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A functional variant in the promoter region of *IGF1* gene is associated with chicken abdominal fat deposition

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ABSTRACT

Insulin-like growth factor 1 (*IGF1*) plays an important role in the regulation of cell growth, proliferation, differentiation, and apoptosis. Previously several studies revealed that genotypes of chicken *IGF1* c.-366A > C were significantly associated with abdominal fat weight and body weight in chickens. But the underlying mechanism is still unknown. To investigate the mechanism underlying the association, herein, we performed *IGF1* gene mRNA expression profiling, a dual-luciferase reporter assay and electrophoretic mobility shift assay (EMSA). Quantitative real-time PCR results showed that *IGF1* gene was widely expressed in 14 tissues. The mRNA expression levels of *IGF1* gene in both abdominal fat and jejunum were significantly higher in fat broilers than in lean broilers. However, the opposite results were observed in the pancreas. The reporter gene assay showed that the promoter luciferase activity of allele A was significantly higher than that of allele C ($P < 0.05$). In addition, the luciferase activity of allele A promoted by the transcription factor AP1 and OCT1 was higher than that of allele C ($P < 0.05$). Electrophoretic mobility shift assay result showed that allele A binding to the transcription factor AP1 and OCT1 was stronger than that of allele C. All in all, our data indicated that the *IGF1* gene c.-366A > C is a functional SNP responsible for chicken adipose deposition.

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1. Introduction

With the rapid development of broiler industry, the problem of excessive accumulation of broiler body fat (especially abdominal fat) is becoming increasingly serious. Excessive fat deposition in broilers has adverse effects on feed conversion, carcass yield, hatching rate, and fertility rate [1]. Controlling the excessive accumulation of fat in chickens and improving the feed conversion efficiency and carcass quality of broilers are major issues that the poultry industry urgently needs to solve [2–4]. However, measurement of chicken abdominal fat is costly and laborious by slaughtering birds, which impede genetic improvement based on birds' abdominal fat measures. Understanding the genetic factors associated with obesity will facilitate genetic improvement via marker-assisted selection (MAS).

Insulin-like growth factor 1 (IGF1) belongs to IGF system and has a structure similar to insulin. Insulin-like growth factor 1 gene is involved in the regulation of cell growth, proliferation, differentiation, and apoptosis [5]. Insulin-like growth factor 1 plays an important role in regulating the growth and development of many tissues, such as muscle,

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bone, and fat tissues [6,7]. For instance, *IGF1* gene is highly expressed in the liver of mice and can inhibit the accumulation of cholesterol in the liver, thus affecting the synthesis and decomposition of fat [8], and it can upregulate *PPAR* by activating IGF-1R and PI3K pathways to promote human fat deposition [9]. Some studies reported that *IGF1* gene can affect economically important traits in farm animals. Islam et al found that c-512C > T in the promoter region of bovine *IGF1* gene is significantly correlated with bovine fat deposition and carcass quality traits [10]. There is numerous evidence suggesting that *IGF1* might influence growth rate, body composition, and lipid metabolism in poultry [11–13].

Our previous study indicated that a polymorphism locus c.-366A > C in the promoter region of *IGF1* gene is significantly associated with body weight at 2 to 12 wk of age [14] and abdominal fat content (data unpublished) in F_2 resource derived from a cross between boiler and layer cross, which is confirmed in another 2 independent studies of the same mutation of *IGF1.* Zhou et al reported *IGF1* SNP1 (namely, c.-366A > C) has a significant effect on body weight and abdominal fat percentage in F_2 population from Leghorn and Fayoumi cross [15]. A significant association of the same *IGF1* mutation with average daily gain at 107 d is observed using 2 genetically diverse maternal and paternal Black Penedesenca chicken strains [16]. These results suggest that *IGF1* c.-366A > C might be a causative mutation responsible for chicken fat deposition.

The objectives of this study are to examine whether c.-366A > C in the promoter of *IGF1* displays functional activity and investigate the effects of the polymorphism on gene expression mechanism.

2. Materials and methods

2.1. Ethics statement

All animal work was conducted in accordance with 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. Plasmid construction and transfection were performed as per the directions of the Regulation on Safety Administration of Agricultural Genetically Modified Organisms established by China (revised version 2017).

2.2. The experimental method

2.2.1. Animal and sample collection

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 wk of age (BW7)] and plasma very low–density lipoprotein levels as selection criteria [17,18]. The NEAUHLF lines were kept under the same environmental conditions and had free access to feed and water. Commercial corn-soybean–based diets that met all National Research Council requirements were provided. From hatch to 3 wk of age, the birds received a starter feed (3,000 kal ME = kg and 210 g = kg CP) and from 4 wk of age to slaughter, the birds were fed a grower diet (3,100 kal ME = kg and 190 g = kg CP) [19].

For RNA experiment, we harvested a total of 14 tissues from 7-wk-age male birds (n = 3, per line) of the 19th generation population of NEAUHLF, including craw fat, gizzard fat, mesenteric fat, heart, cerebrum, liver, spleen, gizzard, duodenum, jejunum, kidney, testis, pancreas, and abdominal fat. The samples are stored in the refrigerator at −80°C until RNA extraction.

