Songklanakarin J. Sci. Technol.
36 (2), 137-142, Mar. - Apr. 2014

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Single nucleotide polymorphisms in BMPR-IB and STAT5B genes and their association with growth and reproductive traits in chicken

Shahram Niknafs[1]*, Ardeshir Nejati Javaremi[2], and Mostafa Sadeghi[2]

[1] *Young Researchers and Elites Club, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.*

[2] *Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.*

Received 10 May 2013; Accepted 10 December 2013

Abstract

The aim of the current study was to investigate the association of G4533815A SNP in STAT5B and A287G SNP in BMPR-IB genes with growth and reproduction related traits in chicken. A sample of 205 individuals from breeding station of Mazandaran native chicken population was selected randomly. All of the individuals were genotyped for both SNPs using PCR-RFLP technique. Marker-trait association analyses were performed using estimated breeding value of the traits as dependent variable in GLM procedure of SAS 9.1. Results suggested that breeding value least square means for genotypes of G4533815A SNP is significantly differed from each other for traits of body weight at 8 and 12 weeks ($P<0.01$). In the case of BMPR-IB gene, no significant difference was found. In conclusion, STAT5B gene may be associated with body growth in chicken and may be considered in Marker Assisted Selection program to improve chicken growth performance.

Keywords: chicken, economic traits, breeding value, SNP

1. Introduction

The genetic resource based on indigenous chickens could form the basis for genetic improvement and diversification to produce breeds adapted to local conditions (Hoffmann, 2005). Despite their low productivity, the indigenous genotype birds have a number of advantages: broodiness and reproduction without the need for artificial incubation, being agile and escaping predators, and being more resistant to diseases and infestations compared to commercial broilers or layers (Pym, 2010).

Growth and reproductive performance controlled by many genes are important and economic traits in the poultry industry. Integration of novel technologies with traditional methods and identification of effective genes have provided possibilities of more balanced selection. The use of DNA markers to define the genotype and predict the performance of an animal is a powerful aid to animal breeding (Gholizadeh *et al.*, 2008).

Signal transducers and activators of transcriptions (STAT) are a family of latent cytoplasmic proteins that are involved in the signal transduction pathway of multiple cytokines and peptide hormones (Darnell, 1997). Two members of this family, STAT5A and STAT5B are two closely related members which are activated by a wide variety of cytokines such as interleukins, erythropoietin, growth hormone, and prolactin (Hennighausen *et al.*, 2008). STAT5B gene disruption leads to loss of the GH pulse-regulated male mouse pattern of postpubertal body growth rate and liver gene expression (Park *et al.*, 1999). In addition to mediating the actions of growth hormone, STAT5B is also important for the actions of many other growth factors and cytokines

* Corresponding author.
Email address: niknafssh@gmail.com

(interleukins 26 and interferons 20) (Kofoed *et al.*, 2003).

The bone morphogenetic proteins (BMPs) belonging to the transforming growth factor-β (TGF-β) family play a key role in ovarian physiology of domestic animals (Dube *et al.*, 1998; Shimasaki *et al.*, 1999). A nonconservative substitution (Q249R) in the BMPR-IB sequence was associated with the proliferation characters of some ewe breeds (Wilson *et al.*, 2001; Mulsant *et al.*, 2001; Souza *et al.*, 2001). In the chicken ovary, granulosa cells are major target for BMPs and it was suggested that mRNA levels for BMPR-IB in granulosa cells are higher than in theca cells (Onagbesan *et al.*, 2003). BMPR are also involved in the formation of primordial follicles in hamster ovary (Wang *et al.*, 2009).

As the objective of the current study, we investigated the association of G4533815A SNP in STAT5B gene and A287G SNP in BMPR-IB gene with growth and reproductive related traits in chicken.

