cacctccactctttcttgtcctcgcccagcgdgccggccgcg P G Q R E R A T A I L L A C R</pre>
T V K S Å Å K E S L Y C T Q R 1801 aacccqcqgatcccataqcacaagttcatcaqqqqtcqqq N P A D P I A Q V H Q A F C E 1846 aacctqctqqaqaqaqcqtqqatcccttqqaqacctcaqac N L E R A V D S L V K P Q T 1891 aqqaaqaqqtqqtqqqacaaqaqqaqqacaacqtqqtqtq R E V V G Q E D E P C E F 1936 tccaqcqqatqqaatcctqaattqctcaacttttcctqqac S S A M E Y L K L N S F L D 1951 tcatqqqaqtqqqacacqcctttqccaqttctt S M G S G A P P F A S S S M L 2026 aactaqccqcqctqqdqtqqqtqtqqtqtqqtqqtqqtqqtqqtqqtqqtqq	<pre>2386 agcctgtgcagaagcaggcagcagcagccagctggtgtag S L W Q K Q A S S S Q A L G E 2431 acctaccatgcctcacccccaggctcactggcttcaggctgc T Y H A S P P E L T G F Q R D 2476 ctcggcagcctgcgcaagctggcccacggctcaggcccgcagc L G S L R K L A H G F R P A Y 2521 cgcaaggtcttcccgagcaccatgcgcgcgcgcgg R K V F L H E A T V R L M A G 2566 gccagccctaccgcaccaccagctgctggagaacagtctcagg A S P T R T H Q L L E H S L R 2611 cgtcgcaaccccagagagagcagggcgtggaccaggcgg R R T Q N S K Q G E L D T L 2656 cccagggcagagggagcagccactgccagcagcggcgcgc C C C C C C C C C C C C C C C C C C C</pre>
T V K S A A K E S L Y C T Q R 1801 aacccqcqqatcccatqqcacaaqttcatcaqqqtttqtqq N P A D P I A Q V H Q A F C E 1846 aactqctqqaqaqaqtqqattccttgtqaqqctcaqac N L E R A V D S L V K P Q T 1891 aqqaaaqaqqtqqtqqqacaqaqqqqaqacqaccqtqcqaqttc R K E V V G Q E E D E P C E F 1936 tccaqcqqtqtqqatacctqaattqctaattqttq S A M E Y L K L L N S F L D 1981 tcastqqaqqtqqqacacqcacttttactqqc S K G S G A P P F A S S S M L 2026 aaattaqcctqqqcqqtqqtqcaqqtqcaqqtqc K S A L G P D V V C R W W S A 2071 gcaqtcqctatgqcattqqqtqqqqqcacqcqt A V M M A I G W L R G D D T A 2116 qtqqqtacqtttcaqctatqqqqqcccqcq	2386 agcctgtggcagaagcaggcaggcaggcaggcaggtgggggggg
T V K S Å Å K E S L Y C T Q R 1801 aaccegeggateceatageacaagtteateatgeggteteggag N P Å D P I Å Q V H Q Å F C E 1846 aacetgetggagagaegaeggtggatteeettggaaggeete N L L E R Å V D S L V K P Q T 1891 aggaaagagtggtgggaeaagagaggaegaaeggtggagte K E V V G Q E E D E P C E F 1936 teageggatggaateetgaatteettaetteettgae S Å K E V V G Q E L D P C E F 1938 teastggaatggageaegeettteeagetteatteetg S Å K E V L K L L N S F L D 1981 teastggaatggaegaegeetttggetgeaggtgetgetegea K S Å L G P D V V C R W W S Å 2026 aastaageeetggeeettggetgetgaggatgaeagetegatag K S Å L G P D V V C R W W S Å 2071 geettegettegeetaggtgetggetggetggetggetageaegte A V Å M Å I G W L R G D D T Å 2116 gtgaggteaegtteageatagtggageeettg	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
T V K S A A K E S L Y C T Q R 1801 aacccqcqqqacccataqcacaaqttcatcaqqqqttqqq N P A D P I A Q V H Q A F C E 1846 aacctqctqqaqaqqqcqtqqattccttqqaqactcaqac N L E R A V D S L V K P Q T 1891 aqqaaqaqqtqqtqqqcaaqqqqqaqacaacqqtqqaqttc R K E V V G Q E E D E P C E F 1936 tccaqcqcqtqqaatacctqaaattqctaactctttcctqqac S S A M E Y L K L L N S F L D 1981 tcaatqqqaqtqqqqcacqcctttgcaqqttct S M G S G A P P F A S S S M L 2026 aaatcaqcctqgtqqcatqqtqqtqqtqqtqqtqqtqqtqqtqqtqqtqqtqqtqq	2386 agcctgtggcagaagcaggcaggcaggcaggcaggcgggggggg
T V K S Å Å K E S L Y C T Q R 1801 aacceqcqqqacceataqcacaaqttcatcaqqqqtqqq N P Å D P I Å Q V H Q Å F C E 1846 aacctqctqqaqaqaqcqqtqqtcctctqtqaqacctaqacc N L E R Å V D S L V K P Q T 1891 aqqaaqaqqtqqtqqacaaqaqqaqacaqacqqtqqtqtq k E V V G Q E E D E P C E F 1936 tccaqcqqqtqqatacctqaattqtcaactttcctqqac S Å K E V V G Q E L D F C E F 1931 tcaatqqqaqtqqaccqqcctttqccaqcqttcatqctq S Å G S G Å P P F Å S S K L 2026 aactaqcccqqcqqcqqtqtqcaagtqqqqtqcaacaqt K S Å L G P D V V C R W W S Å 2021 cqutcqttqqcqctqqqqqqqqqqqqtqacaqct K S Å L G P D V V C R W S Å 2011 cqctqctqtqqccatqqtqtqctqaqqqqqtqcacaqt K S Å L G P D V V C R W S Å 2011 cqtqcqttqqtqqcatqqqctqcqaqtqcqqctqt A V Å M Å I G W L R G D D T Å 2116 gtqqqtcaqttqqqaqccctqqqqqqcccqcatqtqqqcccqc K S R F S I V E R L P K S L 2161 qqqqtcqcttqqqaqccctqqtqqaaqccctqcqc K S R F S I V E R L P K S L 2161 qqqttqqatqqaqccctqqtqqaaqccctqcqc	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
T V K S A A K E S L Y C T Q R 1801 aacccqcqqqacccataqcccataqtcatcaqqqqtcqqq N P A D P I A Q V H Q A F C E 1846 aacctqctqqaqaqqcqtqqtcatccttqqaqacctcaqacc N L E R A V D S L V K P Q T 1891 aqqaaqaqqtqqtqqqcaaqaqqqaqaacqcqqcqqtcq R K E V V G Q E E D E P C E F 1936 tccaqcqcqtqqaqtcqaattqctaattqctq S A K E Y L K L L N S F L D 1981 tcataqqqaqtqqqacccqcctttqccaqttcttqctq S A K E Y L K L L N S F L D 1981 tcataqqqaqtqqqacccqcctttqccaqttcttqctq S A K E Y L K L L N S F L D 2026 aattaqqqaqtqqqaccaqqatqqtqqtqqtqqqacaqcaqaqtc A S A L G P D V V C R W W S A 2071 qcaqtcqcttqqcattqqqatqqqqcccqcqctqcqaqttct A V A M A I G W L R G D D T A 2116 qtqqqtctqqqatqqccctqgtqaaqcccttqccaqagtqctqqcctqc V R S R F S I V E R L P K S L 2161 qaqqtcqctqqqatqqccctqtcqaaqqcqctcctqcqaqqccqct E M S E N A L V K A T F H L C 206 aatqccctqqqqtqtqtqtqqqqaqqqcaqqacqqcqcqq	2386 agcctgtggcagaagcaggcaggcaggcaggcgggtgggggggg