2.2.2. Primer design

As per GenBank accession numbers of chicken *IGF1, TBP, AP1, OCT1,* and *β-actin* genes, we designed their primers (Table 1) using Primer Premier 5.0 software (Premier, Canada). The primers of *IGF1* and *TBP* were used for detecting the relative expression levels of genes by RT-qPCR; the primers of *AP1* and *OCT1* were used for constructing their eukaryotic expression vectors; *β-actin was* used as a loading control in western blot analysis.

2.2.3. RNA extraction and RT-qPCR

Total RNA was extracted from each tissue (100 mg each) using TRIzol reagent (Invitrogen Corp, Carlsbad, CA) in accordance with the manufacturer's protocol. Ribonucleic acid quality was assessed by visualization of the 18S and 28S ribosomal RNA bands on a denaturing formaldehyde agarose gel. Only RNA with a 28S:18S ratio between 1.8 and 2.1 was used for reverse transcription. In particular, total RNA of each pooled sample was extracted from 3 lean male birds and 3 fat male birds (randomly selected from the 6 sampled birds in each line) and mixed in equal amounts. The pooled sample of each tissue was used for detection of tissue *IGF1* mRNA expression. Tissue samples from individual birds were used for the comparison of *IGF1* mRNA expression levels between fat and lean birds. Reverse transcription was performed as per the directions of the PrimeScript TM RT reagent Kit with gDNA Eraser (Takara, Dalian, China).

Quantitative reverse-transcription PCR (RT-qPCR) was used to analyze gene expression levels using TATA box binding protein gene (*TBP*) as an internal reference. RT-qPCR was performed using FastStart Universal SYBR Green Master (ROX) (Roche Life Science, Indianapolis, IN) on a 7,500 Real-Time PCR System (Applied Biosystems, Foster City, CA). A 1-μL aliquot of each reverse-transcription reaction product was amplified in a 10-μL PCR reaction mixture. The following PCR conditions were used: incubation for 1 cycle at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Dissociation curves were analyzed using Dissociation Curve 1.0 software (Applied Biosystems Inc) for each PCR reaction to detect and eliminate possible primer dimer artifacts. The relative expression level of the target gene to *TBP* was determined using the $2^{-\Delta CT}$ method, in which C_T is the cycle threshold value and $\Delta C_T = C_T$(*IGF1*) – CT(*TBP*). The statistical significance of the comparison of *IGF1* mRNA expression levels between fat and lean birds was evaluated using the 2-tailed Student's *t*-test.

2.2.4. Construction of IGF1 promoter luciferase reporter

As per GenBank chicken *IGF1* gene (NC_006088.4), the DNA fragment containing SNP locus was synthesized by

Table 1
Primers used in this study.

GenBank accession no.	Primer name	Primer sequence (5′~3′)	Purpose
NM_001004384.2	*IGF1-F*	5′-GCTGGTTGATGCTCTTCAGTTCG-3′	RT-qPCR
	IGF1-R	5′-GCGTGCAGATTTAGGTGGCTTT-3′	
NM_205103.1	*TBP-F*	5′-GCGTTTTGCTGCTGTTATTATGAG-3′	internal reference
	TBP-R	5′-TCCTTGCTGCCAGTCTGGAC-3′	
NM_015296163.1	*AP1-F*	5′-TGGCCATGGAGGCCCGAATTCAGATGGAGCCTACTTTCTACGAG-3′	Construction of eukaryotic expression vector
	AP1-R	5′-CCGCGGCCGCGGTACCTCGAGGCTTTTGGTTTGTTGTTTGG-3′	
XM_419622.5	*OCT1-F*	5′-TGGCCATGGAGGCCCGAATTCCCAAAATGCCAACCCT-3′	
	OCT1-R	5′-CCGCGGCCGCGGTACCTCGAGACTGCCCAGAAAGTTGTG-3′	
NM_205518.1	*β-actin-F*	5′-TGGCCATGGAGGCCCGAATTCCCAAAATGCCAACCCT-3′	loading control
	β-actin-R	5′-CCGCGGCCGCGGTACCTCGAGACTGCCCAGAAAGTTGTG-3′	

GENEWIZ company, and the DNA fragment of *IGF1* c.-366A > C was cloned into pGL3-Basic Vector, named pGL3-*IGF1*-CC, and pGL3-*IGF1*-AA.

2.2.5. Bioinformatics analysis of chicken IGF1 gene c.-366A > C

To investigate potential molecular mechanism underlying the association of abdominal fat content with chicken *IGF1* gene c.-366A > C, we carried out in silico analysis of the transcription factor binding site of *IGF1* promoter using 3 bioinformatic tools, including JASPAR (http://jaspar.binf.ku.dk/), TFBIND (http://tfbind.hgc.jp/), and Mulan (http://mulan.dcode.org/).

2.2.6. Construction of eukaryotic expression vector

The total RNA of chicken fat and liver tissues was extracted by TRIzol method (Invitrogen Corp, Carlsbad, CA) and reverse-transcribed into cDNA (Takara, Dalian, China). The coding sequence (CDS)fragments of AP1 and OCT1 genes were obtained by PCR amplification with the mixed cDNA as template (primers shown in Table 1). The AP1 and OCT1 expression vectors were obtained by inserting the amplified CDS fragments to pCMV-HA vector (Promega) using ClonExpress II One Step Cloning Kit (Vazyme Biotech Co, Ltd). The recombinant plasmids were identified by double enzyme digestion of *XhoI* and *EcoRI*, and the eukaryotic expression vectors were named pCMV-AP1 and pCMV-OCT1.

