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Full Length Research Paper

Identification of a single nucleotide polymorphism of the pituitary-specific transcriptional factor 1 (Pit 1) gene and its association with body composition trait in Iranian commercial broiler line

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Pit-1 is a pituitary-specific transcriptional factor that has been shown to play a critical role both in cell differentiation during organogenesis of the anterior pituitary and as a transcriptional activator for pituitary gene transcription. This study was designed to investigate the associations of Pit-1 gene polymorphism on chicken body growth and body composition traits. Genomic DNA was extracted from 120 chickens from Iranian commercial broiler line. Two polymorphisms of the Pit-1 gene were found with restriction fragment length polymorphisms. The association between these polymorphisms with chicken growth and body composition traits were analyzed using single marker analysis. Polymorphisms in Pit-1 gene were significantly (P<0.1) associated with body growth and body composition traits. This study suggests that Pit-1 gene could be a candidate locus or linked to major gene(s) that affects growth and body composition traits in the chicken.

Key words: Iranian broiler lines, growth and body composition traits, pituitary-specific transcription factor gene (Pit 1), polymorphism, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

INTROUDUCTION

Commercial selection of meat-type chickens has become complex as it must take into consideration a great number of objectives, all related to the reduction of costs and the improvement of quality of the final product (Barton, 1994). Many economically important traits of domestic animals, such as growth, egg production and body composition are controlled by quantitative trait loci. Classical quantitative genetics can not independently decompose individual gene effects from the multiple genes associated with the variation of complex and quantitative traits. With the advances of the cutting edge molecular biology, sequencing of the entire genome, comparative genomics and candidate gene approach, facilitated by results of the comparative genomic study, has been proven to be powerful for studying the genetic architecture of complex traits and is a far more effective and economical method for direct gene discovery. It has been widely applied in identification of genes responsible for economically important traits in animals. Marker-assisted selection (MAS) can be used to increase selection efficiency and make further improvements in production traits. Genetic markers linked with QTL allow for direct selection of genotype (Lamont et al., 1996). To improve production and fitness traits simultaneously, molecular markers associated with one or both sets of traits may be useful. Understanding the genetic control of growth in chickens will provide an opportunity for genetic improvement of production performance and physiology (Li et al., 2003).

The pituitary-specific transcription factor (POU1F1) is a protein which binds to and transactivates promoters of growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone chain (TSHB)-encoding genes (Bodner et al., 1988; Ingraham et al., 1988; Steinfelder et al., 1992) and the pituitary-specific transcription factor gene (Pit 1)

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Table 1. Genotype and gene frequency of Pit1-*Taq1* loci in chicken population.

Genotype frequency			Gene frequency		Chi-square test (χ^2) p<0.05
AA	AB	BB	A	B	
0.61	0.32	0.07	0.00	0.23	0.00

itself in animal anterior pituitary (McCormick et al., 1990; Sornson et al., 1996). There are seven exons in Pit 1 and mutations in these exons cause hypoplasia of the pituitary gland and deficiencies of GH, PRL and TSHB (Aarskog et al., 1997; Holl et al., 1997). The chicken Pit 1 has been cloned and its cDNA sequence reported (Van As et al., 2000). Mutations in the chicken Pit 1 may regulate the expression of GH by altering the POU1F1 binding ability to the promoter of GH gene, which ultimately resulted in growth variation. With crucial role in the differentiation of anterior pituitary and the regulation of the PRL, GH and TSH genes, the chicken Pit 1 gene is regarded as a key candidate gene for production traits (Nie et al., 2008). The main goal of this study was to identify single nucleotide polymorphisms (SNP) in the Pit 1 gene, develop PCR-RFLP methods to detect the DNA polymorphisms in Iranian commercial broiler line, and evaluate associations between Pit 1 SNP and growth and body composition traits.

MATERIALS AND METHODS

Chicken populations

Iranian commercial broiler line were used in this study. All birds had free access to feed and water. The individuals were raised in floor pens and fed comercial corn-soybean diets that met NRC requirements. The fifteen generation individuals from Iranian commercial broiler line (n = 120) was used in this study. Live BW was measured at 6 weeks of age. Chickens were slaughtered, carcasses were eviscerated and dissected. Carcass weight (CW), breast muscle weight (BMW), drumstick weight (DW), back weight (BAKW), wing weight (WINW) and abdominal fat weight (AFW) traits were determined.

DNA extraction

Whole blood samples were collected from 120 chickens at 6 weeks of age. Genomic DNA were extracted using salting-out method with some modifications (Javanrouh et al., 2006). Optimization includes utilization of separate buffer instead of buffy coat isolation, in that chloroform is for DNA phase isolation and is used to purify DNA and sodium accetate for more concentrated DNA. The optimize porotocol would be more safe, simple, cheap and rapid.