Figure 1. Nucleotide and deduced amino acid sequences of chicken SREBF2.

Table 2. Predicted chicken SREB	F2 phosphory	lation sites.				
Amino acid acids Phosphorylation of amino acid	Locus position	Point score	Locus position	Point score	Locus position	Point score
Serine	155	0.995	322	0.959	661	0.509
	156	0.982	328	0.884	711	0.990
	222	0.987	379	0.898	719	0.993
	243	0.665	382	0.986	723	0.516
	247	0.948	422	0.972	743	0.987
	249	0.623	453	0.767	744	0.828
	256	0.991	465	0.979	754	0.981
	259	0.648	472	0.997	761	0.905
	262	0.791	494	0.631	774	0.823
	266	0.927	496	0.810	805	0.696
	280	0.980	521	0.597	857	0.914
	310	0.980	558	0.966	877	0.588
	317	0.996	567	0.895	905	0.651
	320	0.983	594	0.670	911	0.676
Threonine	5	0.888	462	0.702	704	0.799
	92	0.708	471	0.713	820	0.692
	111	0.728	586	0.919	873	0.605
	145	0.780	630	0.985	874	0.983
	923	0.584				
Tyrosine	596	0.693				

Genetics and Molecular Research 15 (3): gmr.15038514



Figure 2. Schematic representation of the secondary structure of the chicken SREBF2 protein.

The deduced Erlang Mountain chicken *SREBF2* AA and gene sequences were compared with six *SREBF2* sequences from mammals and *Gallus gallus* using DNAMAN 6.0 (Table 3). The Erlang Mountain chicken *SREBF2* CDS was 99.8, 77.0, 76.2, 75.7, 78.2, 77.0, and 60.7% identical to that of *G. gallus, Homo sapiens, Mus musculus, Rattus norvegicus, Bos taurus, Canis lupus familiaris*, and *Danio rerio*, respectively. The deduced AA sequence of the Erlang Mountain chicken SREBF2 protein was 100, 82.4, 81.9, 81.7, 83.1, 82.3, and 53.8% identical to that of *G. gallus, H. sapiens, M. musculus, R. norvegicus, B. taurus, C. lupus familiaris*, and *D. rerio*, respectively.

Table 3. Similarity (%) of SREBF2 mRNA and amino acid sequences.										
Species	Bos taurus	Canis lupus familiaris	Danio rerio	Erlang Mountain chicken	Gallus gallus	Homo sapiens	Mus musculus	Rattus norvegicus		
Bos taurus	-	93.7	52.6	83.1	83.1	91.7	91.3	91.2		
Canis lupus familiaris	85.5	-	53.4	82.3	82.3	92.8	92.6	92.4		
Danio rerio	56.1	55.7	-	53.8	53.8	52.9	52.7	52.9		
Erlang Mountain chicken	78.2	77.0	60.7	-	100.0	82.4	81.9	81.7		
Gallus gallus	75.5	76.4	59.8	99.8	-	82.4	81.9	81.7		
Homo sapiens	84.6	86.5	56.1	77.0	76.3	-	92.7	92.9		
Mus musculus	81.2	83.3	55.4	76.2	75.4	84.7	-	96.5		
Rattus norvegicus	76.1	83.3	55.2	75.7	75.1	84.8	92.9	-		

Similarity of nucleotide sequences are shown below the diagonal and similarity of amino acid sequences are shown above the diagonal.