2.2.7. Cell culture

Two cell lines were used for promoter luciferase reporter assay; one is DF1 cell line that is widely used for cellular and molecular studies in chickens, including the luciferase reporter assay [20,21], and the other is an immortalized chicken preadipocyte cell line (ICP-1) from our laboratory [22].

Cells were cultured in DMEM/F12 medium (Gibco, New York, NY) supplemented with 10% fetal bovine serum (BI, Germany), 100 units/ml penicillin, and 100 mg/mL streptomycin, and incubated at 37°C, 5% CO_2.

2.2.8. Luciferase reporter gene assay

For transfection of the luciferase reporter plasmid, cells were seeded in 24-well plates, at 70%–80% confluence transfected with pGL3-Basic Vector containing the SNP pGL3-*IGF1*-CC or pGL3-*IGF1*-AA and pRL-TK Renilla luciferase vector (Promega) as an internal control using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). For the cotransfection of luciferase reporter plasmid and the expression plasmid, cells were seeded in 24-well plates, transfected with pGL3-Basic Vector containing the SNP pGL3-*IGF1*-CC or pGL3-*IGF1*-AA, the pRL-TK Renilla luciferase vector, and pCMV-AP1 or pCMV-OCT1 expression vector using Lipofectamine 2000. After 48 h, cells were collected and luciferase activity was measured using the

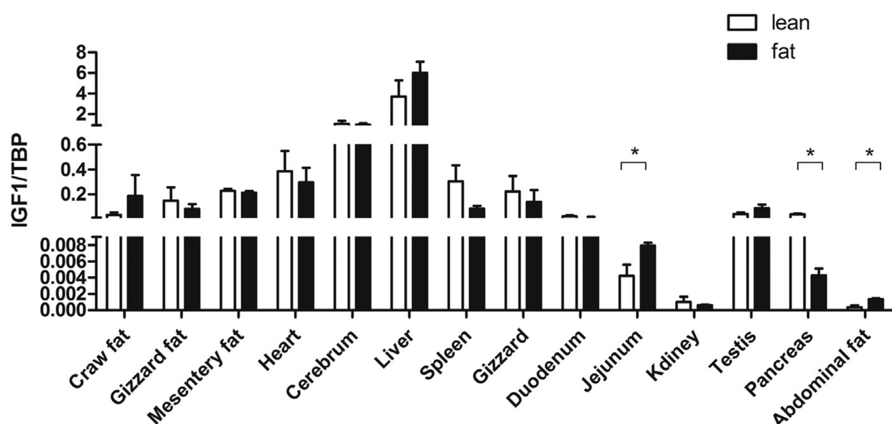

Fig. 1. Expression pattern of the chicken *IGF1* gene in various tissues. *TBP* was used as an internal reference gene. * means the difference is significant ($P < 0.05$).

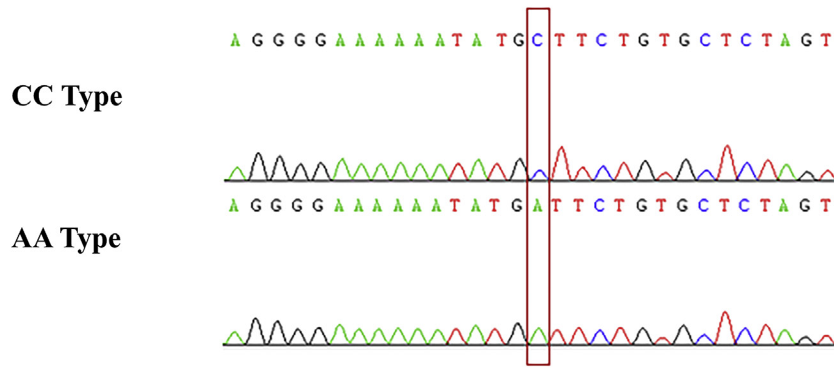

Fig. 2. The sequencing map of luciferase reporter gene vectors of different alleles. The sequencing results were homologous compared with known sequences to determine the correctness of the target fragment.

dual-luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to Renilla luciferase.

2.2.9. Western blot and EMSA

To validate the constructed eukaryotic expression plasmids pCMV-AP1 and pCMV-OCT1, we transfected pCMV-AP1 and pCMV-OCT1 into chicken DF1 cell lines, respectively, and extracted the total cell protein 48 h after transfection. After being mixed with 6 × denaturing loading buffer and boiled for 5 min, total cell proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). Western blotting was performed using antibody that recognizes HA-tag (1:1,000; TransGen Biotech, Beijing, China), a secondary horseradish peroxide–conjugated antibody was added with enhanced chemiluminescence (Beyotime Institute of Biotechnology).

To prepare nuclear extracts capable of interacting with SNP probes, the constructed pCMV-OCT1 and pCMV-AP1 eukaryotic expression vectors were transfected into DF1 cells, respectively. After 48 h transfection, NE-PER extraction reagent was used to collect the nuclear extract (Pierce, Waltham, MA).