2. Material and Methods

2.1 Experimental population

In 1986 about 5000 cocks and hens were purchased from rural regions across the Mazandaran province and kept in a quarantine farm for a year. In 1987 about 2500 birds of two sexes were kept to produce hatching eggs and chicks produced from these eggs were transferred to the station in 1988. Since then birds have been individually tagged and trap nest has been used for pedigree recording. Genetic evaluation of the birds for body weight at 8 weeks, age of the hens at first egg, average egg weight and total number of eggs laid during first 12 weeks after flock maturity (when 5% of the flock are in egg production) have been performed. Economic indices were calculated for these traits and birds of two sexes were selected based on their aggregate genotypes for these traits (Khadem *et al.*, 2003). Parents of each generation (100 cocks and 800 hens) were selected from among 6000 pedigreed and performance recorded birds produced each generation (Enayati *et al.*, 2009). A total of 205 individuals from Mazandaran Native Chicken, including 10 males and 195 females which were reared in Native chicken breeding station of Mazandaran were selected. The individuals belonged to generation 17 of the breeding station pedigreed animals which were developed by crossing 80 sires and 751dams from generation 16.

2.2 Traits measured

Whole information data file consisted of three registered fixed effect factors (generation, sex and hatch) and 11 recorded traits including body weight at hatch (BW1), body weight at age of 8 (BW8), 12 (BW12) weeks, body weight at sex maturation (WSM), age at first egg (ASM), egg number (EN), first egg weight (EW1), average egg weight at age of 28 (EW28), 30 (EW30), 32 (EW32) weeks and average egg weight for the first 12 weeks of production (EW12). BW1, BW8 and BW12 were measured in both male and female chicken. Moreover, three combined traits consisting of AV (average of EW28, EW30 and EW32), intensity of egg production [EINT = (Egg Number/Days Recording) × 100] and egg mass (EM = EN × EW12) were analyzed and variance components were also estimated for them.

2.3 PCR-RFLP assay

Blood was sampled from plumage veins and was preserved in tubes containing EDTA as an anticoagulant. DNA was isolated by a slightly modified standard salting out procedure described by Miller *et al.* (1988); 554 (including G4533815A SNP) and 581 (including A287G SNP) base pairs (bp) fragments of the STAT5B and BMPR-IB genes were respectively amplified by polymerase chain reaction. For STAT5B gene, PCR in a total volume of 25 µl included 1 µL of pooled DNA (80-100 ngµL), 1 µL (10 µM) of each primer (Table 1), 0.5 µL mix dNTP (10mM), 2.5 µL of 10 × PCR buffer, and 1 U of Taq DNA polymerase. Thermal-temporal protocol was as follows: initial denaturation was at 95°C for 5 min; followed by 34 cycles. Each cycle consisted of 30 s at 94°C, 45 s at 58°C, 50 s at 72°C for denaturation, annealing and extension steps respectively; and final extension was at 72°C for 5 min. In the case of BMPR-IB gene, total volume of 25 µl was as for STAT5B. But the Thermal-temporal protocol was: initial denaturation at 94°C for 5 min; followed by 35 cycles. Each cycle consisted of 30 s at 94°C, 30 s at 58°C, 30 s at 72°C for denaturation, annealing and extension steps respectively; and final extension at 72°C for 10 min. PCR products of STAT5B and BMPR-IB were respectively digested by restriction enzymes *Msp*I and *Hind*III for 14-16 hours at a temperature of 37°C. Finally, digestion products were electrophoresed on 2% agarose gel and stained with ethidium bromide.

