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Single nucleotide Polymorphisms in intron 1 of growth hormone gene and it's association with economic important traits in Iranian Fars native fowl

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ABSTRACT

The chicken growth hormone (cGH) gene has an important function in chicken growth and reproduction. Single nucleotide polymorphisms (SNPs) are the most frequently found DNA sequence variations in the animal genome and can be used as genetic markers for association analysis with economic traits. The primary aim of this study was to identify SNPs in cGH as potential candidate genes for growth and production traits in Iranian chicken. In this study 150 blood samples were collected and a specific primer was designed for amplifying a fragment of cGH gene using polymerase chain reaction (PCR). The PCR products were sequenced. Sequencing was compared and aligned with the sequence obtained from the gene bank (accession number AY461843). Eleven SNPs were identified, one of which was common to all individuals. The association of other SNPs with the breeding values of individuals for five traits including body weight at 1 day of age (BW1), body weight at 8 weeks (BW8), body weight at 12 weeks (BW12), egg number (EGG NO), and age of sexual maturity (ASM), which were predicted by univariate animal model using ASREML, was investigated. This analysis showed that SNPs in 844 C/T were significantly associated with body weight at all ages measured. SNPs in 662 G/A were mainly related to BW1 and BW8. SNPs in 1025 T/C were significant affected on BW1 and SNPs in 762 C/A were significantly associated with ASM and EGGNO.

Key words: cGH, Single nucleotide polymorphisms, association, indigenous chicken.

INTRODUCTION

The chicken growth hormone (cGH) gene is one of the effective genes that influenced the chicken performance traits because it plays a crucial role in growth and metabolism [1, 2]. The products of cGH gene include a 191– amino acid mature growth hormone protein and a 25–amino acid signal peptide. In birds a complex pattern of structural variants in GH has been found [3]. The cGH gene is located at in the end of the long arm of chromosome 1 which consists of 5 exons and 4 introns with a whole length of 4.1 kb [4, 5, 8, 7].

Studies of polymorphisms of cGH gene were carried out by new approaches such as RFLP technique and sequencing of DNA [7, 8, 9]. Research on Chinese native Taihe Silkies chickens indicated that a 50 bp deletion occurred in intron 4 of the cGH gene [2]. Assessment of intron 4 of the cGH gene has demonstrated that polymorphisms were related to certain characteristics of Chinese native chickens [10]. Molecular studies have shown that substitution, deletion or insertion of a single nucleotide lead to variations in a gene, called single nucleotide polymorphisms (SNPs). Investigation of the whole chicken genome identified over 2.8 million SNPs [11].

A single SNP can influence the production and reproduction traits. For example, the chickens, sex-linked dwarf trait is a single nucleotide mutation at an exon-intron junction of the GH receptor gene [12]. Studies show significant progress in identifying the relationship between quantitative trait loci (QTL) and SNPs in domestic animals.

According to the findings of Van Laere *et al*. (2003), there is a variation in the QTL for muscle growth in pigs that it caused by SNPs in intron 3 of IGF2 gene[13]. Three SNPs in IGF1 and IGF2 genes of chicken were detected by Amills et al. (2003), which were associated with performance traits [14]. SNPs can be determined by new methods such as PCR-RFLP analysis, DHPLC (Denaturing high-performance liquid chromatography) [2, 15].

DNA sequencing is another technique that has been used to identify great numbers of SNP positions in the genome. For instance, one thousand SNPs in chicken were observed in a survey of 31 000 bases analyzed from broiler and layer lines [16].

The purpose of the present research was to detect the SNPs in intron 1 of cGH gene and to investigate their associations with the growth and production traits of the Fars native chicken breed in Iran.

MATERIALS AND METHODS

Experimental population

In this experiment, data were obtained from 10809 Iranian Fars native chickens belonging to the Breeding Center for Fars Native Chicken. These data were used for prediction of chickens breeding value and genetic parameters of body weight at 1 day (BW1); body weight at 8 weeks (BW8); body weight at 12 weeks (BW12); egg number (EGG NO) and age of sexual maturity(ASM).

Sampling and DNA extraction

Blood samples (2-4 mL) were collected from the wing vein and transferred to tubes containing EDTA and then preserved at −80 ◦C. Genomic DNA was extracted using the Salting Out method [17]. The quantity and quality of the extracted DNA was checked by spectrophotometer and Agarose gel electrophoreses, respectively.