For assaying the binding of nuclear proteins to the sequence of c.-366A > C, 2 double-stranded oligonucleotides with the primers 5′-AAAAATATGCTTCTGTGCTCTAA-3′ and 5′-GAAAAAATATGCTTCTGTGCT-3′ were biotin-labeled. Nuclear extracts were incubated with a biotin-labeled DNA probe for 20 min at room temperature and then, separated by electrophoresis on a 5% nondenaturing polyacrylamide gel with 0.5 × TBE running buffer. DNA-protein complexes were transferred onto nylon membranes (Pierce), and then cross-linked for 1 min with a UV cross-linker. The signal was detected as per the manufacturer's instructions for the LightShift Chemiluminescent EMSA Kit (Pierce, Waltham, MA). For the competition assay, nuclear extracts were incubated with unlabeled probes (Invitrogen) for 10 min at room temperature before the addition of biotin-labeled oligonucleotide. The density of the gel shift bands was quantified using ImageJ software (NIH Bethesda, MA). The EMSA experiment was replicated twice.

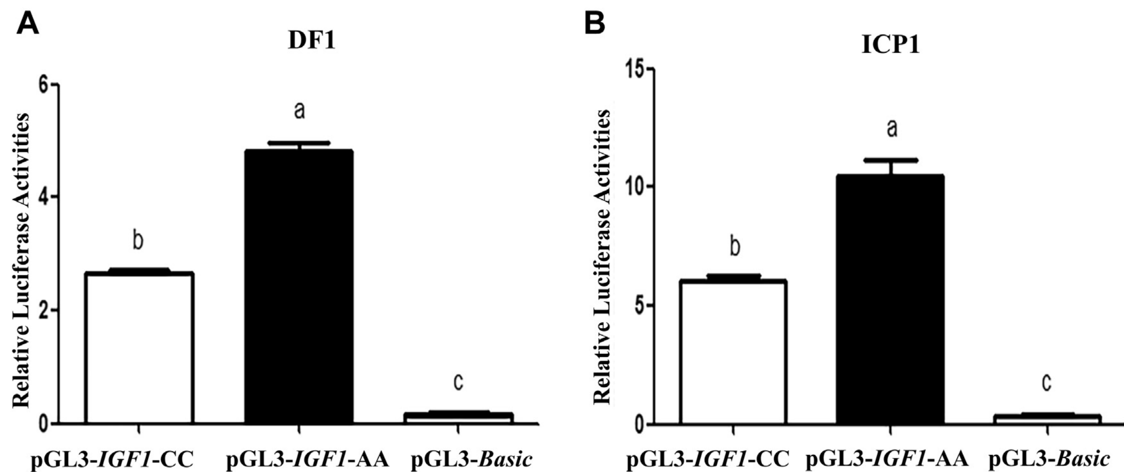

Fig. 3. Luciferase activity of different allele in DF1 and ICP-1 cells. (A) Luciferase assays in DF1 cells. (B) Luciferase assays in ICP-1 cells. Differences between means in relative luminescence units (RLUs) for the contrast among constructs with alternative alleles of the c.-366A > C SNP and the pGL3-Basic Vector in the luciferase assay. Values are shown as the mean ± SD (n = 3). Note: Different letters indicate significant difference ($P < 0.05$).

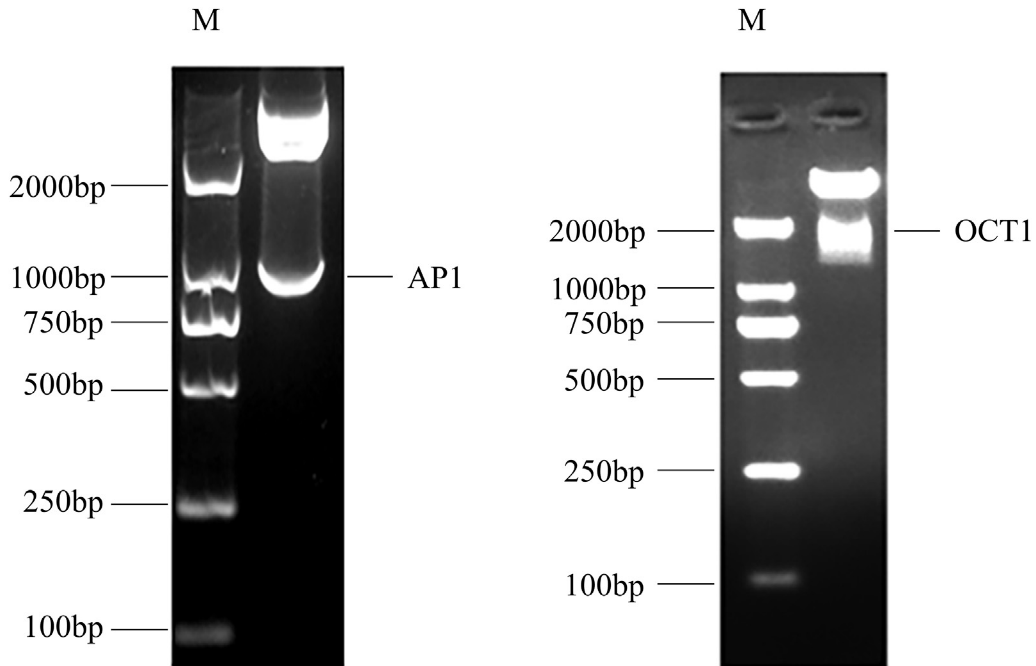

Fig. 4. Characterization of pCMV-AP1 and pCMV-OTC1 plasmids by restriction enzyme digestion via XhoI and EcoRI double enzyme digestion.

