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Association of *PCSK1* gene polymorphisms with abdominal fat content in broilers

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Abstract

Protein proteolytic enzymes (Proprotein Convertase, PC) is a Ca^{2+}-dependent serine protease family, whose main function is to cleave precursors of biologically inactive proteins or peptide chains into active functional molecules. Proprotein convertase subtilisin/kexin type 1 (*PCSK1*) gene is mainly expressed in nerve and endocrine tissues. In this study, *PCSK1* was selected as an important candidate gene for abdominal fat content in broilers. We cloned the exon region of chicken *PCSK1* gene and found six single-nucleotide polymorphisms (SNPs). Association analysis was carried out and we found that the polymorphisms of these six SNPs were significantly associated with abdominal fat content in G19 and G20 populations. Five of these SNPs were significantly associated with abdominal fat content in G19 and G20 combined population. The polymorphism of these five SNPs was significantly correlated with the abdominal fat content of AA broilers. Together, our study demonstrated that c.927T>C, c.1880C>T, c.*900G>A, and c.*1164C>T were significantly associated with abdominal fat content in populations used in this study, which means that these SNPs in *PCSK1* gene could be used as candidate markers to select lean broiler lines.

KEYWORDS

abdominal fat, association analysis, broilers, *PCSK1* gene, single-nucleotide polymorphism

1 | INTRODUCTION

The chicken (*Gallus gallus*) is an important model animal that can bridge the evolutionary gap between mammals and other vertebrates (International Chicken Genome Sequencing Consortium, 2004). For more than 60 years, substantial advances have been made in the improvement of body weight in chicken by artificial selection and will continue to be one of the most important economic traits in broiler breeding programs. Progress in rapid growth has been accompanied by an increase in abdominal fat deposition in the broilers. Abdominal fat is considered as a by-product with very low commercial value, and a large amount of abdominal fat deposition can decrease feed efficiency (Li et al., 2003). Furthermore, the overweight of female birds may seriously affect egg production percentage, fertilization percentage, and hatching percentage, and may induce the occurrence of fatty liver syndrome. The overweight of male birds may affect the amount of semen and reduce the quality of semen. At the same time, some diseases caused by obesity might increase the death and culling percentage during the laying period and reduce economic benefits. Therefore, selecting lean broiler lines is one of the important goals of broiler breeding in the world (Demeure et al., 2013; Zerehdaran, Vereijken, Arendonk, & Van, d.W.E.H., 2004). The abdominal fat weight (AFW) can be measured directly, and experiments could be designed to identify genetic variants associated with abdominal fat content. The results from this type of experiment may be useful for marker-assisted

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selection (MAS) and for understanding the genetic background of abdominal fat deposition in broilers.

Our team has been concerned on the study of chicken abdominal fat trait for a long time and has obtained some important results. In our previous study, we used chicken 60K SNP chip and two broilers lines divergently selected for abdominal fat content to carry out the selection signature analysis and we found ten important chromosome regions that under harbor selection signatures (Zhang et al., 2012). These important chromosome regions were mainly distributed on chicken chromosomes 1, 2, 4, 5, 11, 15, 20, 26, and Z. Among them, the most significant selection signature was located on 0.73 Mb (55.43–56.16 Mb) region of chromosome Z, in which the proprotein convertase subtilisin/kexin type 1 (*PCSK1*) gene was located (Zhang et al., 2012). This result indicated that *PCSK1* gene may play an important role in the chicken abdominal fat deposition. Therefore, they selected *PCSK1* gene as an important candidate gene for abdominal fat deposition in broilers. The single-nucleotide polymorphisms (SNPs) are detected in exon regions of *PCSK1* gene. The aim of this study is to identify important SNPs for abdominal fat content in *PCSK1* gene by analyzing the associations between polymorphisms of SNPs in *PCSK1* gene and abdominal fat content in chicken. The results of this study may supply genetic markers that may have important application value to select lean broiler lines.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All animal work was conducted according to the guidelines for the Care and Use of Experimental Animals established by the Ministry of Science and Technology of the People's Republic of China (approval number: 2006–398), and was approved by the Laboratory Animal Management Committee of Northeast Agricultural University.

2.2 | Experimental populations and phenotype measurements

The Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF) have been established since 1996, using abdominal fat percentage (AFP) [AFP = abdominal fat weight (AFW)/body weight at 7 weeks of age (BW7)] and plasma very low-density lipoprotein (VLDL) levels as selection criteria. Details on these two lines were described in Zhang et al. (2012).

After 20 generations, the average AFP was 0.8% in the lean line and 5.0% in the fat line. A total of 542 and 685 male birds from the 19th and 20th generation (G19 and G20) of NEAUHLF were used to carry out the association study.

Another population, an Arbor Acres (AA) commercial broiler population containing 370 birds (170 male birds and 200 female birds), was also used to carry out the association study.

All birds were kept in similar environmental conditions and had free access to feed and water. Commercial corn–soybean–based diets that met all NRC (1994) requirements were provided in the study. The birds were fed starter feed (3,100 kcal ME/kg and 210 g/kg CP) from hatching to 3 weeks of age and then a grower diet (3,000 kcal ME/kg and 190 g/kg CP) from 4 to 7 weeks of age.

All these birds were slaughtered at 7 weeks of age. The blood was collected from wing veins before slaughtered and genomic DNA was extracted and properly kept. The body weight at 7 weeks of age (BW7) was measured before slaughtered and abdominal fat weight (AFW) were measured after slaughter. Abdominal fat percentage was calculated as the ratio of AFW to BW7 (AFP = AFW/BW7).