PCR amplifications and genotyping

The Pit 1 primers (5' GGA CCC TCT CTA ACA GCT CTC 3'; 5' GGG AAG AAT ACA GGG AAA GG 3') were chosen based on the primers design by Nie et al. (2008), to ampify a 599 bp within intron 5 of the Pit 1 gene. Fifteen microliters of each PCR reaction contained: 1X PCR buffer; 2 mM $MgCl_2$; 0.25 µM primers; 200 µM dNTPs; 1 unit of Taq polymerase; 150 ng/reaction genomic DNA and ddH_2O. Thermal cycling included initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 1 min, 61°C for 1 min, 72°C for 1 min, and an extension at 72°C for 7 min. Two single nucleotide polymorphism (SNP) of the Pit 1 gene were detected by digesting 10 µl of the PCR product with *MspI* and *TaqI* restriction endonuclease at 37 and 65°C overnight, respectively. Restriction patterns were visualized by agarose gel electrophoresis and ethidium bromide staining.

Statistical analysis

Data were subjected to the MIXED precedures of SAS (SAS Inst. Inc., CARY, NC) with genotype, line and sex as fixed effects; Sire and Dam as random effects according to the models:

$$y_{ijkl} = \mu + Genotype_i + Sex_j + Line_k + Sire(Line) + Dam(Line\ Sire) + e_{ijkl}$$

In the formula, Y is the response variable; µ represents population mean and e stands for the random error. Significant differences between least-squares means of the different genotypes and haplotypes were calculated using a contrast test.

RESULTS

Allele frequency

The genotype and allele frequencies at Pit1-*Taq1* loci calculated by PopGene.S2 software, are shown in Table 1. The A allele was more frequent than B allele and AA genotype was more frequent than other genotypes in this population. The Chi-square test (P<0.05) indicated that the genotype distributions were not in Hardy–Weinberg equilibrium (Table 1). The genotype and allele frequencies at Pit1-*MspI* loci calculated by PopGene.S2 software, are shown in Table 2. The A allele was more frequent than B and C alleles, and AA genotype was more frequent than other genotypes in this population. The Chi-square test (P<0.05) indicated that the genotype distributions were not in Hardy–Weinberg equilibrium (Table 2). Disagreement of the genotype frequencies with the Hardy–Weinberg equilibrium expections tested indicated that Pit1 gene frequency was significantly different (P<0.05) in this population.

Identification of polymorphism and PCR-RFLP analysis]

The transition of C into T SNP, located at the intron 5 of the Pit 1 gene creates a restriction site for *Taq1*

Table 2. Genotype and gene frequency of Pit1-*Msp1* loci in chicken population.

Genotype frequency						Gene frequency			Chi-square test (χ^2)
AA	AB	BB	AC	BC	CC	A	B	C	p< 0.05
0.55	0.083	0.3	0	0.008	0.058	0.592	0.346	0.062	0.00