Primer design and PCR conditions

Based on cGH gene sequence (GenBank accession number: AY461843) primers were designed using the Allele ID 6 software. The specificity of primer pairs was confirmed by BLASTN with all the nucleotide sequences available for chicken at the National Center of Biotechnology Information (NCBI). The forward and reverse primers were 5'-AAGCAACACCTGAGCAAC-3' and 5'- CTCTCTGGGACACACCTG -3' respectively and they were synthesized by Bioneer Company (Seoul, Korea). The PCR reactions were performed with a total volume of 30 mL. The cycling conditions were as follow: 5 min at 94°C for initiation of denaturation, then 45 s at 94°C (denaturation), 45 s at 58°C (annealing), 60 s at 72°C for extension (30 cycles), followed by 5 min at 72°C for final extension. The PCR products were purified and sequenced (Bioneer Co., Seoul, Korea). Subsequently, the cGH gene nucleotide sequences were analyzed using the Vector NTI Advance 10 Aligner software.

Statistical Analysis

Pedigree and records data of traits were edited by CFC and Excel 2007 software. We also analyzed the fixed effects with JMP (SAS Institute [18]) and SPSS software. Subsequently, based on the suitable single-trait animal model using ASREML software [19], breeding values of chicken were estimated. In order to select the suitable model, 6 different animal models were fitted. The animal models in matrix notations are presented in Table 1.

Table 1. Studied models

Models Number	Models Matrix Notations	
1	$y_i = X_ib_i + Z_ia_i + e_i$	
2	$y_i = X_ib_i + Z_ia_i + W_ic_i + e_i$	
3	$y_i = X_i\, b_i + Z1_i\, a_i + Z2_im_i + e_i$	$cov_{am} = 0$
4	$y_i = X_i\, b_i + Z1_i\, a_i + Z2_im_i + e_i$	$cov_{am} \neq 0$
7	$y_i = X_ib_i + Z1_ia_i + Z2m_i + Wc_i + e_i$	$cov_{am} = 0$
8	$y_i = X_ib_i + Z1_ia_i + Z2_im_i + W_ic_i + e_i$	$cov_{am} \neq 0$

In these models, y_i is vector of observations, b_i is a vector of fixed effects on observations of the ith trait (Generation-Hatch for all traits and Sex effect for body weight traits), a_i is an additive genetic effect, m_i is a vector of maternal additive genetic effect, c_i is a maternal environmental effect and e_i is a residuals for the ith trait and i = 1,…., 5 consists of BW1, BW8, BW12, ASM and EGG No. X, Z1, Z2 and W are incidence matrices relating observations to b_i, a_i, m_i and c_i, respectively. Cov_{am} is the covariance between direct and maternal additive genetic effects. Estimates of genetic parameters and (co)variance components were obtained by restricted maximum likelihood (REML) method, using the ASREML software [19]. Determination of superiority of one model than another was made by likelihood ratio test.

Association Analysis

In order to investigate the association between SNPs and breeding values of each individual for each trait, analysis was carried out using the GLM procedure of JMP software (SAS Institute, [18]).

The following linear model was used:

$y_{ij} = \mu + SNP_i + e_j$

in which y_{ij} is the predicted breeding value for each trait, μ is the population mean, SNP_i is the ith allele of the SNP and e_j is the random error and $P < 0.05$ indicates a significant difference.

RESULTS AND DISSCUSION

PCR product

We used the sequencing method in order to assess and detect SNPs in Iranian native chicken. We successfully amplified a fragment of intron 1 of the cGH gene with 621 bp in length in Fars native chicken. Our results showed 11 SNPs in a part of intron 1 of the cGH gene in Fars native chicken (Table 2). Out of these SNPs, 1 insertion was observed in all individuals: insertion of thymine, which occurred between nucleotides 647 and 648. The other SNPs were nucleotide substitutions, which included transition and transversion, the frequency of transition being higher than transversion. Six transitions were cytosine–thymine and guanine-adenine and the four transversions were cytosine- adenine. The frequency of the observed SNPs in this fragment was one SNP per 62 bp on average.

Breeding values

A summary of the number of animals recorded, mean, standard deviation, minimum and maximum for each of the traits is presented in Table 3. Breeding values were predicted for all chickens base on fitted models.

Association of cGH Gene SNP with Traits

Association analysis of cGH SNPs showed that four SNPs have a significant (P < 0.05) effect on age of sexual maturity, egg number and body weight traits in this study (Table 4). The SNPs in locus 662G/A with BW1 and BW8 traits, SNPs in 762C/A with ASM and EGGNO traits, SNPs in 844C/T with BW1, BW8 and BW12 traits and SNPs in base number 1025T/C with BW1 trait, have significant association (P < 0.05).The other SNPs didn't have significant associated with growth and production traits.