Table 1. Primers used for amplification of the genes

Gene	SNP	Primer Sequence	
STAT5B	G4533815A	5‘-CCATCCCTTCCTGGTGCAGT-3‘	Forward
		5‘-ACTGCTGCCATTTCCCTTTG-3‘	Reverse
BMPR-IB	A287G	5‘- GCTATGGGGAAGTCTGGATG-3‘	Forward
		5‘- TGCCTTTAATGTCTGCCGC-3‘	Reverse

2.4 Genetic analysis and breeding value estimation

Visual FoxPro 9.0 software, the relational database management system, was used to construct the reference file including pedigree and records. Descriptive statistics and model fitting were carried out using SAS 9.1 package. Fixed effect factors and their interactions were considered in utilized animal model provided if they had significant effect. Genetic analyses were performed using ASREML software (Gilmour *et al.*, 2006). Breeding values of growth and egg production traits was estimated by univariate animal model. The model used in matrix notation was as follows: $y = Xb + Za + e$ Where: y = vector of observations; b = vector of fixed effects of generation, sex and hatch; a = vector of random direct genetic effects; e = vector of random residual effects; X and Z are incidence matrices relating the observations to the respective fixed and direct genetic effects.

2.5 Marker-trait association analysis

Allelic and genotypic frequencies of the SNPs were calculated and Chi-Square test was performed to examine Hardy-Weinberg equilibrium. Marker-trait association analysis was conducted using the following model including breeding values (as dependent variables) and genotypes (as independent variables) in GLM procedure of SAS 9.1. The significant differences of least squares means (LS means) were tested with Tukey–Kramer's multiple range tests, and a P-value of ≤ 0.05 was considered statistically significant. $Y_{ijk} = \mu + S_i + B_j + e_{ijk}$ where: Y_{ijk} = estimated breeding values of the traits, μ = population mean, Si = fixed effect of G4533815A SNP genotypes, B_j = fixed effect of A287G SNP genotypes, e_{ijk} =residual random error. There was no significant interaction between the genes additive effects so interactions were not considered in the model.

3. Results

3.1 Genotyping and frequencies

For the STAT5B SNP, non-digested (554 bp) and digested (477/77 bp) fragments were considered respectively as alleles B and C. As for BMPR-IB SNP, non-digested (581 bp) and digested (294/287 bp) fragments were considered respectively as alleles G and A. Genotypic and allelic frequencies are presented in Table 2. Genotypic frequencies of BB, BC and CC (STAT5B genotypes) were 0.063, 0.348 and 0.589 respectively, and for AA, AG and GG (BMPR-IB genotypes) were 0.349, 0.544 and 0.107.

3.2 Marker-trait association

Statistical description of data set is presented in Table 3. Least square means of breeding value for the G4533815A SNP (STAT5B) and A287G SNP (BMPR-IB) genotypes can be seen in Table 4. Marker-trait association analysis results showed that there were significant differences among breeding value LSmeans of the G4533815A SNP genotypes

Table 2. Genotypic and allelic frequencies

Gene	Genotype	Frequency	Allele	Frequency
STAT5B	BB	0.063	B	0.237
	BC	0.348		
	CC	0.589	C	0.763
BMPR-IB	AA	0.349	A	0.621
	AG	0.544		
	GG	0.107	G	0.379

Table 3. Statistical description of data set for growth and egg production traits

Traits	No of Animal in Data File	Mean	Coefficient of Variation
BW1	35287	35.53	8.15
BW8	43067	563.7	17.09
BW12	38297	953.9	14.49
WSM	31147	1694	11.90
ASM	31349	165.5	9.23
EN	31349	36.66	39.78
EW1	27294	41.21	15.74
EW28	17225	46.91	8.48
EW30	19031	48.12	8.50
EW32	18955	49.22	8.29
EW12	18847	46.62	9.28
AV	28725	46.84	13.05
EM	28725	1768	39.89
EINT	31349	57.05	33.29

for body weight at 8 and 12 weeks of age (P<0.01). For both traits homozygote genotype of CC showed higher average breeding value than BB genotype, while differences between CC and BC were not significant (Table 4). No significant association between BMPR-IB SNP and breeding value of the investigated traits was found.