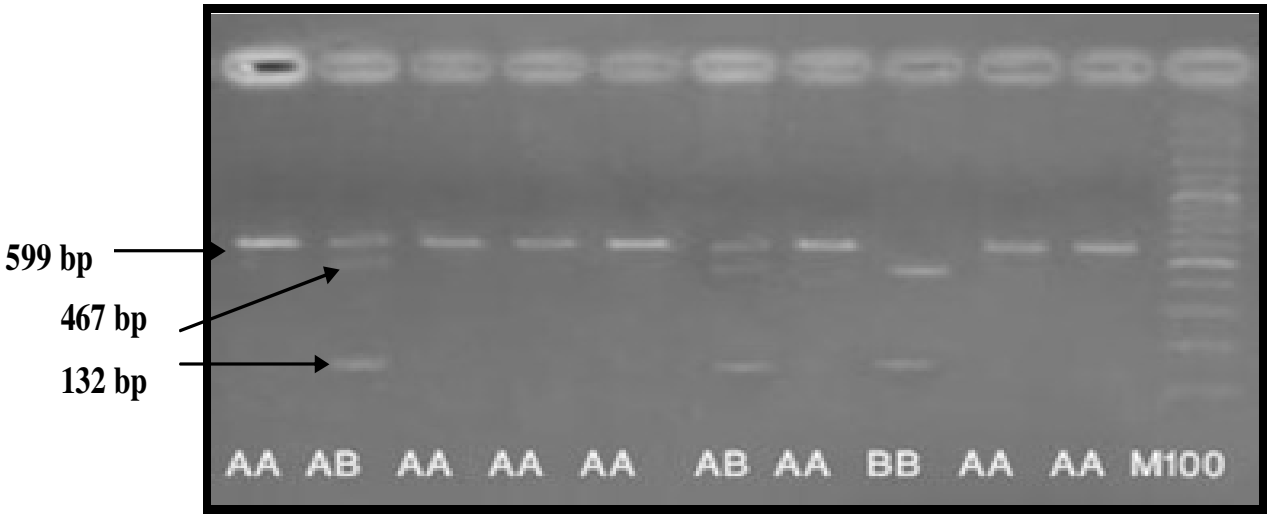

Figure 1. PCR-RFLP pattern for Pit 1 gene with *Taq1*digestion.

Table 3. Effects of Pit1-*Taq1* genotype on growth and body composition (least squares means).

Trait	P-value	AA	AB	BB
BW6 (g)	0.065	2649.7± 34.58[a]	2557.85± 42.07[b]	2729.65± 83.28[a]
CW (g)	0.213	1827.1± 23.01	1788.35± 28.76	1886.95± 56.95
BMW (g)	0.067	600.74± 9.67[ab]	579.3±10.79[b]	627.35± 21.6[a]
DW (g)	0.607	527.3± 7.8	519.65± 9.54	537.80± 18.74
WINW (g)	0.075	209.63± 2.54[ab]	202.46± 3.10[b]	213.98± 6[a]
BAKW (g)	0.034	410.6± 6.18[ab]	390. 7± 7.35[b]	420± 25.06[a]
AFW (g)	0.418	23.93± 1.56	21.88± 1.70	26.85± 3.09

[a,b]Means with no common superscripts differ significantly (P<0.1). [1]BW6 (g) = Body weight at 6 week; CW = carcass weight ; BMW = breast muscle weight. DW = drumstick weight; WINW = wing weight; BAKW = back weight, AFW = abdominal fat weight

endonuclease. The 599-bp fragment was digested with *Taq1* restriction enzyme. The restriction enzyme *Taq1* digested PCR product had fragments of 599 bp for AA homozygotes, fragments of 599, 467 and 132 bp for AB heterozygotes and 467 and 132 bp for BB homozygotes (Figure 1).

There were significant associations between the genotypes of Pit1-*Taq1* loci and BW6, BAKWT, BMW and WINW (P≤0.1). There were no significant associations between the genotypes of Pit1-*Taq1* loci and CW, DW and AFW (P>0.1; Table 3).

The transition of A into G SNP and transversion C into G SNP, located at the intron 5 of the Pit 1 gene creates a two restriction site for *Msp1* endonuclease. The 599-bp fragment was digested with *Msp1* restriction enzyme. The restriction enzyme *Msp1* digested PCR product had fragments of 599 bp for AA homozygotes, fragments of 599, 500 and 99 bp for AB heterozygotes, fragments of 500 and 99 bp for BB homozygotes, fragments of 599, 321 and 278 bp for AC heterozygotes, fragments of 500, 321, 278 and 99 bp for BC heterozygotes and fragments of 321 and 278 bp for CC homozygotes (Figure 2).

There were significant associations between the genotypes of Pit1-*Msp1* loci and CW, DW, BAKWT and WINW (P≤0.1). There were no significant associations between the genotypes of Pit1-*Msp1* loci and BW6, BMW

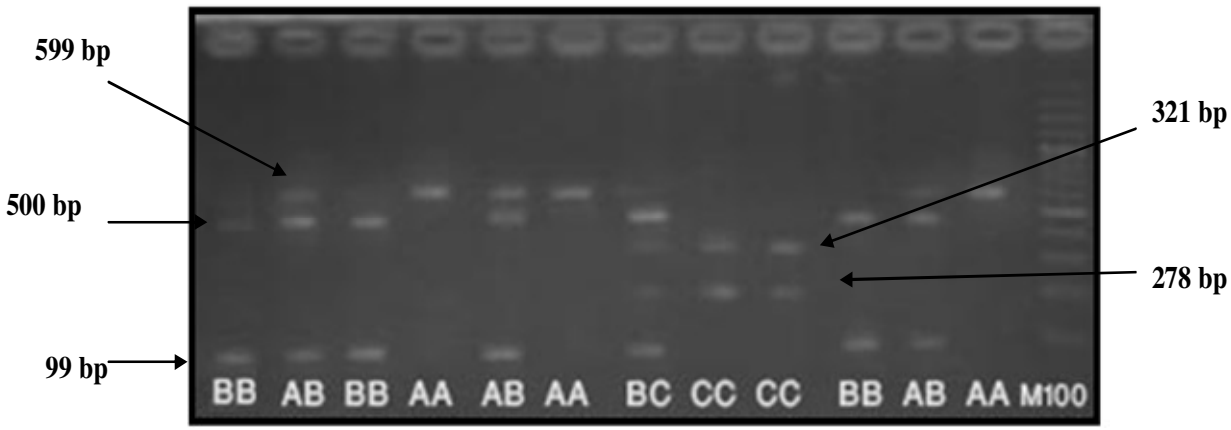

Figure 2. PCR-RFLP pattern for Pit 1 gene with *Msp1*digestion.