Table 2. Single nucleotide polymorphisms (SNPs) detected by sequencing in intron 1 of the chicken growth hormone gene

SNP	C→T	C→A	Insertion	G→A	T→C	C→A	A→C	C→A	C→T	C→T	T→C
Location	620	635	648	662	712	762	805	806	844	928	1025
%Frequency	36%	21%	100%	20%	8%	17%	22%	21%	16%	10%	12%

Table 3: Number of chickens, mean, minimum, maximum and standard deviation for the data used for the estimation of genetic parameters

Traits	No. of records	Mean	Minimum	Maximum	standard deviations
BW1	10287	33.8010	22.80	46.30	3.01406
BW8	10247	614.95	300.00	1100.00	110.99426
BW12	10142	1025.73	500.00	1900.00	171.30066
EGG NO	6179	49.79	1	81	12.402
ASM	6194	141.51	110	207	12.361

BW1= body weight at 1 days of age; Bw8= body weight at 8 weeks; BW12= body weight at 12 weeks; EGG NO= egg number; ASM=Age of sexual maturity

Table 4: P-Values of Identified SNPs on Breeding Value of BW1, BW8, BW12, ASM and EGGNO Traits.

Traits	SNP									
	C/T 620	**C/A 635**	**G/A 662**	**T/C 712**	**C/A 762**	**A/C 805**	**C/A 806**	**C/T 844**	**C/T 928**	**T/C 1025**
BW1	n.s	n.s	**0.041**	n.s	n.s	n.s	n.s	**0.048**	n.s	**0.022**
BW8	n.s	n.s	**0.046**	n.s	n.s	n.s	n.s	**0.045**	n.s	n.s
BW12	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**0.015**	n.s	n.s
ASM	n.s	n.s	n.s	n.s	**0.004**	n.s	n.s	n.s	n.s	n.s
EGGNO	n.s	n.s	n.s	n.s	**0.006**	n.s	n.s	n.s	n.s	n.s

DISCUSSION

In recent years, DNA polymorphisms have been widely studied in the GH gene of various animals. In this study, we detected SNPs in cGH gene and analyzed their association with economic traits in Fars indigenous chicken. Eleven point mutations were identified in interon 1 of the cGH gene. Nie *et al*. (2005) also detected 11 SNPs in intron 1, but in other studies only a few cGH gene SNPs in this region had been found [2, 20, 21, 22].

Out of these SNPs, the insertion of T between nucleotide number 647 and 648 was observed in all individuals. Ip *et al*. (2001) found three insertions and 12 substitutions in intron 1 region of the cGH gene of Chinese native chicken [9]. In this study, SNPs at G/A762 showed the significant association with egg production and age of sexual maturity and SNPs at G/A662, C/T844 and T/C1025 of cGH intron 1 have a significant effect on body weight traits via an influence on cGH gene expression. By the other hand, these SNPs might have caused linkage disequilibrium among some other causative polymorphism that influences egg production, age of sexual maturity and body weight at population under study. The other SNPs that we tested failed to exhibit a significant effect with traits.

Previous studies on polymorphisms in cGH introns indicated associations with chicken growth, fat deposition and egg production [2, 21, 23]. An SNP in intron 1 (G/A 119) was reported to be significantly associated with abdominal fat weight and length of small intestine [2]. Another study reported that an SNP with G to A substitution in GH gene was significantly associated with abdominal fat pad weight, abdominal fat pad ratio, and crude fatty content of the breast muscle [24].

A polymorphism located in cGH intron 1 was found to be associated with the selection of growth and production traits [9].

The four polymorphisms identified in SNP of the cGH intron 1 region, which were associated with traits, could be used to select the chicken for growth and egg production traits in molecular marker assisted selection programs.

In this study, one SNP per 62 bp occurred while Nie *et al*. (2005) reported one SNP per 86 bp on average [2]. In the cGH gene, most of these SNPs were located in introns and there was one SNP per 75 bp in introns. In our study, SNP frequency of the cGH gene was higher than several other chicken genes [2]. The high amounts of changes in sequences could be due to the long period of time over which the traits affected by the cGH gene in the population.

CONCLUSION

In conclusion this study showed that there were rich polymorphisms in the intron 1 of chicken GH gene. Four SNPs associated with age of sexual maturity, egg number and body weight traits in Fars native chickens.