2.3 | Genotyping of SNPs

Six SNPs were detected in all exons of *PCSK1* gene previously (Zhang et al., 2017). These SNPs were named as c.927T>C, c.1880C>T, c.*856G>A, c.*900G>A, c.*1164C>T, and c.*896G>A. Among these SNPs, two SNPs are located in the coding sequence (CDS) region (c.927T>C and c.1880C>T) and four others in the 3' untranslated region (UTR) (c.*856G>A, c.*900G>A, c.*1164C>T, and c.*896G>A). The c.927T>C is a silent mutation and c.1880C>T is a missense mutation that the corresponding amino acid is mutated from alanine (Ala) to valine (Val). These SNPs were genotyped with the PCR-restriction fragment length polymorphism (PCR-RFLP) method. The primers of these SNPs are shown in Table 1.

The PCR amplification system included: 50 μg/μl genomic DNA 1 μl, 10 mmol/L dNTP 0.8 μl, 10 × PCR Buffer 1 μl, 10 mol/L upstream and downstream primers each 0.2 μl, 5 U/μl Taq DNA polymerase 0.1 μl, deionized water 6.7 μl.

The PCR reaction conditions were 94°C for 5 min, 30 cycles of 94°C for 30 s, denaturing conditions listed in Table 1 for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min.

The digestion reaction system consisted of 2 μl of PCR product, 1 μl of Cutsmart Buffer, 6.8 μl of deionized water, and 0.2 μl of endonuclease, which were digested overnight at 37°C. The endonuclease used for every SNP is listed in Table 1.

2.4 | Statistical analyses

The linkage disequilibrium (LD) of the six SNPs of *PCSK1* gene was measured by coefficient r^2 using Haploview software version 4.2.

Allele frequencies were calculated and the difference of allele frequencies between the lean and fat lines were analyzed by Chi-square test. A value of $p < .05$ was used as the significant difference between the lean and fat lines.

The associations between the SNPs and abdominal fat content (AFW and AFP) were analyzed by Generalized Linear Mixed Model using JMP 7.0 software (SAS Inst. Inc.). $p < .05$ was used as the significant association between the SNPs (or haplotypes) and abdominal fat content (AFW and AFP). Significant differences ($p < .05$) between

TABLE 1 Primers used to genotype the SNPs in *PCSK1* gene

SNPs	Primers (5′–3′)	Size of product (bp)	Denaturing conditions	Cycle number	Endonuclease
c.927T>C	F:ATTTTTGTGTGGGCTTCCGGGAT R:TTGTTTTACGTTAGTAGATGTTCGGGAAT	391	57.8°C	30	DpnII
c.1880C>T	F:TAAATCCCACCTTCTGATAAGTTCTGTCCCT R:GCACAAAGAAAAGCATTAATGAGACACTA CCTGT	383	55°C	30	BsrGI-HF
c.*856G>A	F:CCATGGGGATTCTCACCACCTAACT R:CAGGAGGTTGGTTCTTTCAAGTGCA	206	57.8°C	30	ApaLI
c.*896G>A	F:ACTATGTGGACTTGAAAGAACCAACCTCCTG CCTTTGGAGCCTCC R:GGTACAGTTTTCCTTTCTATACCACTATGGTC ACAGGTTAAGGATGGG	181	63.1°C	35	BccI
c.*900G>A	F:GGTTTAGACTAGTGACAGTCATATTC R:CCTTCCCTCTATTTATGACTCT	565	55°C	30	Hpy188I
c.*1164C>T	F:GTTAAGTAATATAAATGCCTCCTGAG R:CCACTTTGGATAGGGTGATAGAT	506	55°C	30	HinfI

least squares means of different genotypes were calculated using a contrast test. The models used were as follows:

$$Y=\mu+G+F+D(F)+\mathrm{BW7}+e \quad [1]$$

$$Y=\mu+G+Q+F+D(F)+\mathrm{BW7}+e \quad [2]$$

$$Y=\mu+G+S+G\times S+F+D(F)+\mathrm{BW7}+e \quad [3]$$

In these models, *Y* was the dependent variable for the traits measured in the population (AFW and AFP), μ was the overall population mean of the traits, *G* was the genotype fixed effect, *S* was the gender fixed effect, *Q* was the generation fixed effect, $G \times S$ was the interaction effect of genotype by gender, *F* was the random effect of the family, *D(F)* was the random effect of dam nested within family, BW7 was the covariance variable, and *e* was the residual random error. Model [1] was used to calculated the association between SNP polymorphisms and abdominal fat content (AFW and AFP) in G19 and G20 populations of the lean and fat lines. Model [2] was used to calculated the association between SNP polymorphisms and abdominal fat content (AFW and AFP) in G19 and G20 combined population. Model [3] was used to calculated the association between SNP polymorphisms and abdominal fat content (AFW and AFP) in AA commercial broiler population.

3 | RESULTS

3.1 | Association of PCSK1 polymorphisms and abdominal fat content in G19 and G20 populations

The genotype frequencies and allele frequencies of the six SNPs in G19 and G20 populations of NEAUHLF were analyzed, and the chi-square independence test was used to calculate the difference of allele frequencies between the lean and fat lines. The results indicated that the allele frequencies of the six SNPs were extremely significantly different between the lean and fat lines in both G19 and G20 populations of NEAUHLF ($p < .05$; Table 2).

The difference in AFW and AFP in G19 and G20 populations between the lean and fat lines were analyzed and the results indicated that AFW and AFP in lean line were significantly lower than that in the fat line (Table 3).