Tissue expression patterns of chicken SREBF2

We evaluated the relative RNA expression levels of chicken *SREBF2* in different tissues using the quantitative PCR method (Figure 3). Statistical analysis demonstrated that there were highly significant differences in *SREBF2* transcript levels among the six tissues tested (P < 0.01). In 91-day-old chickens, *SREBF2* mRNA was most abundant in the uropygial gland, followed by the liver, breast muscle, and leg muscle, and was at extremely low levels in the sebum cutaneum and abdominal fat.

Genetics and Molecular Research 15 (3): gmr.15038514



Figure 3. *SREBF2* expression in six tissues of the Erlang Mountain chicken as detected by quantitative polymerase chain reaction. Expression levels were normalized against β -*actin* and measured as 2^(-AACI) values. The results were averaged from three independent replicates that were measured at the 91-day-old stage. *Significant difference compared with other tissues.

Polymorphisms and genetic diversity

We investigated chicken *SREBF2* sequence variants by comparing our sequencing results with those of the chicken *SREBF2* sequence published in Ensemble (reverse-strand, Ensemble No. ENSGALG00000011916). Ten SNPs were identified; eight were in exons and two in introns. By comparing these results with those on the dbSNP database (http://www.ncbi.nlm.nih.gov/snp/), SNP1 (intron 6, g.49363077T>A), SNP5 (intron 12, g.49357503C>T), SNP7 (exon 14, g.49355533G>A), and SNP8 (exon 15, g.49354641G>A) were identified as novel SNPs, while the remaining SNPs were deposited as rs318011316 (SNP2, exon 7), rs13864629 (SNP3, exon 8), rs317877794 (SNP4, exon 11), rs10730451 (SNP6, exon 13), rs313438447 (SNP9, exon 16), and rs13864614 (SNP10, exon 19). Detailed information about the SNPs and AA changes are shown in Table 4. The genotyping of these SNPs was successfully performed using DNA sequencing and the PCR-SSCP method (Figure 4).

Table 4.	Genetic variation in chicken	n <i>SREBF2</i> .			
SNP	Position on chromosome	Location	Allele	AA exchange	Study result
SNP1	49363077	Intron 6	T>A	-	This study
SNP2	49362808	Exon7	C>T	-	rs318011316
SNP3	49361809	Exon8	T>C	-	rs13864629
SNP4	49357915	Exon11	G>A	-	rs317877794
SNP5	49357503	Intron12	C>T	-	This study
SNP6	49356963	Exon13	G>A	-	rs10730451
SNP7	49355533	Exon14	G>A	-	This study
SNP8	49354641	Exon15	G>A	-	This study
SNP9	49354218	Exon16	G>A	-	rs313438447
SNP10	49351627	Exon19	C>G	-	rs13864614

SNP = single nucleotide polymorphism; AA = amino acid.

Population genetic diversity parameters were estimated for the 10 populations (data not shown), and the statistical analysis revealed that A, C, A, A, C, G, G, G, and C alleles were predominant in the seven loci examined that contained SNP1, SNP2, SNP3, SNP4, SNP6, SNP7, SNP8, SNP9, and SNP10, respectively, in all 10 populations. Interestingly, unlike in the other populations, allele T was predominant at the SNP5 locus in population SD99. Four of the

Genetics and Molecular Research 15 (3): gmr.15038514

SNPs (SNP3, SNP4, SNP5, and SNP7) in the 10 experimental lines were in HWE (P > 0.05) with a medium amount of genetic diversity (0.25 < PIC < 0.50). Three of the SNPs (SNP1, SNP2, and SNP10) were also in HWE (P > 0.05) but exhibited low genetic diversity (PIC < 0.25). However, SNP6 and SNP9 were not in HWE in the S01 and SD02 populations or the S05 and S06 populations, respectively (P < 0.05 or 0.01, respectively); the other populations had no SNP6 or SNP9 polymorphisms. Unlike the other mutation loci, SNP8 was in HWE in all of the populations except for S01 and S02 (P > 0.05), and the mean PIC was less than 0.1. Therefore, these three markers (SNP6, SNP8, and SNP9) were excluded from later analyses.



Figure 4. Polymerase chain reaction-single-strand conformation polymorphism patterns of *SREBF2* in the Erlang Mountain chicken. a. SNP1; b. SNP2; c. SNP3; d. SNP4; e. SNP5; f. SNP6; g. SNP7; h. SNP8; i. SNP9; j. SNP10.

Linkage and haplotype reconstruction of chicken SREBF2

D' and r^2 are the main parameters in LD analysis. Several researchers have found that r^2 is not as sensitive as D' to allelic frequency (Zhao et al., 2007; Marty et al., 2010). In cases where $r^2 > 0.33$, a sufficiently strong LD is available for mapping (Ardlie et al., 2002). The LDs of the seven SNPs in the 10 chicken populations were estimated, and the D' values ranged from 0.000 to 1.000 and the r^2 values from 0.000 to 0.320 (Table 5). Therefore, the results confirmed that the seven SNPs had little linkage disequilibrium.

Table 5. Estimated values of linkage disequilibrium between seven mutation sites in chicken SREBF2.										
Locus	SNP1	SNP2	SNP3	SNP4	SNP5	SNP7	SNP10			
SNP1		D' = 0.780	D' = 1.000	D' = 0.520	D' = 0.900	D' = 0.000	D' = 0.680			
SNP2	$r^2 = 0.320$		D' = 0.121	D' = 0.020	D' = 0.820	D' = 0.000	D' = 0.254			
SNP3	$r^2 = 0.051$	$r^2 = 0.000$		D' = 0.321	D' = 0.235	D' = 0.715	D' = 0.310			
SNP4	$r^2 = 0.040$	$r^2 = 0.000$	$r^2 = 0.031$		D' = 0.740	D' = 0.721	D' = 0.334			
SNP5	$r^2 = 0.040$	$r^2 = 0.071$	$r^2 = 0.052$	$r^2 = 0.193$		D' = 0.630	D' = 0.540			
SNP7	$r^2 = 0.012$	$r^2 = 0.030$	$r^2 = 0.164$	$r^2 = 0.050$	$r^2 = 0.126$		D' = 0.710			
SNP10	$r^2 = 0.140$	$r^2 = 0.040$	$r^2 = 0.010$	$r^2 = 0.050$	$r^2 = 0.050$	$r^2 = 0.023$				