2.2.10. Statistical analysis

The experimental data were expressed as mean ± standard deviation (SD). JMP 11.0 (SAS Inst. Inc, Cary, NC) was used to compare the differences between the 2 groups of data using Student's *t*-test. $P < 0.05$ is considered statistically significant.

3. Results

3.1. Expression pattern of chicken IGF1 gene in various tissues

We detected the relative expression of *IGF1* gene mRNA in 14 different tissues of fat and lean chicken lines by RT-qPCR (Fig. 1). The results showed that *IGF1* gene was widely expressed in chicken tissues, and highly expressed in the brain and liver, and moderately expressed in craw fat, mesenteric fat, gizzard fat, heart, spleen, testis, muscle stomach, duodenum, and pancreas. The low expression level of *IGF1* gene was observed in jejunum, kidney, and abdominal fat. Furthermore, the mRNA expression levels of *IGF1* gene of fat line broilers were significantly higher than those of lean line broilers in abdominal fat and jejunum. However, the expression levels of lean line broilers were significantly higher than that in fat line birds in the pancreas ($P < 0.05$).

3.2. Bioinformatics analysis

To analyzing the effect of this SNP c.-366A > C on the binding of transcription factors, we carried out in silico analysis using 3 bioinformatic tools. The consistent prediction result indicated that the SNP could change the binding sites of some transcription factors. When the allele was A, the predicted transcription factors were AP1 and OCT1 that can bind to the DNA sequence of chicken *IGF1* gene c.-366A > C. The result implicated that SNP c.-366A > C may affect *IGF1* expression by regulating its promoter activity via AP1 and OCT1.

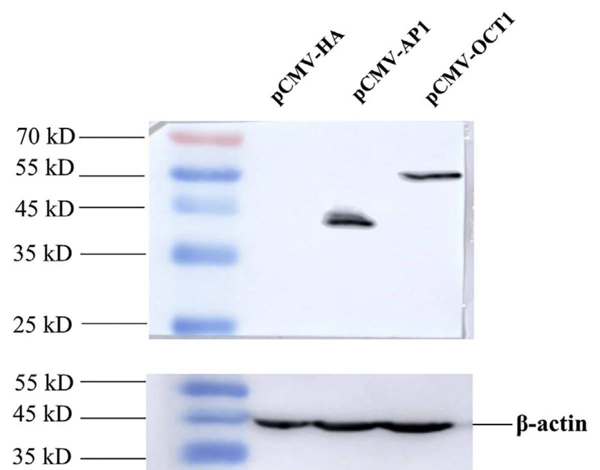

Fig. 5. Western blot to detect the protein expression of pCMV-AP1 and pCMV-OCT1. DF 1 cells were transfected with the pCMV-AP1 and pCMV-OCT1 vector. The total cell protein was harvested 48 h after transfection and immunoblotted with a HA-labeled antibody.

3.3. Dual-luciferase report gene assay

To identify the function of chicken *IGF1* gene c.-366A > C, we inserted the *IGF1* promoter fragment containing different alleles of this SNP into the pGL3-Basic Vector and constructed the dual luciferase reporter vectors pGL3-IGF1-CC and pGL3-IGF1-AA with different alleles of this SNP. These 2 reporter constructs were verified by sequencing (Fig. 2). To ensure the accuracy of the experiment, pGL3-IGF1-CC and pGL3-IGF1-AA were transfected into 2 types of chicken ICP-1 cells and DF1 cells, respectively. The results showed that the luciferase reporter gene activity of different alleles was significantly different in ICP-1 and DF1 cells, and the luciferase activity of pGL3-IGF1-AA was significantly higher than that of pGL3-IGF1-CC ($P < 0.05$, Fig. 3A and B).

3.4. Transcription factors AP1 and OCT1 regulate the expression of IGF1 c.-366A > C

We constructed the gene eukaryotic expression vector of AP1 and OCT1 and verified by *XhoI* and *EcoRI* double enzyme digestion (Fig. 4). Western blot was used to confirm their protein expression of pCMV-AP1 and pCMV-OCT1 by HA-labeled antibody. The results showed that both eukaryotic expression plasmids pCMV-AP1 and pCMV-OCT1 could express corresponding proteins in cells (Fig. 5).