The association between the polymorphisms of six SNPs and the abdominal fat content (AFW and AFP) in G19 and G20 populations of the lean and fat lines, respectively, were analyzed. In both G19 and G20 populations, the polymorphisms all these six SNPs were significantly associated ($p < .05$) with AFW and AFP (Table 4). The two generations were combined together and the polymorphisms of five SNPs were significantly associated with AFW and AFP. The polymorphism of c.*856G>A was not significantly associated with AFW and AFP in G19 and G20 combined population, however it was significantly associated with AFW and AFP in both G19 and G20 populations, respectively. This maybe because that different models (Model [1] for single generation and Model [2] for combined population) were used to carry out the associated analysis and the effect of c.*856G>A was not big enough to be detected by both of these two models. The least square means of different genotypes for these five SNPs were compared (Table 5). The results showed that in G19, G20, and two generations combined populations, birds with TT genotype had significantly lower AFW and AFP than those with CC genotype for c.927T>C. For c.1880C>T and c.*1164C>T, birds with CC genotype had significantly lower AFW and AFP than those with TT genotype. For c.*896G>A and c.*900G>A, birds with GG genotype had significantly lower AFW and AFP than those with AA genotype.

The LD level of six SNPs in G19 and G20 populations of NEAUHLF was calculated. The results showed that these six SNPs were in strong LD (r^2>0.67; Figure 1).

TABLE 2 Genotype and allele frequencies of six SNPs in lean and fat lines of G19 and G20 populations

SNPs	Populations	Lines (No. of birds)	Genotype frequencies			Allele frequencies		χ^2
c.927T>C			TT	CT	CC	T	C	
	G19	Lean (281)	0.971	0.029	0.000	0.986	0.014	631.792
		Fat (253)	0.068	0.349	0.583	0.242	0.758	($p < .05$)
	G20	Lean (360)	1.000	0.000	0.000	1.000	0.000	1,149.694
		Fat (328)	0.003	0.174	0.823	0.009	0.910	($p < .05$)
c.1880C>T			CC	CT	TT	C	T	
	G19	Lean (281)	0.975	0.025	0.000	0.987	0.013	805.114
		Fat (253)	0.051	0.154	0.795	0.128	0.872	($p < .05$)
	G20	Lean (360)	1.000	0.000	0.000	1.000	0.000	1,176.170
		Fat (328)	0.006	0.149	0.845	0.081	0.919	($p < .05$)
c.*856G>A			GG	AG	AA	G	A	
	G19	Lean (281)	0.954	0.046	0.000	0.977	0.023	862.327
		Fat (253)	0.004	0.154	0.842	0.081	0.919	($p < .05$)
	G20	Lean (360)	1.000	0.000	0.000	1.000	0.000	1,146.281
		Fat (328)	0.000	0.183	0.817	0.091	0.909	($p < .05$)
c.*896G>A			GG	AG	AA	G	A	
	G19	Lean (281)	1.000	0.000	0.000	1.000	0.000	867.059
		Fat (253)	0.047	0.123	0.830	0.109	0.891	($p < .05$)
	G20	Lean (360)	1.000	0.000	0.000	1.000	0.000	1,146.281
		Fat (328)	0.000	0.183	0.817	0.091	0.909	($p < .05$)
c.*900G>A			GG	AG	AA	G	A	
	G19	Lean (281)	0.957	0.043	0.000	0.979	0.021	866.111
		Fat (253)	0.004	0.154	0.842	0.081	0.919	($p < .05$)
	G20	Lean (360)	0.991	0.006	0.003	0.994	0.006	1,177.780
		Fat (328)	0.000	0.146	0.854	0.073	0.927	($p < .05$)
c.*1164C>T			CC	CT	TT	C	T	
	G19	Lean (281)	0.972	0.028	0.000	0.986	0.014	877.827
		Fat (253)	0.004	0.159	0.837	0.083	0.917	($p < .05$)
	G20	Lean (360)	0.997	0.000	0.003	0.997	0.003	1,168.219
		Fat (328)	0.003	0.156	0.841	0.081	0.919	($p < .05$)

3.2 | Association of PCSK1 polymorphisms and abdominal fat content in AA commercial broiler population

The genotype frequencies and allele frequencies of the six SNPs in AA commercial broilers were also calculated (Table 6). Phenotype information of AFW and AFP in AA commercial broilers is shown in Table 3. The AFW and AFP in AA commercial broilers were higher than that in the lean line and lower than that in the fat line (Table 3). Associations between *PCSK1* polymorphisms and abdominal fat trait were carried out and the results indicated that in AA commercial broiler population, the polymorphisms of five SNPs (c.927T>C, c.1880C>T, c.*856G>A, c.*900G>A, and c.*1164C>T) were significantly associated ($p < .05$) with AFW and AFP (Table 4). The least square means of different genotypes for every SNPs were compared (Table 7). The results showed that in AA commercial broiler population, birds with CT genotype had significantly lower AFW and AFP than those with TT genotype for c.927T>C. For c.1880C>T and c.*1164C>T, birds with CT genotype had significantly lower AFW and AFP than those with CC genotype. For c.*856G>A and c.*900G>A, birds with AG genotype had significantly lower AFW and AFP than those with GG genotype.

4 | DISCUSSION

Most of the economic traits in livestock and poultry are quantitative traits, which are affected by genetic and environmental factors (Giunta, Vita, Mastrangelo, Sanna, & Motzo, 2018; Selvaggi, D'Alessandro, & Dario, 2017). Identifying the major genes controlling quantitative traits, and even the single-nucleotide polymorphisms in the gene could be better serving the breeding of livestock and poultry.

In the process of natural and artificial selection, frequencies of the alleles that beneficial to human are more likely to be increased rapidly, whereas unfavorable alleles are easily likely to be eliminated. The lean and fat lines used in this study was selected for abdominal fat content for about 20 generations. The lean and fat lines were originated from the same population and divergently selected for abdominal fat content only. Therefore, the alleles for low AFW may be increased in the lean line rapidly and alleles for high AFW may be increased in the fat line. Therefore, we compared the allele frequencies of SNPs in *PCSK1* gene and found that the allele frequencies of the six SNPs were all significantly different between the lean and fat lines. Specially, one allele of every SNP was nearly fixed in the lean line and the other allele of the same SNP was nearly fixed in the fat line. This result indicated that the selection for abdominal fat content accompanied by the changes in the distribution of the allele frequency of *PCSK1* gene between the lean and fat lines. In the above study, we found that in both G19 and G20 populations, the six SNPs are in a strong linkage state, which means that these six SNPs may transfer to the next generation as a block. Therefore, it was speculated that *PCSK1* gene may be related to abdominal fat deposition directly or *PCSK1* may linked with genes that affect abdominal fat deposition in chicken.