Using the PHASE program, we found 30 haplotypes in the chicken populations tested (Table 6), the following four of which accounted for 49.79% of the estimates: H4 (ACCATAG),

Genetics and Molecular Research 15 (3): gmr.15038514

H10 (ACTACGC), H13 (ACTATGC), and H16 (ACTGCGC). H13 (ACTATGC) had the highest frequency in all of the populations (14.52%), and the following six had frequencies of lower than 0.5%: H20 (ACTGTAC), H22 (ATTACGC), H23 (ATTATGC), H24 (TCTACGG), H28 (TTTATGG), and H29 (TTTGCGC).

Haplotype				Site				Frequency (%)
	SNP1	SNP2	SNP3	SNP4	SNP5	SNP7	SNP10	
H1	Α	С	С	Α	С	G	С	2.86
H2	Α	С	C	Α	С	Α	C	1.99
H3	Α	С	С	Α	Т	G	С	7.57
H4	Α	С	С	Α	Т	Α	С	12.63
H5	Α	С	C	Α	Т	Α	G	0.67
H6	Α	С	С	G	С	G	С	4.74
H7	Α	С	С	G	С	G	G	3.07
H8	Α	С	С	G	Т	G	С	0.79
H9	Α	С	С	G	Т	G	G	0.98
H10	Α	С	Т	Α	С	G	C	11.05
H11	Α	С	Т	Α	С	G	G	4.08
H12	Α	С	Т	Α	С	Α	G	0.65
H13	Α	С	Т	Α	Т	G	C	14.52
H14	Α	С	Т	Α	Т	G	G	1.61
H15	Α	С	Т	Α	Т	Α	C	0.81
H16	Α	С	Т	G	С	G	С	11.59
H17	Α	С	Т	G	С	G	G	2.54
H18	Α	С	Т	G	С	Α	C	1.22
H19	Α	С	Т	G	Т	G	С	1.50
H20	Α	С	Т	G	Т	Α	С	0.42
H21	А	Т	С	А	С	G	С	5.58
H22	А	Т	Т	А	С	G	С	0.34
H23	А	Т	Т	А	Т	G	С	0.41
H24	Т	С	Т	А	С	G	G	0.15
H25	Т	С	Т	G	С	G	С	0.92
H26	Т	Т	Т	А	С	G	С	0.66
H27	Т	Т	Т	А	С	G	G	0.83
H28	Т	Т	Т	А	Т	G	G	0.22
H29	Т	Т	Т	G	С	G	С	0.34
H30	Т	Т	Т	G	С	G	G	3.59

Associations between SNP markers and carcass traits

To investigate possible associations between the different genotypes and carcass traits, the effects of single markers on the carcass traits were analyzed (Table 7). Because breed was not significant, data from the 10 populations were pooled and analyzed together. For SNP1, the statistical analysis revealed that individuals with the genotypes AA or AT had significantly greater EW values than those with the genotype TT (P < 0.01), demonstrating that the A allele might be associated with increases in EW. However, the other traits evaluated had no significant associations with the genotypes (P > 0.05). For SNP2, chickens with CC or CT genotypes had greater EW values than those with the TT genotype (P < 0.05), but the other traits evaluated had no significant associations with any genotypes (P > 0.05). For SNP4, individuals with the AA genotype had higher values for the following carcass traits than individuals with AG or GG genotypes (P < 0.01): LW, CW, EW, SEW, BMW, and LMW. However, the other traits evaluated had no significant associations with any genotypes (P > 0.05). For the remaining markers (SNP3, SNP5, SNP7, and SNP10), none of the carcass traits had significant associations with the genotypes in any of the populations (data not shown) (P > 0.05).

Genetics and Molecular Research 15 (3): gmr.15038514

lab	le 7. Associé	ations between SKE1	BFZ single loci and	chicken carcass trai	ts.				
SNP	Genotype	LW (g)	CW (g)	SEW (g)	EW (g)	BMW (g)	LMW (g)	AW (g)	ST (cm)
SNP1	AA	2481.63 ± 572.52	2202.21 ± 501.88	2059.52 ± 479.26	1683.93 ± 377.43^{A}	139.80 ± 34.35	192.58 ± 52.48	71.89 ± 44.08	0.49 ± 0.17
	AT	2461.43 ± 504.92	2195.00 ± 448.37	2051.11 ± 428.08	1675.30 ± 342.15^{A}	139.21 ± 32.79	191.79 ± 50.10	65.80 ± 37.75	0.48 ± 0.19
	TT	3110.00 ± 636.39	2807.50 ± 611.65	2634.80 ± 544.19	1925.00 ± 460.10^{B}	179.02 ± 23.79	248.10 ± 82.72	80.72 ± 30.72	
SNP2	cc	2438.01 ± 557.09	2167.04 ± 494.87	2025.63 ± 471.84	1661.62 ± 373.42^{a}	136.96 ± 33.31	189.91 ± 51.74	70.43 ± 44.56	0.50 ± 0.17
	CT	2586.44 ± 550.44	2289.47 ± 465.29	2141.88 ± 446.04	1699.60 ± 385.08^{a}	147.57 ± 34.34	197.34 ± 49.84	74.53 ± 39.35	0.47 ± 0.17
	TT	3047.50 ± 560.48	2708.13 ± 497.93	2551.65 ± 468.30	2064.11 ± 388.53^{b}	172.20 ± 37.23	247.70 ± 64.53	66.24 ± 30.96	
SNP4	AA	$2538.69 \pm 568.57^{\rm A}$	2264.44 ± 511.99^{A}	2122.94 ± 486.78^{A}	1734.51 ± 376.93^{A}	143.91 ± 35.45^{A}	201.12 ± 53.07^{A}	73.15 ± 45.68	0.49 ± 0.18
	AG	2428.47 ± 590.46^{B}	2147.12 ± 502.88^{B}	2003.80 ± 482.27^{B}	1623.12 ± 397.43^{B}	135.97 ± 33.95^{B}	185.95 ± 53.75^{B}	68.02 ± 39.36	0.50 ± 0.15
	GG	2453.51 ± 423.46^{AB}	2173.38 ± 376.11^{A}	2025.16 ± 354.09^{AB}	1657.52 ± 295.00^{AB}	138.41 ± 27.87^{AB}	183.94 ± 37.93^{B}	74.57 ± 46.72	0.51 ± 0.19