Table 4. Effects of Pit1-*Msp1* genotype on growth and body composition (least squares means).

Trait	P-value	AA	AB	BB	CC
BW6 (g)	0.258	2590.4±36.58	2704.85±77.07	2664.95±45.28	2538.7±96. 8
CW (g)	0.032	1776.1±23.81^{c}	1872.6±51.36^{ab}	1865.85±56.95^{b}	1879.55±63.95^{a}
BMW (g)	0.24	584.04±9.47	608.3±20.29	518.65±11.6	593.95±25.6
DW (g)	0.087	514.58±7.8^{b}	545.22±17.11^{ab}	534.80±9.9^{b}	555.24±21.3^{a}
WINW (g)	0.055	203.23±2.54^{b}	213.16±3.10^{ab}	211.92±3.3^{ab}	216.48±7.2^{a}
BAKW (g)	0.004	392.3 0.09^{b}	423.8±13.35^{ab}	416.22±7.06 b	435.6±16.32^{a}
AFW (g)	0.948	22.95±1. 6	22.78±3.4	24.15±1.9	24.55±4.2

^{a,b}Means with no common superscripts differ significantly (P<0.1). [1]BW6 (g) = Body weight at 6 week; CW = carcass weight ; BMW = breast muscle weight; DW = drumstick weight; WINW = wing weight; BAKW = back weight, AFW = abdominal fat weight.

and AFW (P>0.1; Table 4).

DISCUSSION

The candidate gene approach is a very powerful method to investigate associations of gene polymorphisms with economically important traits in farm animals (Rothschild and Soller, 1997). Many studies have examined growth, skeletal and immune function traits using the candidate gene approach in chickens (Zhou et al., 2001; Amills et al., 2003; Li et al., 2003). The Pit 1 gene was selected as a candidate gene to investigate associations of gene polymorphisms with growth and body composition in Iranian commercial broiler line. Growth is a composition of complex developments that result from genetic, nutritional and environmental factors (Scanes et al., 1984).

This study reported two mutations of the Pit 1 gene. Disagreement of the genotype frequencies with the Hardy–Weinberg equilibrium expections tested indicated that Pit 1 gene frequency was significantly different (P<0.01) in this population. This may be due to the high selection program done in population as meat chicken.

In this study, For Pit1-*Taq1* loci, there were significantly higher BW6, BMW, WINW and BAKWT in birds that were of the BB genotype than those of the AA and AB genotypes (P≤ 0.1; Table 3). This result is similar to that of Nie et al. (2008). For Pit1-*MspI* loci, there were higher CW, DW, WINW and BAKWT in birds that were of the CC than other genotypes. The other single nucleotide polymorphism in intron 5 of the PIT 1 gene was associated with body composition traits in chicken. The positive relationship between genotype CC and CW, DW, WINW and BAKWT traits indicates that the PIT1 SNP is a potential molecular marker for body composition traits in chicken. Therefore, it was presumed that it might have a QTL that affected the body composition traits in chicken in this region, and allele C was linked with the QTL of high body composition traits.In a previous study, variations of the PIT 1 gene were related to fatty trait in pig (Brunsch et al., 2002). However, none of these polymorphisms was significantly associated with any of chicken fatty trait (P>0.1). This result was similar to that of Nie et al. (2008).

The consistency of identifying significant associations

of the Pit1-*Taq1* and Pit1-*MspI* with growth and body composition traits in multiple independent studies suggests that use of Pit 1 variation may be valuable for efficient genetic selection for growth in broiler chickens.

Conclusion

In summary, the broiler chickens have undergone intensive breeding with so many objectives that should be simultaneously considered to reduce costs, improve health and product quality. So, several traits such as growth and body composition traits have been included in selection indices. In addition to difficulty of measurement of these traits, the corrolations among them are complex. MAS can be an ideal option to improve selection programs. The results from this study indicate that a SNP marker in the Pit 1 gene is associated with growth and body composition traits in chickens growing up to market weight and is therefore, a potential marker for molecular MAS programs in commercial broiler line in Iran.

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