TABLE 3 Phenotype information of different populations

Populations	Lines	Traits (unit)	Mean ± SE	C.V.
G19	Lean line	AFW (g)	13.66 ± 0.42^{B}	0.49
		AFP (%)	0.7 ± 0.02^{B}	0.46
	Fat line	AFW (g)	95.53 ± 0.43^{A}	0.18
		AFP (%)	4.9 ± 0.8^{A}	0.15
G20	Lean line	AFW (g)	13.73 ± 0.5^{B}	0.65
		AFP (%)	0.8 ± 0.02^{B}	0.53
	Fat line	AFW (g)	95.06 ± 1.42^{A}	0.24
		AFP (%)	5.0 ± 0.1^{A}	0.19
AA commercial broilers	/	AFW (g)	59.72 ± 1.01	0.33
	/	AFP (%)	2.2 ± 0.04	0.34

Note: Different letters indicate significant differences between the lean and fat lines.

Furthermore, we also calculated the association between SNP polymorphisms and abdominal fat content (AFW and AFP) in different populations and we found that polymorphisms of four SNPs, including c.927T>C, c.1880C>T, c.*900G>A, and c.*1164C>T, were significantly associated with AFW and AFP in all populations used. This result further indicated *PCSK1* gene was an important gene for abdominal fat deposition in chicken. *PCSK1* was the third member of the proprotein convertase family to be cloned from mammalian organisms (Seidah et al., 1991). The gene is mainly expressed in nervous tissue and some endocrine tissues which regulate energy metabolism (Taylor, Ven, & Creemers, 2003). There are several studies about *PCSK1* mutation of homozygote or complex heterozygote leading to single genotype obesity (Frank et al., 2013; Harter et al., 2016), however, the mechanism of *PCSK1* gene on fat deposition is still unknown. Children with proprotein convertase 1/3 (PC1/3) deficiency are reported to have severe early-onset obesity due to excessive appetite and increased food intake (Farooqi et al., 2007). The previous QTL mapping results in human indicated that *PCSK1* gene was located in a QTL region for obesity (Bell et al., 2004; Chagnon et al., 2001; Chen et al., 2005; Hager et al., 1998). A genome-wide association study revealed that *PCSK1* gene was associated with human obesity (Herbert et al., 2006) and mutations in this gene could cause severe monogenic obesity in human (Chang et al., 2010; Jackson et al., 1997; Kilpeläinen, Bingham, Khaw, Wareham, & Loos, 2009), because the abnormal proprotein convertase 1/3 (PC1/3) hinders maturation of important hormones involved in energy metabolism (Mbikay et al., 2007; Rehfeld et al., 2008). In an obese mouse model, the N222D PC1/3 (proprotein convertase 1/3) mutation caused multiple endocrine defects and increased deposition of fat in white adipose tissue due to a defect in insulin maturation (Lloyd, Bohan, & Gekakis, 2006). In Italian heavy pigs, three SNPs on *PCSK1* gene were found to be significantly associated with the fat deposition (Fontanesi et al., 2012). These results further indicated that *PCSK1* was an important gene for fat deposition.

The four SNPs detected to be significantly associated with AFW and AFP in all populations used in this study are located in CDS

TABLE 4 Effects (*p*-Value) of *PCSK1* polymorphisms on abdominal fat trait (AFW and AFP) in different populations

Populations	Trait (unit)	c.927T>C	c.1880C>T	c.*856G>A	c.*896G>A	c.*900G>A	c.*1164C>T
G19	AFW (g)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	AFP (%)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
G20	AFW (g)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	AFP (%)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
G19 and G20 combined	AFW (g)	**<0.0001**	**<0.0001**	0.3860	**<0.0001**	**<0.0001**	**<0.0001**
	AFP (%)	**<0.0001**	**<0.0001**	0.5566	**<0.0001**	**<0.0001**	**<0.0001**
AA commercial broilers	AFW (g)	**<0.0001**	**0.0001**	**<0.0001**	0.1220	**<0.0001**	**<0.0001**
	AFP (%)	**<0.0001**	**0.0002**	**<0.0001**	0.1557	**<0.0001**	**<0.0001**

Abbreviations: AFP, abdominal fat percentage; AFW, abdominal fat weight.
Bold indicates significantly associated ($p < .05$).

TABLE 5 Effects (LSM ± SE) of SNPs on abdominal fat trait in different populations