Genetics and Molecular Research 15 (3): gmr.15038514

12

Least square mean values within a row of the same SNP locus with different lowercase superscript letters differed significantly at P < 0.05; least square mean values within a row of the same SNP locus with different uppercase superscript letters differed significantly at P < 0.01. SNP = single nucleotide polymorphism; LW = live weight; CW = carcass weight; SEW = semi-eviscerated weight; EW = eviscerated weight; BMW = breast muscle weight; LMW = leg muscle weight; AW = abdominal fat weight; ST = sebum thickness.

Associations between combined genotypes and carcass traits

Haplotype analysis can provide more information than single-marker analysis on genetic diseases and trait associations because of a population's ancestry and demography (Akey et al., 2001). The advantage of using haplotype-based methods is greatest when marker alleles are not in strong LD with each other (Morris and Kaplan, 2002). In the present study, all 30 of the haplotypes evaluated were used for establishing combinations, and 117 haplotype combinations were identified. These combinations were selected for further analysis, except those with percentages lower than 1%. Data of the associations between haplotypes and carcass traits are shown in Table 8.

Table 8. Associations between combined SREBF2 genotypes and chicken carcass traits.										
Combined				Traits						
genotype	LW (g)	CW (g)	SEW (g)	EW (g)	BMW (g)**	LMW (g)	AW (g)	ST (cm)		
H1H4	2553.33 ± 412.88	2270.00 ± 377.46	2116.62 ± 264.35	1717.27 ± 175.62	141.83 ± 17.72	202.91 ± 34.90	73.87 ± 16.52	0.569 ± 0.208		
H3H4	2855.00 ± 332.58	2541.43 ± 362.78	2371.89 ± 234.95	1920.91 ± 206.96	170.72 ± 21.97	226.85 ± 38.96	94.52 ± 28.63	0.450 ± 0.035		
H3H6	2446.25 ± 477.20	2191.25 ± 250.95	2019.08 ± 286.37	1661.09 ± 269.15	145.96 ± 22.74	193.14 ± 30.41	63.56 ± 18.01	0.412 ± 0.160		
H3H10	2401.25 ± 543.02	2137.50 ± 218.58	2020.40 ± 209.67	1674.52 ± 242.34	137.77 ± 27.24	190.59 ± 32.36	78.08 ± 22.97	0.558 ± 0.183		
H3H11	2555.00 ± 357.03	2291.67 ± 328.60	2170.90 ± 339.15	1779.43 ± 305.35	135.85 ± 26.94	208.58 ± 27.94	63.82 ± 23.45	0.416 ± 0.090		
H3H13	1995.00 ± 164.22	1775.00 ± 154.11	1637.50 ± 161.37	1381.25 ± 129.70	100.70 ± 6.59	161.53 ± 18.10	38.85 ± 9.13	0.484 ± 0.155		
H3H16	2333.33 ± 470.62	2080.67 ± 233.33	1959.96 ± 214.72	1635.98 ± 217.20	126.36 ± 20.32	186.34 ± 23.28	48.43 ± 19.23	0.476 ± 0.126		
H4H10	2670.00 ± 411.60	2405.67 ± 200.61	2236.75 ± 260.93	1800.48 ± 272.16	155.31 ± 26.39	207.13 ± 36.58	84.20 ± 26.23	0.569 ± 0.173		
H4H11	2804.00 ± 390.61	2517.00 ± 200.93	2369.18 ± 268.63	1987.21 ± 194.94	154.83 ± 21.51	248.54 ± 26.34	48.70 ± 14.71	0.464 ± 0.251		
H4H13	2376.00 ± 336.46	2135.50 ± 296.64	2006.85 ± 278.50	1639.27 ± 171.45	130.07 ± 25.78	181.99 ± 22.44	79.83 ± 27.52	0.465 ± 0.197		
H4H16	2477.17 ± 620.77	2210.00 ± 246.58	2071.56 ± 225.96	1690.16 ± 209.52	128.94 ± 26.38	191.09 ± 34.96	70.94 ± 27.15	0.437 ± 0.103		
H4H17	2433.33 ± 364.60	2175.00 ± 336.34	1958.33 ± 267.93	1675.00 ± 254.44	141.47 ± 25.02	201.37 ± 28.63	54.00 ± 27.23	0.477 ± 0.116		
H4H21	2739.29 ± 479.90	2457.86 ± 234.22	2337.72 ± 230.05	1906.72 ± 218.77	166.25 ± 28.45	214.45 ± 32.78	67.46 ± 23.36	$\underline{0.401 \pm 0.084}$		
H6H16	2410.00 ± 236.78	2162.50 ± 208.67	2050.00 ± 188.19	1681.25 ± 139.01	135.20 ± 11.70	196.13 ± 14.13	72.25 ± 26.26	0.513 ± 0.226		
H7H10	2870.00 ± 368.16	2546.25 ± 366.74	2385.80 ± 334.81	1970.15 ± 323.07	167.30 ± 21.59	208.85 ± 36.38	60.68 ± 21.63	0.444 ± 0.007		
H7H13	2499.50 ± 635.52	2211.98 ± 296.42	2057.92 ± 259.51	1684.41 ± 224.66	137.50 ± 21.44	191.23 ± 37.17	78.93 ± 24.26	0.655 ± 0.208		
H10H10	2651.67 ± 646.02	2337.50 ± 246.59	2178.67 ± 213.92	1776.18 ± 262.45	147.99 ± 27.26	199.67 ± 24.62	109.50 ± 21.23	0.579 ± 0.179		
H10H13	2180.71 ± 408.97	1947.50 ± 185.22	1828.72 ± 164.26	1494.19 ± 270.59	121.86 ± 24.48	167.13 ± 22.76	81.80 ± 21.27	0.551 ± 0.188		
H10H16	2659.29 ± 494.79	2244.29 ± 304.78	2084.63 ± 288.39	1712.68 ± 277.18	138.50 ± 22.23	202.62 ± 33.55	78.33 ± 20.19	0.538 ± 0.135		
H11H13	2593.75 ± 432.06	2313.75 ± 362.15	2178.01 ± 316.39	1785.91 ± 252.48	161.28 ± 27.46	213.35 ± 42.95	55.22 ± 13.20	0.442 ± 0.058		
H13H13	2552.14 ± 448.29	2247.14 ± 327.82	2097.66 ± 363.68	1714.60 ± 270.62	149.77 ± 28.04	186.74 ± 30.83	78.15 ± 25.58	0.443 ± 0.133		
H13H16	2244.23 ± 474.45	1987.69 ± 238.15	1849.20 ± 215.29	1489.79 ± 201.08	126.48 ± 21.32	166.12 ± 26.52	71.61 ± 27.23	0.511 ± 0.108		
H13H17	2535.71 ± 330.23	2242.14 ± 227.82	2104.00 ± 203.92	1719.92 ± 204.67	145.85 ± 29.74	195.89 ± 30.44	92.40 ± 32.65	0.607 ± 0.112		
H13H21	2890.00 ± 342.69	2517.86 ± 340.04	2395.48 ± 317.10	1926.37 ± 267.14	173.78 ± 27.80	230.23 ± 31.44	101.28 ± 27.75	-		
H13H30	2516.00 ± 423.12	2227.00 ± 377.47	2082.88 ± 336.65	1688.76 ± 282.34	152.81 ± 29.54	170.27 ± 30.59	105.62 ± 17.46	0.588 ± 0.111		
H16H16	2416.67 ± 247.20	2179.17 ± 235.81	2029.17 ± 225.51	1695.83 ± 207.01	144.87 ± 22.96	178.97 ± 30.29	97.12 ± 33.46	0.652 ± 0.167		
H21H21	3115.00 ± 358.71	2770.00 ± 266.85	2603.73 ± 254.35	2090.78 ± 267.71	172.67 ± 26.41	257.38 ± 34.31	90.52 ± 8.63	-		