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REFERENCES

[1] R Vasilatos-Younken, Y Zhou, X Wang, JP McMurtry, W Rosebrough, E Decuypere, N Buys, VM Darras, V Geyten, Tomas F. *Journal Endocrinol*, **2000**, 166, 609–620.
[2] Q Nie, B Sun, D Zhang, C Luo, NA Ishag, M Lei, G Yang, Zhang X. *Journal of Heredity*, **2005**, 96, 698–703.
[3] AA Alaw Qotbi, Z Ansari Pirsaraei, Seidavi AR. *Annals Biol. Res*, **2012**, 3(2), 1102-1108
[4] EM Shaw, RN Shoffner , DN Foster , Guise KS. *Journal of Heredity*, **1991**, 82, 505-508.
[5] VK Saxena, AK Sachdev; R gopal; Pramod AB. *World's Poultry Science Journal*, **2009**, 65, 37-50.
[6] N Kansaku, G Hiyama, T Sasanami, Zadworny D. *Journal of Poultry Science*, **2008**, 45, 1-6.
[7] B Enayati, Rahimi-Mianji G. *African Journal of Biotechnology*, **2009**, 8 (14), 3154-3159.
[8] B Yan, X Deng, Q Fei, X Hu, C Wu, Li N. *Sci. Bull. Sin*, **2003**, 48, 1304- 1307.
[9] SC Ip, X Zhang, Leung FC. *Exp Biol Med*, **2001**, 226, 458–462.
[10] Q Nie, CY Stephen, X Zhang, FC Leung, Yang G. *Journal of Heredity*, **2002,** 93, 277-279.
[11] International Chicken Polymorphism Map Consortium. *Nature*, **2004**, 432: 717–722.
[12] N Huang, LA Cogburn, SK Agarwal, MARKS HL. *Molecular Endocrinology*, **1993**, 7, 1391-1398.
[13] AS Van Laere, M Nguyen, M Braunschweig, C Nezer, C Collette ,L Moreau, AL Archibald, CS Haley, N Buys, M Tally, G Andersson, M Georges, Andersson L. *Nature*, **2003**. 425, 832–836.
[14] M Amills, N Jimenez , D Villalba, M Tor, E Molina, D Cubilo, C Marcos, A Francesch, A Sanchez, Estany J. *Poultry Science*, **2003**, 82,1485–1493.

[15] A Vignal, D Milan, M SanCristobal, Eggen A. *Genet Sel Evol*, **2002**, 34, 275–305.
[16] Schmid M, Nanda I, Hoehn H, Schartl M, Haaf T, Buerstedde JM, Arakawa H, Caldwell RB, Weigend S, Burt DW, Smith J, Griffin DK, Masabanda JS, Groenen MA, Crooijmans RZ, Vignal A, Fillon V, Morisson M, Pitel F, Vignoles M, Garrigues A, Gellin J, Rodionov AV, Galkina SA, Lukina NA, Ben-Ari G, Blum S, Hillel J, Twito T, Lavi U, David L, Feldman MW, Delany ME, Conley CA, Fowler VM, Hedges SB, Godbout R, Katyal S, Smith C, Hudson Q, Sinclair A, Mizuno S. *Cytogen & Genome Res*, **2005**, 109: 415–479.
[17] SA Mille, DD Dykes, Polesky HF. *Nucl. Acids Res*, **1988**, 16, 1215.
[18] SAS Institute. SAS Users guide: Statistics, Version 7, edition. SAS Institute, Inc. Cary, NC. Pp, **1998**, 113-137.
[19] AR Gilmour, BJ Gogel, BR Cullis, Thompson R. ASReml User Guide, Release 2.0, (NSW Department of Primary Industries, Australia) **2006**.
[20] N Fotouhi, CN Karatzas , U Kuhnlein, Zadworny D. *Theor Appl Genet*, **1993**, 85, 931–936.
[21] U Kuhnlein, L Ni, S Weigend, JS Gavora, W Fairfull, Zadworny D. *Anim. Genet*, **1997**, 28, 116-123.
[22] HC Liu, HJ Kung, JE Fulton, RW Morgan, Cheng HH. Proc. *Natl. Acad. Sci*, **2001**, 98, 9203-9208.
[23] XP Feng, U Kuhnlein, SE Aggrey, JS Gavora, Zadworny D. *Poultry Science*, **1997**, 76, 1770-1775.
[24] M Lei, C Luo, X Peng, M Fang, Q Nie, D Zhang, G Yang, Zhang X. *Poultry Science*, **2007**. 86: 835-842.