4. Discussion

Application of MAS in breeding programs requires advances in some areas like detection and estimation of associations of identified genes and genetic markers with economic traits. Candidate gene approach directly tests the effects of genetic variants of a potentially contributing gene in an association study (Jennifer *et al.*, 2000). Phenotypic evaluation is critical to establish marker-assisted associations or perform the candidate gene validations required to conduct MAS. Here, high quality phenotyping is necessary (Guimarães *et al.*, 2007). Up to now, a majority of association studies, especially in chicken, have been performed using phenotypic information. Phenotypic performance includes two major genetic (additive and non-additive) and environmental factors (Bourdon, 1997), of which the non-heritable portion (non-additive and environmental) may affect marker-trait association analysis results. Therefore, using estimated breeding value instead of phenotypic performances in such studies probably suggests more reliable and replicable results. Fortunately, we could access pedigree information for our genetic analyses.

Two SNP makers of G4533815A and A287G respectively in STAT5B and BMPR-IB genes were investigated in the Mazandaran indigenous chicken population. In the case of STAT5B gene, the frequency of allele C in this study is higher than in the population studied by Ou *et al.* (2009). This may be due to implementation of 18 generations of selection in our studied population. Concerning the BMPR-IB gene, A and G allele frequencies were in the range reported by Zhang *et al.* (2008). They investigated the frequency of these alleles in 4 Chinese native chickens and a synthetic broiler line.

Our results showed that G4533815A SNP of chicken STAT5B gene is associated significantly with additive genetic effect of body weight at 8 and 12 weeks of age (Table 4). Homozygote genotype of CC had a higher average breeding value than BB genotypes. Significant effect of this SNP on age at first egg was reported previously by Ou *et al.* (2009). Generally, previous studies showed that STAT5B may be involved physiologically in growth hormone actions and body growth (Park *et al.*, 1999; Udy *et al.*, 1997; Rosenfeld *et al.*, 2007; Teglund *et al.*, 1998). Contrary to expectation, we found no significant association between BMPR-IB, as a well-known effective gene for reproductive traits (Zhang *et al.*, 2008; Wilson *et al.*, 2001; Mulsant *et al.*, 2001; Souza *et al.*, 2001), and reproductive as growth traits. Zhang *et al.*

Table 4. Breeding value LSmeans for genotypes of G4533815A SNP (STAT5B) and A287G SNP (BMPR-IB) [least squares means ± SEM]. BW1, BW8, BW12 and WSM are Body Weight at Hatch, 8, 12 weeks of age and sexual maturity respectively. ASM (Age at first egg), EN (Egg Number), EW1 (Weight of First Egg). EW28, EW30 and EW32 are Average Egg Weight at 28, 30 and 32 weeks of age respectively. EW12 (Average egg weight for First 12 weeks of production), AV (Average for EW28, 30 and 32), EM [Egg Mass (=EN×EW12)], EINT [Egg Production Intensity = (Egg Number/Days Recording)× 100]. ^{ab} Means within a row with no common superscript differ significantly (P < 0.05)

Gene	STAT5B			BMPR-IB		
Genotype	BB	BC	CC	AA	AG	GG
BW1	-0.18±0.159	0.137±0.208	0.229±0.458	0.101±0.226	-0.073±0.195	0.158±0.361
BW8	61.03±6.64^{b}	66.02±7.62^{ab}	72.09±3.47^{a}	63.69±3.75	64.18±3.24	71.27±6.00
BW12	105.21±3.79^{b}	112.05±5.9^{ab}	119.68±4.97^{a}	109.65±5.4	113.05±4.6	114.24±8.6
WSM	-12.24±2.9	-19.17±2.7	-20.21±2.7	-16.38±3.7	-16.33±2.93	-18.91±1.28
ASM	-22.38±0.64	-23.64±0.84	-25.10±2.85	-23.81±2.6	-24.12±1.78	-23.2±2.28
EN	14.17±0.18	14.08±0.23	14.01±0.51	14.14±0.25	14.33±0.22	13.79±0.40
EW1	-1.98±0.126	-2.07±0.165	-2.04±0.36	-2.21±0.178	-2.04±0.154	-1.83±0.286
EW28	1.00±0.13	0.768±0.17	1.38±0.377	1.06±0.186	0.966±0.16	1.13±0.297
EW30	0.62±0.15	0.414±0.20	0.975±0.45	0.610±0.222	0.658±0.192	0.744±0.355
EW32	0.465±0.167	0.277±0.220	0.610±0.483	0.293±0.238	0.210±0.205	0.848±0.380
EW12	1.25±0.145	1.17±0.190	1.66±0.419	1.21±0.206	1.26±0.178	1.60±0.330
AV	1.40±0.110	1.22±0.145	1.63±0.319	1.35±0.157	1.32±0.135	1.57±0.251
EM	657.25±7.9	649.0±10.4	669.15±12.7	657.06±11.2	665.04±9.67	653.30±1.9
EINT	21.86±0.33	21.78±0.44	20.97±0.97	21.38±0.476	21.93±0.411	21.31±0.762