**Significant difference between least mean squares at P < 0.01, respectively. LW = live weight; CW = carcass weight; EW = eviscerated weight; SEW = semi-eviscerated weight; BMW = breast muscle weight; LMW = leg muscle weight; AW = abdominal fat weight; ST = sebum thickness. Underlined values present the lowest value and the bolded values present the highest value.

Chickens with the combined genotype H13H21 had the highest BMW and those with the combined genotype H3H13 had the lowest BMW, and all of the combined genotypes were

Genetics and Molecular Research 15 (3): gmr.15038514

significantly associated with this trait (P = 0.006). The combined genotype H21H21 had the highest LW, CW, EW, and SEW values, H13H21 had the highest BMW value, H10H10 had the highest AW value, and H7H13 had the highest ST value. H3H13 had a negative effect on LW, CW, EW, SEW, BMW, LMW, and AW.

DISCUSSION

The *SREBF2* nucleotide sequence alignment results revealed that the Erlang Mountain chicken *SREBF2* CDS was similar to that of most mammals. We found one transmembrane domain in Erlang Mountain chicken *SREBF2*; however, Brown and Goldstein (1997) reported *SREBF2* as having two transmembrane domains.

The mRNA expression levels found in this study were consistent with those reported by previous studies. Assaf et al. (2003) reported chicken *SREBF2* as being highly expressed in the uropygial gland and liver, with comparatively lower expression levels in adipose tissue and skeletal muscle. In addition, Gondret et al. (2001) found that *SREBF2* expression levels in pig, rabbit, and chicken livers were twice as high as those in adipose tissue. Interestingly, we found that Erlang Mountain chicken *SREBF2* was highly expressed in breast muscle, suggesting that *SREBF2* might play an important role in meat quality. Whether the SREBF2 protein also regulates muscle fiber growth in chickens is unclear. Further studies of the function of the SREBF2 protein in the Erlang Mountain chicken are warranted to determine its role in the growth of muscle fiber.

Marker-assisted selection is a more accurate and convenient method of selection than traditional selection. Therefore, in the present study, the genomic DNA sequences of 10 chicken populations were successfully amplified using primer pairs for *SREBF2*. Based on previously reported sequences (Ensemble number: ENSGALG00000011916), 10 SNPs were identified in chicken *SREBF2* by sequencing, four of which were novel mutations. We combined the DNA sequencing results with those generated by the PCR-SSCP method, which accurately detected SNPs in chicken *SREBF2*.