SNPs	Trait	G19			G20			G19 and G20 combined		
c.927T>C		TT	CT	CC	TT	CT	CC	TT	CT	CC
	AFW (g)	29.82 ± 1.91^{C}	75.94 ± 2.39^{B}	81.40 ± 2.17^{A}	15.46 ± 0.91^{B}	90.14 ± 2.13^{A}	95.49 ± 1.05^{A}	19.93 ± 1.02^{C}	83.82 ± 1.67^{B}	92.21 ± 1.18^{A}
	AFP (%)	1.9 ± 0.12^{B}	3.6 ± 0.14^{A}	3.8 ± 0.13^{A}	0.8 ± 0.04^{B}	5.0 ± 0.11^{A}	5.3 ± 0.05^{A}	1.1 ± 0.06^{C}	4.5 ± 0.09^{B}	4.9 ± 0.07^{A}
c.1880C>T		CC	CT	TT	CC	CT	TT	CC	CT	TT
	AFW (g)	22.43 ± 1.42^{C}	77.50 ± 2.88^{B}	89.54 ± 1.60^{A}	15.87 ± 0.95^{B}	89.88 ± 2.37^{A}	95.37 ± 1.10^{A}	18.73 ± 0.88^{C}	82.8 ± 1.98^{B}	93.13 ± 0.99^{A}
	AFP (%)	1.1 ± 0.07^{C}	4.1 ± 0.15^{B}	4.7 ± 0.08^{A}	0.8 ± 0.05^{B}	5.0 ± 0.12^{A}	5.3 ± 0.05^{A}	0.9 ± 0.05^{C}	4.5 ± 0.1^{B}	5.0 ± 0.05^{A}
c.*896G>A		GG	AG	AA	GG	AG	AA	GG	AG	AA
	AFW (g)	92.75 ± 1.29^{B}	87.5 ± 2.99^{A}	19.63 ± 1.14^{A}	94.91 ± 1.09^{B}	90.57 ± 2.21^{A}	15.27 ± 0.97^{A}	94.01 ± 0.84^{B}	90.11 ± 1.87^{A}	17.39 ± 0.74^{A}
	AFP (%)	4.9 ± 0.07^{B}	4.6 ± 0.15^{A}	0.97 ± 0.06^{A}	5.26 ± 0.05^{B}	5.04 ± 0.11^{A}	0.78 ± 0.05^{A}	5.0 ± 0.04^{B}	5.0 ± 0.1^{A}	0.9 ± 0.04^{A}
c.*900G>A		GG	AG	AA	GG	AG	AA	GG	AG	AA
	AFW (g)	16.73 ± 1.21^{C}	72.19 ± 2.26^{B}	92.29 ± 1.29^{A}	15.29 ± 0.99^{C}	87.55 ± 2.38^{B}	94.71 ± 1.12^{A}	16.03 ± 0.81^{C}	77.73 ± 1.74^{B}	93.54 ± 0.88^{A}
	AFP (%)	0.8 ± 0.06^{C}	3.8 ± 0.11^{B}	4.9 ± 0.07^{A}	0.8 ± 0.05^{C}	4.9 ± 0.12^{B}	5.2 ± 0.06^{A}	0.8 ± 0.04^{C}	4.1 ± 0.09^{B}	5.0 ± 0.04^{A}
c.*1164C>T		CC	CT	TT	CC	CT	TT	CC	CT	TT
	AFW (g)	15.91 ± 1.15^{C}	79.49 ± 2.19^{B}	92.67 ± 1.22^{A}	15.46 ± 1.00^{B}	91.84 ± 2.34^{A}	94.46 ± 1.13^{A}	15.62 ± 0.77^{C}	83.63 ± 1.71^{B}	93.57 ± 0.84^{A}
	AFP (%)	0.78 ± 0.06^{C}	4.2 ± 0.11^{B}	4.9 ± 0.07^{A}	0.8 ± 0.05^{B}	5.1 ± 0.12^{A}	5.2 ± 0.05^{A}	8.0 ± 0.04^{C}	5.0 ± 0.09^{B}	0.5 ± 0.04^{A}

Note: Different letters indicate significant differences among three genotypes.

Abbreviations: AFP, abdominal fat percentage; AFW, abdominal fat weight.

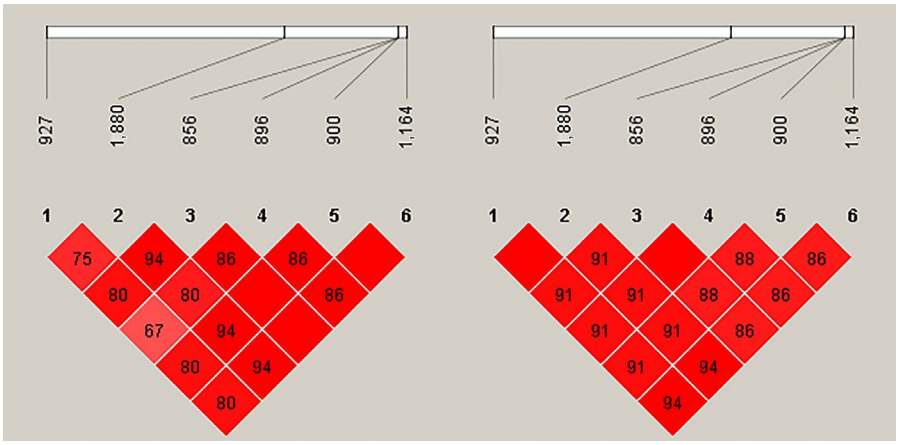

FIGURE 1 LD analysis (r^2) of the six SNPs of *PCSK1* gene in G19 (left) and G20 (right) populations

TABLE 6 The genotype and allele frequencies of SNPs in AA commercial broilers

SNPs	No. of birds	Genotype frequencies			Allele frequencies	
c.927T>C	344	TT	CT	CC	T	C
		0.500	0.413	0.087	0.706	0.294
c.1880C>T	343	CC	CT	TT	C	T
		0.490	0.408	0.102	0.694	0.306
c.*856G>A	348	GG	AG	AA	G	A
		0.529	0.428	0.043	0.743	0.257
c.*896G>A	339	GG	AG	AA	G	A
		0.935	0.056	0.009	0.963	0.370
c.*900G>A	348	GG	AG	AA	G	A
		0.529	0.422	0.049	0.740	0.260
c.*1164C>T	348	CC	CT	TT	C	T
		0.526	0.417	0.570	0.734	0.266

TABLE 7 Effects (LSM ± SE) of SNPs on abdominal fat trait in AA commercial broilers