(2008) stated that A287G SNP of chicken BMPR-IB is associated with egg production from 47 to 56 weeks. Regarding BMPR-IB gene, obtaining no result may due to the small size of the investigated sample or method of sampling. Moreover, this gene may not affect reproductive traits of chicken as it does in sheep, in which effectiveness of BMPR-IB on reproduction has been proved frequently (Wilson *et al.*, 2001; Mulsant *et al.*, 2001; Souza *et al.*, 2001).

5. Conclusion

We conclude that G4533815A SNP of chicken STAT5B gene is associated with body growth and it can be an informative SNP marker for use in the MAS program.

Acknowledgements

We sincerely thank the farm attendants and staff who worked on data collection and let us to provide blood samples at the breeding station of Mazandaran native chicken.

References

Bourdon, M.R. 1997. Undestanding Animal Breeding. 1st ed. Prentice Hall, Upper saddle River, New Jersey 07458.

Darnell, J.E. 1997. STATs and gene regulation. Science. 277 (5332), 1630-5.

Dube, J.L., Wang, P., Elvin, J., Lyons, K.M., and Celeste, A.J., Matzuk, M.M. 1998. The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Molecular Endocrinology. 12 (12), 1809-17.

Enayati, B. and Rahimi, G. 2009. Genomic growth hormone, growth hormone receptor and transforming growth facor β-3 gene polymorphism in breeder hens of Mazandaran native fowls. African Journal of Biotechnology. 8 (14), 3154-3159.

Gholizadeh, M., Rahimi, M.G. and Sayahzadeh, H. 2008. Potential use of molecular markers in the genetic improvement of livestock. Asian Journal of Animal and Veterinary Advances. 3 (3), 120-128.

Gilmour, A.R., Gogel, B.J., Cullis, B.R. and Thompson, R. 2006. ASReml User Guide, Release 2.0, (NSW Department of Primary Industries, Australia).

Guimarães, E.P., Ruane, J., Scherf, B.D., Sonnino, A. and Dargie, J.D. 2007. Marker-assisted selection. Food and agriculture organization of the United Nations (FAO).

Hennighausen, L. and Robinson, G.W. 2008. Interpretation of cytokine signaling through the transcription factors STAT5A and STAT5B. Genes Development. 22 (6), 711-21.

Hoffmann, I. 2005. Research and investment in poultry genetic resources? challenges and options for sustainable use. World's Poultry Science Journal. 61 (1), 57-70.

Jennifer, M., Kwon, M.D., Alison, M. and Goate, D.P. 2000. The candidate gene approach. Alcohol Research and Health. 24 (3), 164-168.

Kamalzadeh, A., Rajabbaigy, M. and Kiasat, A. 2008. Livestock production systems and trends in livestock industry in Iran. Journal of Agriculture and Social Sciences. 4, 183-188.

Khadem, A., Hafezian, H. and Rahimi-Mianji, G. 2010. Association of single nucleotide polymorphisms in IGFI, IGF-II and IGFBP-II with production traits in breeder hens of Mazandaran native fowls breeding station. African Journal of Biotechnology. 9 (6), 805-810.