Bioinformatics prediction showed that the substitution of C to A of *IGF1* c.-366A > C eliminated the AP1 or OCT1 binding sites in the chicken *IGF1* gene promoter region. To further examine the effects of AP1 and OCT1 on transcriptional activity of the reporter gene carrying c.-366A > C, we

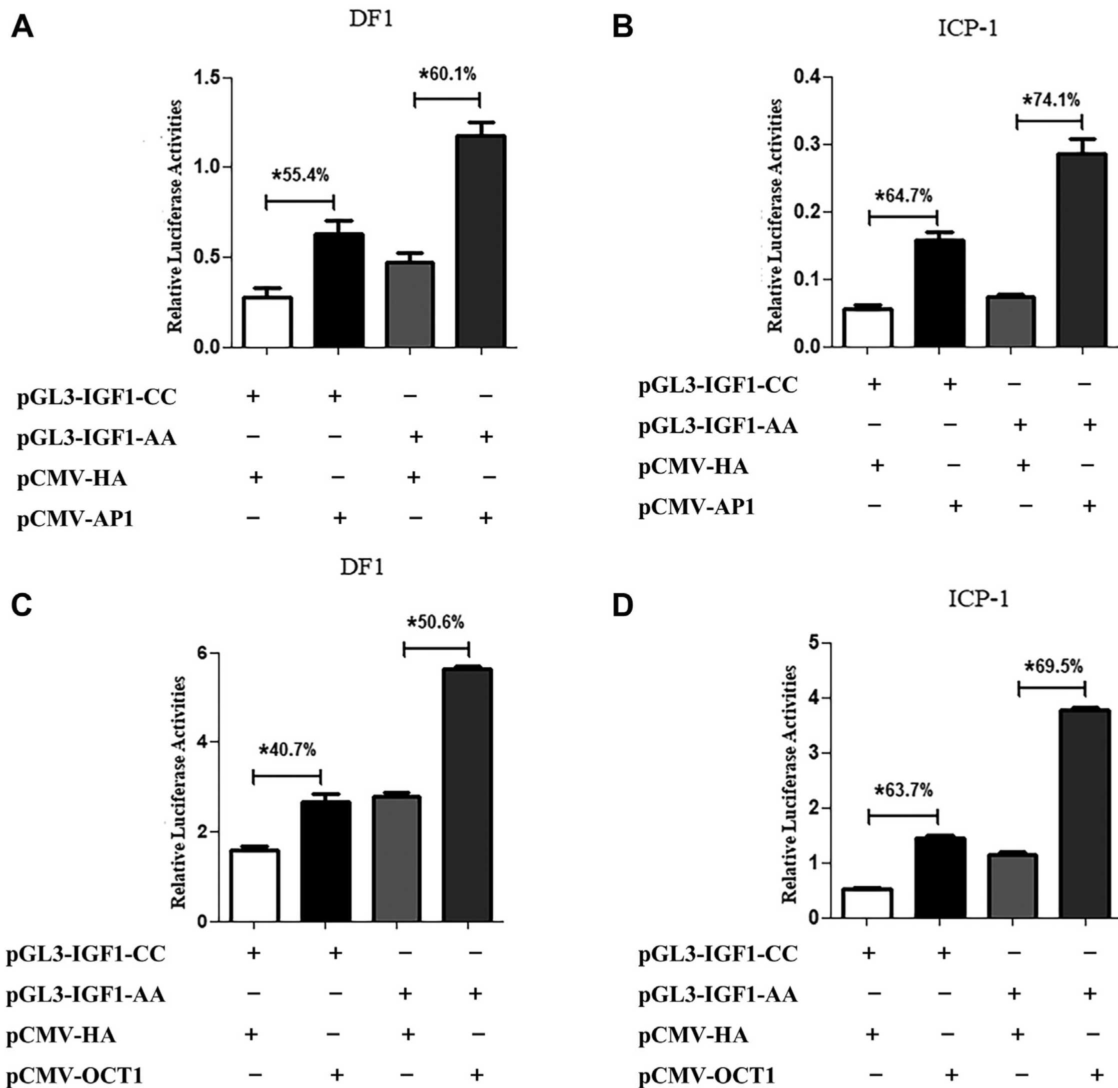

Fig. 6. The influence of AP1 and OCT1 on expression of different alleles of *IGF1* c.-366A > C in DF1 and ICP-1 cell. DF1 cells were cotransfected with a luciferase reporter construct containing pGL3-IGF1-CC or pGL3-IGF1-AA and AP1 expression vector (A) or OCT1 expression vector (C). The same as in ICP-1 cell is (B and D). Values are shown as the mean ± SD (n = 3). *$P < 0.05$.