SNPs	Trait	LSM ± SE		
c.927T>C		TT	CT	CC
	AFW (g)	66.18 ± 2.27^{A}	52.44 ± 2.38^{B}	58.42 ± 3.81^{AB}
	AFP (%)	2.4 ± 0.09^{A}	2.0 ± 0.09^{B}	2.1 ± 0.15^{AB}
c.1880C>T		CC	CT	TT
	AFW (g)	65.02 ± 2.24^{A}	55.33 ± 2.28^{B}	59.06 ± 3.27^{AB}
	AFP (%)	2.4 ± 0.09^{A}	2.0 ± 0.09^{B}	2.2 ± 0.12^{AB}
c.*856G>A		GG	AG	AA
	AFW (g)	67.27 ± 2.18^{A}	51.57 ± 2.31^{B}	61.89 ± 5.79^{AB}
	AFP (%)	2.5 ± 0.08^{A}	1.9 ± 0.09^{B}	2.2 ± 0.22^{AB}
c.*900G>A		GG	AG	AA
	AFW (g)	67.36 ± 2.14^{A}	51.54 ± 2.25^{B}	60.79 ± 4.87^{AB}
	AFP (%)	2.5 ± 0.08^{A}	1.9 ± 0.09^{B}	2.2 ± 0.19^{AB}
c.*1164C>T		CC	CT	TT
	AFW (g)	67.73 ± 2.22^{A}	49.22 ± 2.30^{B}	62.23 ± 4.05^{A}
	AFP (%)	2.5 ± 0.09^{A}	1.9 ± 0.09^{B}	2.2 ± 0.16^{AB}

Note: Different letters indicate significant differences among three genotypes.

Abbreviations: AFP, abdominal fat percentage; AFW, abdominal fat weight.

(c.927T>C, c.1880C>T) and 3'UTR (c.*900G>A and c.*1164C>T) regions of *PCSK1* gene. c.927T>C is a synonym SNP (sSNP) which involve a nucleotide and codon change, but not an amino acid change. Although this kind of SNPs do not change the amino acid sequence in proteins, they can affect certain traits by affecting the RNAs splicing, structure, folding, and stability, the response to medications, and interaction with different RNA-binding proteins (Hunt, Sauna, Ambudkar, Gottesman, & Kimchi-Sarfaty, 2009; Ramensky, Bork, & Sunyaev, 2002; Sauna, Kimchi-Sarfaty, Ambudkar, & Gottesman, 2007; Shabalina, Spiridonov, & Kashina, 2013; Tuller & Zur, 2015; Zhao, Fu, Hewett-Emmett, & Boerwinkle, 2003). c.1880C>T is a missense non-synonym SNP (nsSNP) which generate an amino acid change (Ala to Val). The nsSNPs can affect different proteins' (or enzymes) structure, folding, stability, interaction with other proteins, function, and activity, as well as the response to medications (Haraksingh & Snyder, 2013; Sauna et al., 2007; Stitziel et al., 2003; Waldman, Tuller, Keinan, & Ruppin, 2011; Yates & Sternberg, 2013). c.*900G>A and c.*1164C>T are located in 3'UTR region of *PCSK1* gene and this kind of SNPs may have a function by regulating mRNA expression and stability (Pugal et al., 2005; Seneviratne, Huang, Ait-Daoud, Li, & Johnson, 2009).

Additionally, the SNPs on *PCSK1* gene may have different effects on AFW in different populations. For example, in G19 and

G20 populations, TT individuals show significantly lower AFW than CC individuals for c.927T>C, however, in AA commercial broiler population, TT individuals show higher AFW than CT individuals. This discrepancy may be because that these SNPs may not be causal mutations, but link with the causal mutation for abdominal fat content. The lean and fat lines used in this study was selected for abdominal fat content for about 20 generations. The patterns of linkage disequilibrium (LD) in this population is different from the AA commercial broiler population which is not selected for abdominal fat content for many years. Therefore, the same genotypes show different effects in these two populations. The different populations may have different patterns of linkage disequilibrium in both domestic animals and human (López et al., 2013; Upadhyay et al., 2019). Therefore, we could use the SNPs detected in the study to reduce AFW in MAS in the lean and fat lines, however, some further studies need to be carried out to verify if these SNPs could be used in other populations.

5 | CONCLUSIONS

The polymorphisms of four SNPs, including c.927T>C, c.1880C>T, c.*900G>A, and c.*1164C>T were significantly associated with AFW and AFP in all populations used in this study. This result indicated that *PCSK1* gene may be the major gene affecting the abdominal fat deposition in chicken.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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REFERENCES

Bell, C. G., Benzinou, M., Siddiq, A., Lecoeur, C., Dina, C., Lemainque, A., … Froguel, P. (2004). Genome-wide linkage analysis for severe obesity in french caucasians finds significant susceptibility locus on chromosome 19q. *Diabetes*, *53*(7), 1857–1865. https://doi.org/10.2337/diabetes.53.7.1857

Chagnon, Y. C., Rice, T., Pérusse, L., Borecki, I. B., Ho-Kim, M.-A., Lacaille, M., … Bouchard, C. (2001). Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE. *Journal of Applied Physiology.*, *90*(5), 1777–1787. https://doi.org/10.1152/jappl.2001.90.5.1777

Chang, Y.-C., Chiu, Y.-F., Shih, K.-C., Lin, M.-W., Sheu, W.-H., Donlon, T., … Chuang, L.-M. (2010). Common PCSK1 haplotypes are associated with obesity in the Chinese population. *Obesity (Silver Spring)*, *18*(7), 1404–1409. https://doi.org/10.1038/oby.2009.390

Chen, G., Adeyemo, A. A., Johnson, T., Zhou, J., Amoah, A., Owusu, S., … Rotimi, C. (2005). A genome-wide scan for quantitative trait loci linked to obesity phenotypes among West Africans. *International Journal of Obesity*, *29*(3), 255–259. https://doi.org/10.1038/sj.ijo.0802873