Kofoed, E.M., Hwa, V., Little, B., Woods, K.A., Buckway, C.K., Tsubaki, J., Pratt, K.L., Bezrodnik, L., Jasper, H., Tepper, A., Heinrich, J.J. and Rosenfeld, R.G. 2003. Growth hormone insensitivity associated with a STAT5b mutation. The New England Journal of Medicine. 349 (12), 1139-47.

Miller, S.A., Dykes, D.D. and Polesky, H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research. 16 (3), 12-15.

Mulsant, P., Lecerf, F., Fabre, S., Schibler, L., Monget, P., Lanneluc, I., Pisselet, C., Riquet, J., Monniaux, D., Callebaut, I., Cribiu, E., Thimonier, J., Teyssier, J., Bodin, L., Cognie, Y., Chitour, N. and Elsen, J.M. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceeding of the National Academy of Science of the USA. 98(9), 5104-9.

Onagbesan, O.M., Bruggeman, V., Van As, P., Tona, K., Williams, J. and Decuypere, E. 2003. BMPs and BMPRs in chicken ovary and effects of BMP-4 and -7 on granulosa cell proliferation and progesterone production in vitro. The American Journal of Physiology - Endocrinology and Metabolism. 285(5), 973-83.

Ou, J.T., Tang, S.Q., Sun, D.X. and Zhang, Y. 2009. Polymorphisms of three neuroendocrine-correlated genes associated with growth and reproductive traits in the chicken. Poultry Science. 88(4), 722-727.

Park, S.H., Liu, X., Hennighausen, L., Davey, H.W. and Waxman, D.J. 1999. Distinctive roles of STAT5a and STAT5b in sexual dimorphism of hepatic P450 gene expression, Impact of STAT5a gene disruption. Journal of Biological Chemistry. 274(11), 7421-30.

Pym, R. 2010. Poultry genetics and breeding in developing countries. FAO, Poultry Development Review.

Rosenfeld, R.G., Belgorosky, A., Camacho-Hubner, C., Savage, M.O., Wit, J.M. and Hwa, V. 2007. Defects in growth hormone receptor signaling. Trends in Endocrinology & Metabolism. 18(4), 134-41.

Shimasaki, S., Zachow, R.J., Li, D., Kim, H., Iemura, S., Ueno, N., Sampath, K., Chang, R.J. and Erickson, G.F. 1999. A functional bone morphogenetic protein system in the ovary. Proceeding of the National Academy of Science. 96(13), 7282-7.

Souza, C.J., MacDougall, C., Campbell, B.K., McNeilly, A.S. and Baird, D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. Journal of Endocrinology. 169(2), R1-6.

Teglund, S., McKay, C., Schuetz, E., van Deursen, J.M., Stravopodis, D., Wang, D., Brown, M., Bodner, S., Grosveld, G. and Ihle, J.N. 1998. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell. 93(5), 841-50.

Udy, G.B., Towers, R.P., Snell, R.G., Wilkins, R.J., Park, S.H., Ram, P.A., Waxman, D.J. and Davey, H.W. 1997. Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proceeding of the National Academy of Science. 94(14), 7239-44.

Wang, C. and Roy, S.K. 2009. Expression of bone morphogenetic protein receptor (BMPR) during perinatal ovary development and primordial follicle formation in the hamster: Possible Regulation by FSH. Endocrinology. 150(4), 1886-1896.

Wilson, T., Wu, X.Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., McEwan, J.C., O'Connell, A.R., McNatty, K.P. and Montgomery, G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction. 64 (4), 1225-35.

Zhang, N.B., Tang, H., Kang, L., Ma, Y.H., Cao, D.G., Lu, Y., Hou, M. and Jiang, Y.L. 2008. Associations of single nucleotide polymorphisms in BMPR-IB gene with egg production in a synthetic broiler line. Asian Australian Journal of Animal Science. 21 (5), 628-632.