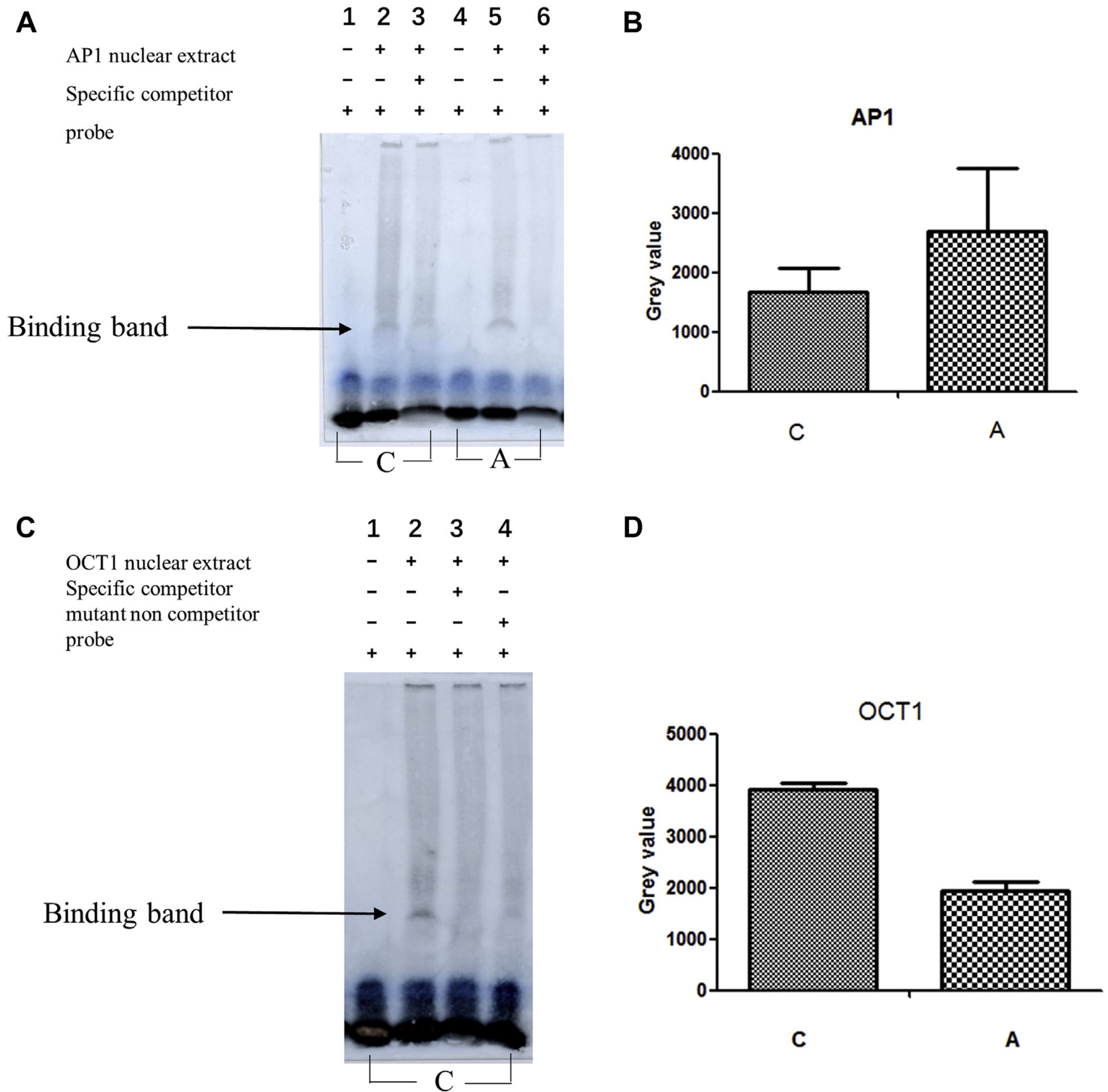

Fig. 7. EMSA assays for the c.-366A > C polymorphic site. (A) Assays in DF1 cells transfected with an AP1 expression vector. (B) The C allele gel shift band density and the A allele gel shift band density. (C) Assays in DF1 cells transfected with an OCT1 expression vector. (D) The C allele gel shift band density and the A allele gel shift band density. Only free probe containing the C allele is observed (lane 1). Binding of nuclear proteins is observed with the probe containing the C allele (lane 2). Binding was competed by a 50-fold excess of unlabeled probe (lane 3). (C) Binding of nuclear proteins is observed with the probe containing the A allele (lane 4).

cotransfected the AP1 or OCT1 expression vector with the luciferase reporter containing the SNP pGL3-IGF1-CC or pGL3-IGF1-AA into DF1 cells and ICP-1 cells, respectively. The reporter gene assay results indicated that both transcription factors AP1 and OCT1 promoted reporter gene activity in ICP-1 cells and DF1 cells, and the promoting effect of AP1 and OCT1 on A allele was significantly higher than that of the C allele ($P < 0.05$, Fig. 6).

3.5. EMSA experiment

To validate the reporter gene results, we performed an in vitro DNA-protein binding assay using EMSA. A protein-DNA complex band was detected when the probe was incubated with the nuclear protein extracts (binding band indicated by the arrow in Fig. 7A and C). In contrast with the C allele, the A allele of c.-366A > C showed stronger

binding properties with nuclear extracts rich in both AP1 (Fig. 7A and B) and OCT1 (Fig. 7C and D). In competition experiments, both allele shift band densities decreased with a 50-fold excess of unlabeled A allele oligonucleotides.

4. Discussion

IGF1 gene is widely implicated in the regulation of cell growth, proliferation, differentiation, and apoptosis [5], and plays an important role in the regulation of animal growth and metabolism. Prior multiple independent studies revealed that genotypes of chicken *IGF1* c.-366A > C is associated with abdominal fat deposition in chickens [14–16], indicating that it could act as a potential molecular marker for the breeding of high-quality broilers with low abdominal fat content. It is, however, exceedingly necessary to perform further confirmation of functionality and disentangling of mechanism of effect of the SNP before its application to breeding program.