Demeure, O., Duclos, M. J., Bacciu, N., Le Mignon, G., Filangi, O., Pitel, F., … Le Bihan-Duval, E. (2013). Genome-wide interval mapping using SNPs identifies new QTL for growth, body composition and several physiological variables in an F2 intercross between fat and lean chicken lines. *Genetics Selection Evolution*, *45*(1), 36. https://doi.org/10.1186/1297-9686-45-36

Farooqi, I. S., Volders, K., Stanhope, R., Heuschkel, R., White, A., Lank, E., … Creemers, J. W. M. (2007). Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. *Journal of Clinical Endocrinology & Metabolism*, *92*(9), 3369–3373. https://doi.org/10.1210/jc.2007-0687

Fontanesi, L., Bertolini, F., Scotti, E., Trevisi, P., Buttazzoni, L., Dall'olio, S., … Davoli, R. (2012). Polymorphisms in an obesity-related gene (PCSK1) are associated with fat deposition and production traits in Italian heavy pigs. *Animal*, *6*(12), 1913–1924. https://doi.org/10.1017/S1751731112001280

Frank, G. R., Fox, J., Candela, N., Jovanovic, Z., Bochukova, E., Levine, J., … Farooqi, I. S. (2013). Severe obesity and diabetes insipidus in a patient with *PCSK1* deficiency. *Molecular Genetics and Metabolism*, *110*(1–2), 191–194. https://doi.org/10.1016/j.ymgme.2013.04.005

Giunta, F., De Vita, P., Mastrangelo, A. M., Sanna, G., & Motzo, R. (2018). Environmental and genetic variation for yield-related traits of durum wheat as affected by development. *Frontiers in Plant Science*, *9*, 8. https://doi.org/10.3389/fpls.2018.00008

Hager, J., Dina, C., Francke, S., Dubois, S., Houari, M., Vatin, V., … Froguel, P. (1998). A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. *Nature Genetics*, *20*(3), 304–308. https://doi.org/10.1038/3123

Haraksingh, R. R., & Snyder, M. P. (2013). Impact of variation in the human genome on gene regulation. *Journal of Molecular Biology*, *425*(21), 3970–3977. https://doi.org/10.1016/j.jmb.2013.07.015

Harter, B., Fuchs, I., Muller, T., Akbulut, U. E., Cakir, M., & Janecke, A. R. (2016). Early clinical diagnosis of PC1/3 deficiency in a patient with a novel homozygous *PCSK1* splice-site mutation. *Journal of Pediatric Gastroenterology and Nutrition*, *62*, 577e580. https://doi.org/10.1097/MPG.0000000000001018

Herbert, A., Gerry, N. P., McQueen, M. B., Heid, I. M., Pfeufer, A., Illig, T., … Wichmann, H. E. (2006). A common genetic variant is associated with adult and childhood obesity. *Science*, *312*(5771), 279–283. https://doi.org/10.1126/science.1124779

Hunt, R., Sauna, Z. E., Ambudkar, S. V., Gottesman, M. M., & Kimchi-Sarfaty, C. (2009). Silent (synonymous) SNPs: Should we care about them? *Methods in Molecular Biology*, *578*, 23–39. https://doi.org/10.1007/978-1-60327-411-1_2

International Chicken Genome Sequencing Consortium. (2004). Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature*, *432*(7018), 695–716. https://doi.org/10.1038/nature03154

Jackson, R. S., Creemers, J. W. M., Ohagi, S., Raffin-Sanson, M.-L., Sanders, L., Montague, C. T., … O'Rahilly, S. (1997). Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nature Genetics*, *16*(3), 303–306. https://doi.org/10.1038/ng0797-303

Kilpeläinen, T. O., Bingham, S. A., Khaw, K. T., Wareham, N. J., & Loos, R. J. (2009). Association of variants in the PCSK1 gene with obesity in the EPIC-Norfolk study. *Human Molecular Genetics*, *18*(18), 3496–3501. https://doi.org/10.1093/hmg/ddp280

Li, H., Deeb, N., Zhou, H., Mitchell, A. D., Ashwell, C. M., & Lamont, S. J. (2003). Chicken quantitative trait loci for growth and body composition associated with transforming growth factor-beta genes. *Poultry Science*, *82*(3), 347–356. https://doi.org/10.1093/ps/82.3.347

Lloyd, D. J., Bohan, S., & Gekakis, N. (2006). Obesity, hyperphagia and increased metabolic efficiency in Pc1 mutant mice. *Human Molecular Genetics*, *15*(11), 1884–1893. https://doi.org/10.1093/hmg/ddl111

López, M., Dorado, P., Ortega, A., Peñas-Lledó, E., Monroy, N., Silva-Zolezzi, I., … LLerena, A. (2013). Interethnic differences in UGT1A4 genetic polymorphisms between Mexican Mestizo and Spanish populations. *Molecular Biology Reports*, *40*(4), 3187–3192. https://doi.org/10.1007/s11033-012-2393-2

Mbikay, M., Croissandeau, G., Sirois, F., Anini, Y., Mayne, J., Seidah, N. G., & Chrétien, M. (2007). A targeted deletion/insertion in the mouse Pcsk1 locus is associated with homozygous embryo preimplantation lethality, mutant allele preferential transmission and heterozygous female susceptibility to dietary fat. *Developmental Biology*, *306*(2), 584–598. https://doi.org/10.1016/j.ydbio.2007.03.523

NRC. (1994). *Nutrient Requirements of Poultry*. Washington, DC: Natl Acad Press.