At the SNP6 and SNP8 loci, mutation homozygotes (SNP6-TT and SNP8-AA) were not detected in any of the chicken populations studied. At the SNP1, SNP2, SNP9, and SNP10 loci, the mutation homozygotes TT, TT, AA, and GG were not found in the S01, S02, S03, S06, D99, SD02, or SD03 populations. This demonstrates that frequencies of T, A, T, T, A, and G alleles in the chicken populations decreased during artificial selection, migration, and genetic drift, possibly because these alleles may be negative mutations in certain chicken populations, or through natural selection, individuals with the genotypes that were eliminated caused a decline in the number of T, A, T, T, A, and G alleles in this study. However, the reason why mutations of the homozygotes mentioned above were absent in these chicken populations still needs further investigation.

Haplotype frequencies and LD coefficients were assessed for seven SNPs (SNP6, SNP8, and SNP9 were excluded) in all 10 chicken populations. The *D*' and r^2 values indicated that the seven SNPs in this study had little LD. Based on these results, 30 haplotypes were identified in the chicken populations. Haplotype H13 (ACTATGC) had the highest frequency in the population (14.52%). H20 (ACTGTAC), H22 (ATTACGC), H23 (ATTATGC), H24 (TCTACGG), H28 (TTTATGG), and H29 (TTTGCGC) had frequencies lower than 0.5%. The high-frequency haplotypes probably existed in the population for a long time. Novel variations are derived from common haplotypes, and rare variants represent mutations that are

Genetics and Molecular Research 15 (3): gmr.15038514

more recent and are more likely to be related to common haplotypes than to other rare variants (Huang et al., 2013; He et al., 2014).

To evaluate the effects of the seven SNPs on carcass traits, an association analysis between single SNP genotypes, haplotype combinations, and the carcass traits was conducted, which revealed that SNP4 in chickens with the AA genotype resulted in more desirable LW, CW, EW, SEW, BMW, and LMW values. SNP2, SNP3, SNP4, SNP7, and SNP10 were synonymous mutations that did not cause AA variations, and SNP1 and SNP5 were in introns. However, it has recently been found that silent mutations can affect gene function and phenotype (Ren et al., 2014; Wang et al., 2014). Four silent mutations (g.69307744C>T, g.69355665T>C, g.69340192G>A, and g.69340070C>T) of chicken *TBC1D1* are significantly associated with carcass traits (Wang et al., 2014). One synonymous mutation (g.T1694A) in exon 4 of cattle *CFL2* is significantly associated with growth traits in Qinchuan cattle (Sun et al., 2015). Therefore, it would be interesting to determine the mechanism of the association between these silent mutations and carcass traits in chickens.

Combined genotypes (diplotypes) determine the usefulness of employing closely linked markers to identify genetically superior individuals, and are an essential component of genetic architecture (Stirling and Stear, 2010). To investigate the effects of combined genotypes on carcass traits, we analyzed the combined genotypes present in the chicken populations. The results revealed that the combined genotype H21H21 had the highest LW, CW, EW, and SEW values, H13H21 had the highest BMW value, H10H10 had the highest AW value, and H7H13 had the highest ST value. Therefore, our data demonstrate that associations between combined genotypes and carcass traits are more accurate than those between single SNP genotypes and carcass traits. These results are similar to those of Fallin et al. (2001) and He et al. (2014), who demonstrated that the inheritance of genotype combinations is more effective than that of a single SNP genotype. Therefore, H21H21 may be used as a molecular marker of combined genotypes in the future for the selection of desirable chicken carcass traits.

In summary, this is the first analysis of *SREBF2* polymorphisms in the chicken. Ten *SREBF2* SNPs were validated in Erlang Mountain and Sichuan Daheng chicken populations, three of which were significantly associated with LW, CW, EW, SEW, BMW, and LMW. Thirty haplotypes were identified, and the combined genotype H21H21 had the highest LW, CW, EW, and SEW values while H13H21 had the highest BMW value. Quantitative PCR data suggest that chicken *SREBF2* may play a role in muscle development. Therefore, our results suggest that *SREBF2* could be used as a DNA molecular marker of chicken carcass traits in marker-assisted selection.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Yao Zhang for help in managing the birds and collecting the data. Research supported by the China Agriculture Research System (CARS-41) and The Twelfth Five Year Plan Breeding Program in Sichuan for Selective Breeding of New Breeds and Synthetic Strains of Laying Hens (#2011NZ0099-7).