To understand the function of the *IGF1* gene in chickens, we initially conducted tissue expression profiling to evaluate the differential expression of the gene in 14 tissues between the fat and lean lines. Our data showed that chicken *IGF1* gene was widely expressed in various tissues (Fig. 1). There is numerous evidence suggesting that *IGF1* might influence growth rate, body composition, and lipid metabolism in poultry [11–13], which indicates that *IGF1* gene plays crucial roles in chicken growth and development like mammals. In this study, the mRNA expression levels of *IGF1* gene of fat line birds were significantly higher than those of lean line birds in abdominal fat (Fig. 1), suggesting *IGF1* gene is likely to play a positive role in the fat deposit. Similarly, the mRNA expression levels of *IGF1* gene of fat line birds exhibit strikingly higher than those of lean line birds in jejunum, which seems to imply that *IGF1* gene has a positive effect on the promotion of feed conversion and absorption, contributing to abdominal fat deposit. Actually, a number of studies showed that jejunum is closely related to the digestion and absorption of nutrient in chicken [23–25]. Although there was no significant difference in expression level of *IGF1* gene in liver between fat and lean line birds, the most abundant expression of *IGF1* gene was observed in liver (Fig. 1), which probably indicates that the liver plays a major role in the synthesis and metabolism of fat. Compared with mammals, fat in birds is mainly synthesized in the hepatic tissue. In fact, a host of studies have revealed that liver plays a main role in lipogenesis, providing lipids destined to be used by all tissues and the liver itself [26,27].

A promoter, a sequence of DNA upstream of a gene coding region, contains multiple cis-acting elements, which are specific binding sites of proteins involved in the initiation and regulation of gene transcription [28]. A promoter contains important information of gene expression regulation network, which plays a pivotal role in regulating gene transcription [29]. Single nucleotide polymorphisms in promoter regions can affect gene regulation by altering the binding of transcript factor [30,31]. For instance, Kostek et al reported that a polymorphism in *IGF1* promoter region (rs35767) is significantly associated with total human fat mass [32]. In the present study, c.-366A > C is located in the *IGF1* gene promoter, suggesting that it may affect the efficiency of *IGF1* gene transcription. Our data showed the luciferase activity of allele A was significantly higher than that of allele C in both DF1 and ICP-1 cells (Fig. 3), indicating that this SNP could change the transcription efficiency of *IGF1* gene in vitro. Notably, consistent results of luciferase reporter assay were observed using 2 different types of cell (DF1) and (ICP-1), which assures the reliability of the results. All these results indicate that c.-366A > C is a functional variant that is responsible for chicken obesity.

Transcription factors are trans-acting factors that can bind to cis-acting elements of target genes to regulate gene expression [33,34]. In silico analysis suggested that the SNP could change the binding sites of transcription factors AP1 and OCT1. It was reported that transcription factor AP1 can bind to *IGF1* gene promoter region and enhance the transcriptional activity of *IGF1* gene promoter [35] and affect adipocyte differentiation by regulating PPARγ [36]. Transcription factor OCT1 is highly expressed in mouse liver and plays a major role in glucose and lipid metabolism in liver [37]. Li et al confirmed that A > G mutation in the pig Klotho gene promoter region could regulate the promoter activity through binding to OCT1, thereby affecting the expression of the pig Klotho gene and fat formation [38,39]. Therefore, we speculate that the *IGF1* c.-366A > C might affect abdominal fat content by modulating *IGF1* transcription via transcription factors AP1 and OCT1.

In our study, we confirmed that overexpression of AP1 and OCT1 significantly promoted the transcription efficiency of *IGF1* gene in vitro, and AP1 and OCT1 increased the activity of A allele than that of C allele (Fig. 6), suggesting that the c.-366A > C may influence gene transcription by the alteration of the binding of transcription factors AP1 and OCT1 to *IGF1* promoter. The EMSA showed that the oligonucleotide containing the A allele of the c.-366A > C had higher binding affinity than that containing the C allele. Briefly, the c.-366A > C has an allele-specific effect on *IGF1* expression through varying affinity for AP1 and OCT1. As a result, the findings suggest that c.-366A > C may influence chicken abdominal fat deposition by modulating *IGF1* expression.

5. Conclusions

IGF1 gene c.-366A > C is a functional SNP, which may affect the transcription regulation of *IGF1* gene by influencing the binding of transcription factors AP1 and OCT1 to the *IGF1* gene promoter region. Meanwhile, the findings will provide a reliable functional molecular marker for genetic improvement of abdominal fat content in chicken MAS program and help understand molecular regulatory mechanism of *IGF1* gene on chicken adipose tissue growth and development.

CRediT authorship contribution statement

W.J. Wang: Methodology, Investigation, Validation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Y.Q. Guo:** Validation, Investigation, Writing - review & editing. **K.J. Xie:** Investigation, Writing - review & editing. **Y.D. Li:** Investigation, Writing - review & editing. **Z.W. Li:** Writing - review & editing. **N.**

Wang: Methodology, Writing - review & editing, Formal analysis. **F. Xiao:** Writing - review & editing. **H.S. Guo:** Writing - review & editing. **H. Li:** Supervision. **S.Z. Wang:** Conceptualization, Supervision, Resources, Writing - review & editing, Funding acquisition.

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