Puga, I., Lainez, B., Fernández-Real, J. M., Buxadé, M., Broch, M., Vendrell, J., & Espel, E. (2005). A polymorphism in the 3' untranslated region of the gene for tumor necrosis factor receptor 2 modulates reporter gene expression. *Endocrinology*, *146*(5), 2210–2220. https://doi.org/10.1210/en.2004-1366

Ramensky, V., Bork, P., & Sunyaev, S. (2002). Human non-synonymous SNPs: Server and survey. *Nucleic Acids Research*, *30*(17), 3894–3900. https://doi.org/10.1093/nar/gkf493

Rehfeld, J. F., Bundgaard, J. R., Hannibal, J., Zhu, X., Norrbom, C., Steiner, D. F., & Friis-Hansen, L. (2008). The cell-specific pattern of cholecystokinin peptides in endocrine cells versus neurons is governed by the expression of prohormone convertases 1/3, 2, and 5/6. *Endocrinology*, *149*(4), 1600–1608. https://doi.org/10.1210/en.2007-0278

Sauna, Z. E., Kimchi-Sarfaty, C., Ambudkar, S. V., & Gottesman, M. M. (2007). Silent polymorphisms speak: How they affect pharmacogenomics and the treatment of cancer. *Cancer Research*, *67*(20), 9609–9612. https://doi.org/10.1158/0008-5472.CAN-07-2377

Seidah, N. G., Marcinkiewicz, M., Benjannet, S., Gaspar, L., Beaubien, G., Mattei, M. G., … Chrétien, M. (1991). Cloning and primary sequence of a mouse candidate prohormone convertase PC1 homologous to PC2, Furin, and Kex2: Distinct chromosomal localization and messenger RNA distribution in brain and pituitary compared to PC2. *Molecular Endocrinology*, *5*(1), 111–122. https://doi.org/10.1210/mend-5-1-111

Selvaggi, M., D'Alessandro, A. G., & Dario, C. (2017). Environmental and genetic factors affecting milk yield and quality in three Italian sheep breeds. *Journal of Dairy Research*, *84*(1), 27–31. https://doi.org/10.1017/S0022029916000765

Seneviratne, C., Huang, W., Ait-Daoud, N., Li, M. D., & Johnson, B. A. (2009). Characterization of a functional polymorphism in the 3´ UTR of SLC6A4 and its association with drinking intensity. *Alcoholism: Clinical and Experimental Research*, *33*(2), 332–339. https://doi.org/10.1111/j.1530-0277.2008.00837.x

Shabalina, S. A., Spiridonov, N. A., & Kashina, A. (2013). Sound of silence: Synonymous nucleotides as a key to biological regulation and complexity. *Nucleic Acids Research*, *41*(4), 2073–2094. https://doi.org/10.1093/nar/gks1205

Stitziel, N. O., Tseng, Y. Y., Pervouchine, D., Goddeau, D., Kasif, S., & Liang, J. (2003). Structural location of disease-associated single-nucleotide polymorphisms. *Journal of Molecular Biology*, *327*(5), 1021–1030. https://doi.org/10.1016/s0022-2836(03)00240-7

Taylor, N. A., Van De Ven, W. J., & Creemers, J. W. (2003). Curbing activation:Proprotein convertases in homeostasis and pathology. *The FASEB Journal*, *17*(10), 1215–1227. https://doi.org/10.1096/fj.02-0831rev

Tuller, T., & Zur, H. (2015). Multiple roles of the coding sequence 5´ end in gene expression regulation. *Nucleic Acids Research*, *43*(1), 13–28. https://doi.org/10.1093/nar/gku1313

Upadhyay, M., Bortoluzzi, C., Barbato, M., Ajmone-Marsan, P., Colli, L., Ginja, C., … Crooijmans, R. P. M. A. (2019). Deciphering the patterns of genetic admixture and diversity in southern European cattle using genome-wide SNPs. *Evolutionary Applications*, *12*(5), 951–963. https://doi.org/10.1111/eva.12770

Waldman, Y. Y., Tuller, T., Keinan, A., & Ruppin, E. (2011). Selection for translation efficiency on synonymous polymorphisms in recent human evolution. *Genome Biology and Evolution*, *3*, 749–761. https://doi.org/10.1093/gbe/evr076

Yates, C. M., & Sternberg, M. J. (2013). Proteins and domains vary in their tolerance of non-synonymous single nucleotide polymorphisms (nsSNPs). *Journal of Molecular Biology*, *425*, 1274–1286. https://doi.org/10.1016/j.jmb.2013.01.026

Zerehdaran, S., Vereijken, A. L. J., Van Arendonk, J. A. M., & Van der Waaijt, E.H. (2004). Estimation of genetic parameters for fat deposition and carcass traits in broilers. *Poultry Science*, *83*(4), 521–525. https://doi.org/10.1093/ps/83.4.521

Zhang, H., Hu, X., Wang, Z., Zhang, Y., Wang, S., Wang, N., … Li, H. (2012). Selection signature analysis implicates the PC1/PCSK1 region for chicken abdominal fat content. *PLoS ONE*, *7*(7), e40736. https://doi.org/10.1371/journal.pone.0040736

Zhang, K., Cheng, B. H., Yang, L. L., Wang, Z. P., Zhang, H. L., Xu, S. S., … Wang, S. Z. (2017). Identification of a potential functional single nucleotide polymorphism for fatness and growth traits in the 3'-untranslated region of the PCSK1 gene in chickens. *Journal of Animal Science*, *95*(11), 4776–4786. https://doi.org/10.2527/jas2017.1706

Zhao, Z., Fu, Y. X., Hewett-Emmett, D., & Boerwinkle, E. (2003). Investigating single nucleotide polymorphism (SNP) density in the human genome and its implication for molecular evolution. *Gene*, *312*, 207–213. https://doi.org/10.1016/s0378-1119(03)00670-x

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