Genetics and Molecular Research 15 (3): gmr.15038514

REFERENCES

- Akey J, Jin L and Xiong M (2001). Haplotypes vs single marker linkage disequilibrium tests: what do we gain? *Eur. J. Hum. Genet.* 9: 291-300. http://dx.doi.org/10.1038/sj.ejhg.5200619
- Ardlie KG, Kruglyak L and Seielstad M (2002). Patterns of linkage disequilibrium in the human genome. Nat. Rev. Genet. 3: 299-309. <u>http://dx.doi.org/10.1038/nrg777</u>
- Assaf S, Hazard D, Pitel F, Morisson M, et al. (2003). Cloning of cDNA encoding the nuclear form of chicken sterol response element binding protein-2 (SREBP-2), chromosomal localization, and tissue expression of chicken SREBP-1 and -2 genes. Poult. Sci. 82: 54-61. http://dx.doi.org/10.1093/ps/82.1.54
- Barrett JC, Fry B, Maller J and Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265. <u>http://dx.doi.org/10.1093/bioinformatics/bth457</u>
- Bommer GT and MacDougald OA (2011). Regulation of lipid homeostasis by the bifunctional *SREBF2-miR33a* locus. *Cell Metab.* 13: 241-247. <u>http://dx.doi.org/10.1016/j.cmet.2011.02.004</u>
- Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.
- Brown MS and Goldstein JL (1997). The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 89: 331-340. http://dx.doi.org/10.1016/S0092-8674(00)80213-5
- Edwards PA, Tabor D, Kast HR and Venkateswaran A (2000). Regulation of gene expression by SREBP and SCAP. *Biochim. Biophys. Acta* 1529: 103-113. <u>http://dx.doi.org/10.1016/S1388-1981(00)00140-2</u>
- Fallin D, Cohen A, Essioux L, Chumakov I, et al. (2001). Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res.* 11: 143-151. <u>http://dx.doi.org/10.1101/gr.148401</u>
- Gondret F, Ferré P and Dugail I (2001). ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. J. Lipid Res. 42: 106-113.
- He H, Zhang HL, Li ZX, Liu Y, et al. (2014). Expression, SNV identification, linkage disequilibrium, and combined genotype association analysis of the muscle-specific gene CSRP3 in Chinese cattle. Gene 535: 17-23. <u>http://dx.doi.org/10.1016/j.gene.2013.11.014</u>
- Huang YZ, Wang KY, He H, Shen QW, et al. (2013). Haplotype distribution in the *GLI3* gene and their associations with growth traits in cattle. *Gene* 513: 141-146. <u>http://dx.doi.org/10.1016/j.gene.2012.10.052</u>
- Kaidar-Person O, Bar-Sela G and Person B (2011). The two major epidemics of the twenty-first century: obesity and cancer. Obes. Surg. 21: 1792-1797. <u>http://dx.doi.org/10.1007/s11695-011-0490-2</u>
- Le Hellard S, Mühleisen TW, Djurovic S, Fernø J, et al. (2010). Polymorphisms in SREBF1 and SREBF2, two antipsychotic-activated transcription factors controlling cellular lipogenesis, are associated with schizophrenia in German and Scandinavian samples. *Mol. Psychiatry* 15: 463-472. http://dx.doi.org/10.1038/mp.2008.110
- Liu FH, Song JY, Ma J, Shang XR, et al. (2014). Association of rs2228314 polymorphism in SREBP2 with serum lipid levels and obesity among children and adolescents. *Beijing Da Xue Xue Bao* 46: 355-359.
- Marty A, Amigues Y, Servin B, Renand G, et al. (2010). Genetic variability and linkage disequilibrium patterns in the bovine DNAJA1 gene. Mol. Biotechnol. 44: 190-197. <u>http://dx.doi.org/10.1007/s12033-009-9228-y</u>
- Morris RW and Kaplan NL (2002). On the advantage of haplotype analysis in the presence of multiple disease susceptibility alleles. *Genet. Epidemiol.* 23: 221-233. http://dx.doi.org/10.1002/gepi.10200
- Nei M and Roychoudhury AK (1974). Sampling variances of heterozygosity and genetic distance. Genetics 76: 379-390.
- Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269-5273. <u>http://dx.doi.org/10.1073/pnas.76.10.5269</u>
- Reaven GM (2011). Insulin resistance: the link between obesity and cardiovascular disease. Med. Clin. North Am. 95: 875-892. <u>http://dx.doi.org/10.1016/j.mcna.2011.06.002</u>
- Ren G, Huang YZ, Wei TB, Liu JX, et al. (2014). Linkage disequilibrium and haplotype distribution of the bovine LHX4 gene in relation to growth. Gene 538: 354-360. <u>http://dx.doi.org/10.1016/j.gene.2013.12.037</u>
- Schmittgen TD and Livak KJ (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nat. Protoc.* 3: 1101-1108. http://dx.doi.org/10.1038/nprot.2008.73
- Shimano H, Horton JD, Shimomura I, Hammer RE, et al. (1997). Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J. Clin. Invest. 99: 846-854. <u>http:// dx.doi.org/10.1172/JCI119248</u>
- Stephens M, Smith NJ and Donnelly P (2001). A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68: 978-989. <u>http://dx.doi.org/10.1086/319501</u>

Stirling D and Stear MJ (2010). g you The direct determination of haplotypes from extended regions of genomic DNA. BMC Genomics 11: 223. http://dx.doi.org/10.1186/1471-2164-11-223

Genetics and Molecular Research 15 (3): gmr.15038514

- Sun Y, Lan X, Lei C, Zhang C, et al. (2015). Haplotype combination of the bovine *CFL2* gene sequence variants and association with growth traits in Qinchuan cattle. *Gene* 563: 136-141. <u>http://dx.doi.org/10.1016/j.gene.2015.03.016</u>
- Wang Y, Xu HY, Gilbert ER, Peng X, et al. (2014). Detection of SNPs in the *TBC1D1* gene and their association with carcass traits in chicken. *Gene* 547: 288-294. <u>http://dx.doi.org/10.1016/j.gene.2014.06.061</u>
- Yang L, Chen J, Liu D, Yu S, et al. (2015). Association between SREBF2 gene polymorphisms and metabolic syndrome in clozapine-treated patients with schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 56: 136-141. <u>http:// dx.doi.org/10.1016/j.pnpbp.2014.08.015</u>
- Zhang Y, Li J, Li Y, Shao F, et al. (2014). Analysis on expression characteristics of *CROT, HADHB, SREBP2* genes related with lipid metabolism in embryonic stage of chicken. *China Poult.* 36: 6-9.
- Zhao H, Nettleton D and Dekkers JC (2007). Evaluation of linkage disequilibrium measures between multi-allelic markers as predictors of linkage disequilibrium between single nucleotide polymorphisms. *Genet. Res.* 89: 1-6. <u>http://dx.doi.org/10.1017/S0016672307008634</u>

Zhao XP and Yang FJ (2012). Regulation of SREBP-mediated gene expression. Sheng Wu Wu Li Hsueh Bao 28: 287-294.

Genetics and Molecular Research 15 (3): gmr.15038514